Sudan University of Science and Technology
College of Graduate Studies and Scientific Research

Prevalence, Risk Factors and Public Health Implication of Listeria Monocytogenes in Ready to Eat Camel Meat (Camelus dromedaries) in Tambool Area– Sudan.

الانتشار وعوامل الخطر والتأثير الصحي العام لبكتريا اللستريا مونوسيتوجينس في لحوم الابل الجاهزة للأكل في منطقة تمبول- السودان

By:

MOHAMMED BABIKER MOHAMMED HASSAN

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Supervisor:
Prof. Dr. Mohamed Abdelsalam Abdalla
Department of Veterinary Preventive Medicine and Public Health
Dean College of Veterinary Medicine
Sudan University of Science and Technology

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Dedication

To my mother, father and my brother and sincerely to my sister Dr. Amna Babiker.

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AIDS	Acquired Immune Deficiency Syndrome
ATR	Acid Tolerance Response
CAMP	Christie Atkins Munch Peterson
CCPs	Critical Control Points
CDC	Centers of Disease Control and Prevention
CNS	Central Nervous System
CSF	Cerebro Spinal Fluid
Csps	Cold Shock Proteins
DNA	Deoxyribo Nucleic Acid
ERS	Economic Research Service
FAO	Food and Agricultural Organization
FDA	United State Food and Drug Administration
GAD	Glutamate Decarboxylase
HACCP	Hazard Analysis of Critical Control Points
HIV	Human Immune Deficiency Virus
OXA	Oxford Agar
PALCAM	Polymixin Acriflavin Lithium Chloride Ceftazidime Aesculin Mannitol
PH	Hydrogen ion concentration
RNA	Ribo Nucleic Acid
RTE	Ready-to-Eat

SA	*Staphylococcus aureus*
Spp.	Species
SPSS	Statistical Package for Social Sciences
SSP	Salt Shock Proteins
TSA	Tryptic Soy Agar
USFDA	United States Food and Drug Administration
WHO	World Health Organization
χ^2	Chi-square

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ABSTRACT

Listeriosis is one of the important food-borne bacterial zoonotic diseases caused by Listeria monocytogenes, as a result of food and environmental contamination as well as zoonotic infections. This disease is becoming an emerging bacterial disease, with low incidence but high case fatality rate.

The present study was undertaken to determine the presence of *Listeria monocytogenes* in raw camel meat of Tambool Town market and abattoir. A cross-sectional study was conducted from May to September 2017 to isolate *Listeria monocytogenes* from swab samples from camel meat from abattoir, butcher shops equipments.

A total of 865 swab samples comprising of 380 from the abattoir, 380 from butcher shops were collected using systematic random sampling technique and 105 swabs were collected from equipments.

Questionnaire survey was conducted to assess the hygienic practices of meat production in raw meat of market and abattoir and possible risk factors regarding the contamination of meat. Listeria monocytogenes was isolated and identified using standard bacteriological techniques.

The overall prevalence of Listeria monocytogenes identified was 5.5%, and 6.6%, 2.6% and 7.6% from abattoir, butcher shops and equipments respectively.

In addition, the presence of Listeria monocytogenes attributed to unclean working environment and improper handling of meat till it reaches to the consumer. Preventive measures to avoid the presence of pathogenic Listeria monocytogenes in raw meat and meat products should be undertaken, emphasizing the need for improved hygienic practices during meat production and also during distribution and consumption of the final products.

ملخص الأطروحة

يعد مرض الليستريا واحد من أهم الأمراض الحيوانية المنشأ التي تنقلها الأغذية والتي تسببها الليستيريا مونوسيتوجينيس، نتيجة التلوث الغذائي والبيئي.

هذا المرض أصبح مرض بكتيري ناشئ، مع انخفاض معدل حدوثه ولكن هالك ارتفاع في معدل الموت.

أجريت هذه الدراسة الحالية لتحديد وجود الليستريا مونوسيتوجينس في لحوم الإبل الخام في سوق ومسلخ مدية تمبول.

أجريت دراسة مستعرضة من مايو إلى سبتمبر 2017م لعزل باكتريا الليستريا مونوسيتوجينس من عينات مسحة من لحوم الإبل من المسلخ، ومحلات بيع اللحوم، وكذلك من المعدات المستخدمة في تجهيز اللحوم.

تم جمع عدد 865 عينة مسحة مكونة من 380 من المسلخ ، 380 من محلات بيع اللحوم باستخدام تقنية أخذ العينات العشوائية المنهجية وكذلك تم جمع عدد 105 عينة مسحات من المعدات المستخدمة في تجهيز اللحوم.

أجري مسح استبيان لتقييم الممارسات الصحية لإنتاج اللحوم في اللحوم الجاهزة في السوق والمسلخ وكذلك لمعرفة عوامل الخطر المحتملة فيما يتعلق بتلوث اللحوم.

تم عزل الليستريا مونوسيتوجينيس وتحديدها باستخدام التقنيات البكتريولوجية القياسية.

بلغ معدل انتشار باكتريا الليستريا مونوسيتوجينس 5.5٪. وكذلك 6.6 ٪، 2.6 ٪ و 7.6 ٪ من المسلخ، محلات بيع اللحوم والمعدات الميتخدمة في تجهيز اللحوم على التوالي.

بالإضافة إلى ذلك، فإن وجود الليستيريا مونوسيتوجينيس يعزى إلى بيئة العمل غير النظيفة والتعامل غير السليم مع اللحوم حتى تصل إلى المستهلك.

ينبغي اتخاذ تدابير وقائية لتجنب وجود الليستيريا مونوسيتوجينيس الممرضة في اللحوم النيئة ومنتجات اللحوم، كذلك التأكيد على الحاجة إلى تحسين الممارسات الصحية أثناء إنتاج اللحوم وكذلك خلال توزيع واستهلاك المنتجات النهائية.

Chapter One

Introduction

Animal resources in the Sudan comprise of sheep, goat, cattle, camel, poultry and wild-game. Establishing a hygienic program for exported mutton is required in order to enable the Sudan facing the international trade parameters. This entails a vital need to improve the slaughter houses and to impose strict hygienic measures to provide healthy and wholesome meat to fulfill the international requirements (International Committee of Microbiological Standards of Foods (ICMSF), Gracey 1998; Gracey 1992).

Tambool town is one of the famous town in AlGazera State, it is located in eastern part of AlGazera State, near to Rufaa town -35Kilometers approximately (map). Camel is one of the most fundamental pillars of the national economy and food security for many countries in the world. Camel can provide a substantial amount of high quality meat. The demand for camel meat appears to be increasing due to health reasons, as they produce carcasses with less fat as well as having less cholesterol and relatively high polyunsaturated fatty acids than other meat animals (Elgasim et al., 1987; El-Faer et al.1991; Elgasim and Alkanhal, 1992; Dawood and Alkanhal, 1995).

Meat is one of the highly perishable foods because of its high nutritional contents, enzymatic action and the presence of microorganisms (bacteria, yeasts and molds) which may result in oxidative rancidity, discolouration, mouldiness, off flavour, sliminess. The major source of these deteriorative changes being microorganisms, this renders the meat unacceptable and unfit for human consumption (Ajiboye *et al* 2011).

Meat is an ideal medium for the development and reproduction of micro-organisms particularly bacteria and rapid growth can be expected unless control is affected. Microbial contamination can cause spoilage of meat, reduces shelf life of

meat and causes public health hazards (Rao, 1992). The microbial contamination of carcasses occurs mainly during processing and manipulation such as skinning Evisceration, storage and distribution at slaughterhouses and retail establishments (Gill, 1998; Abdalla *et al*., 2009). In developing countries, some traditional methods of handling, processing and marketing of meat undermine quality whereas poor Sanitation leads to considerable loss of product as well as to the risk of food-borne disease (Garcia de siles *etal*., 1997).

Bacteria which are responsible for the most food borne disease contaminate meat directly and indirectly from animal excreta at slaughter process; also they may be transferred from the surfaces, utensils and other equipment (Arnold International Students, 1993). The external contamination of meat constitutes a constant problem in most developing countries abattoirs where they are potential sources of infection (Lawrie, 1979). The microbial surface contamination of carcasses has been repeatedly reported to have a significant effect on the meat shelf life. Moreover, Contaminants may also include pathogens which can penetrate into the meat (Elmossalami and Wassef, 1971).

Slaughtering is a suitable progress for the contamination of the carcass by partially pathogenic bacteria (Forsythe and Hayes, 1998) so that all surfaces in contact with meat should be taken under control or kept clean to minimize the risk of bacterial contamination (Butterorth-Heineinann, 2000). Unsanitary methods spread such diseases as Salmonellosis, Cholera, *E.coli* food poisoning and Listeriosis that cause Contamination of the meat, a serious public health concern (Neil Trent *et al*., 2002). Fecal matter is a major source of contamination and could reached carcasses through direct deposition as well as by indirect contact through contaminated and unclean carcasses equipment, surfaces, workers, installations and air (Borch and Arnder, 2002).

The microbiological contamination of carcasses occurs mainly during processing and manipulation, such as skinning, evisceration, storage and distribution at slaughterhouses and retail establishments (Gill, 1998; Abdalla et al., 2009). Most microbial contaminants of carcasses represent commensal bacteria, some microorganisms such as *Salmonella* spp., *Escherichia coli*O157::H7, and *Listeria monocytogenes* pose a threat toconsumer health (Gustavsson and Borch, 1993; Samelis et al., 2001). There were significant increases in total bacterial countsat skinning points than that at washing operations; also, dirty workers hands, clothes and equipments of the slaughterhouse acted as intermediate sources of contamination of meat (Gill, 1998; Gilmour et al., 2004; AbdelSadig, 2006; Abdalla et al., 2009).Ali (2007), recorded high contamination level on flanksite and lower contamination level on rump sites during skinning.

Listeria are Gram-positive, non-spore-forming, and non-acid-fast rods.Six species of *Listeria* are recognized *L. murrayi, L. grayi, L. ivanovii, L. innocua,L. welshimeri* and *L. monocytogenes.* The primary pathogenic species is *L. monocytogenes*.

Listeria monocytogenes and other *Listeria* species are widely spread in the environment. Due to the fact that ten people in Austria and Germany died in 2009 and 2010 after eating the deadly cheese and several more were taken ill owing to *Listeria* contamination, the routes of Listeria spreading in the food processing plants should be investigated more intensely in the future. The risk of contamination with *Listeria* in the red meat processing industry has to be considered as rather probable. Possible *Listeria* cross-contamination by employees, equipment, and environment surfaces, animal skin, food additives, packing material and many other sources has been reported (Grif K., 2003), thus effective sanitation programmes in the slaughtering and meat processing plants are strongly recommended. The carcasses and their products may be contaminated during slaughtering and meat processing,

thus they can be recognized as feasible transmission routes of *Listeria* to humans (Nesbakken *et al.* 1996; EFSA 2007). However, relatively small attention is given to the air acting as a potential vector of contaminants of carcasses and equipment (Kang & Frank 1989). *Listeria* can potentially become airborne owing to the sanitation maintenance and meat processing, especially within solid particles suspended into the air, as single organisms or in droplets in the form of aerosols created by the use of water sprayers (Zottola 1991). Therefore, it could be potentially transmitted by air and colonise various surfaces including raw and ready-to-eat meat products (Burfoot *et al.* 2000). Furthermore, recent investigations in aerosol studies have made this theory even more credible since McEvoy *etal.* (1999) reported the possibility of the airborne.

In Sudan, hygienic measures to control microbial contamination of meat are unsatisfactorily applied. Storage at refrigerator temperatures is still one of the most effective practices for improving the safety of fresh meat. However, some Butcheries still use poor refrigeration, in addition, the retail raw meat in most of Butcheries is presented exposed to environmental pollution which might lead to increased bacterial contamination.

Objectives:

General Objective:

To determine the prevalence and Risk factors of *L. monocytogenes* in ready to eat Camel meat in Tambool Town Slaughter point (Algazera State, Central Sudan)".

Specific Objectives:

1. To identify the main points of contamination of camel carcasses during slaughtering operations.
2. To assess the hygienic practices of meat production in market and abattoir and possible risk factors regarding the contamination of meat.

Chapter Two

Literature Review

1.1. Listeria Genus and Taxonomy

The genus *Listeria* includes Gram-positive, non-spore forming, catalase positive rod shaped bacteria, which were once classified into the family Corynebacteriaceae. It was named Bacterium monocytogenes by Murray et al. (1926), whom isolated a 1–2 μm long and 0.5 μm wide round-ended Gram-positive rod in dead laboratory rabbits and guinea-pigs in Cambridge, United Kingdom (Farber and Peterkin, 1991). Following unusual deaths of gerbils in South Africa in the late 1920s, this bacterium was named *Listerella hepatolytica* by Pirie in honor of Lord Joseph Lister who determined that in order to prevent infections, surgeons need to sterilize their instruments before each operation (Ryser, 1999a). Because the strains isolated by Murray et al. (1926) and Pirie (1927) showed great similarity, the bacterium was renamed *Listerella monocytogenes*. However, the generic name Listerella had previously been used for a protozoa and in 1940, Pirie thus proposed changing the name to *Listeria monocytogenes*. This name was accepted, even though the genus name already existed in botanical taxonomy, including an orchid named Listeria, and in zoology, including a diptera called Listeria (Seeliger, 1961). Genera of Listeria and Brochothrix are members of the family 2 Listeriaceae, the order Bacillales, the class Bacilli and the phylum Firmicutes (Ludwig et al., 2009). Currently, it is widely accepted that the core phylogeny of *Listeria* consists of six different species: *L. monocytogenes, L. innocua, L. seeligeri, L. welshimeri, L. grayii and L. ivanovii* (Wiedmann, 2002). A seventh species, Listeria murrayi, was previously recognized in the Listeria genus; however, DNA-DNA hybridization analysis, multiolocus enzyme electrophoresis, and rRNA restriction fragment length polymorphism analysis, proved that L. murrayi appeared to be subspecies within L. grayii.

(Boerlin et al. 1991, 1992; Rocourt et al. 1992). In addition, recent studies described the occurrence of atypical hemolytic L. innocua strains that carry the *Listeria* pathogenicity island I (Graves et al., 2009; Johnson et al., 2004). Most recently, studies have proposed recognition of two novel species within the *Listeria* genus, including Listeria marthii and Listeria rocourtiae (Graves et al., 2009).

Listeria species appear as small rods ranging in size from 0.4 to 0.5 by 1-2µm, and sometimes are found to be arranged in short chains when viewed under the microscope. A coccoid appearance may be seen in direct smears. Listeria produces flagella at room temperature and exhibit a tumbling motion when examined in broth and a swarming motility can be observed in semi-soft agar at 30°C (Roberts et al., 2009), but flagella are not produced at 37°C (Peel et al., 1988).

1.2. Listeria in Nature

All Listeria species are ubiquitous in nature and the bacterium often is described to demonstrate a plant saprophyte lifestyle. Listeria commonly is detected in soil (Weis *et al.*, 1975), water (Watkins *et al.*, 1981), manure, sewage (Colburn *et al.*, 1990; Watkins *et al.*, 1981), vegetation (Weis et al., 1975), animal feed (Wiedmann *et al.*, 1996), and farm environments (Fenlon *et al*., 1996; Nightingale *et al.*, 2004). L. monocytogenes has also been isolated from at least 42 species of wild and domestic mammals and 17 avian species, including domestic and game fowl as well as crustaceans, fish, oysters, ticks, and flies (Schuchat *et al.*, 1992). Also, this pathogen may be carried in the intestinal tracts of a small percentage of the human population without apparent symptoms (Rouquette et al, 1996; Grif et al., 2003).

Studies aimed at isolating Listeria in natural environments not associated with domestic livestock indicated that other Listeria spp. were detected at

higher frequencies than L. monocytogenes (MacGowan et al., 1994). The study reported a high incidence of L. seeligeri in samples collected from the general environment. In another study where samples of grass, leaves, stems, and roots were sampled, L. monocytogenes was detected in nine of 10 samples of wilting grass; however, no L. monocytogenes was isolated from samples taken of the roots or stems (Fenlon et al., 1996). Listeria has also been shown to be common and present in surface samples from natural water sources, such as lakes, rivers, and streams (Dijkstra et al., 1982). Next, animal feeds, and improperly fermented ensiled feeds in particular, have been associated with listeriosis outbreaks in sheep and cattle (Fenlon et al., 1986; Gitter et al., 1986). The contaminated 4 silage has been the outcome of poor silage quality such as inadequate moisture content, which may facilitate the survival and growth of L. monocytogenes (Grønstøl et al., 1979). The wide distribution of L. monocytogenes in nature allows this bacterium to be easily spread and cause infection. Listeria monocytogenes can cause infection by several transmission routes such as ingestion of contaminated foods (e.g. unpasteurized milk or contaminated ready-to-eat foods; (Schlech et al. 1983, Fleming et al. 1985, Linnan et al. 1988), transmission of the organism from mother to fetus in utero (McLauchlin, 1990), directly to the fetus at the time of birth, or by direct contact with the organism which can cause lesions on the skin (McLauchlin 1990). The ability of L. monocytogenes to survive and multiply in many non-host habitats and host species, and the number of possible transmission routes, makes this pathogen difficult to control in its natural environment.

1.3. L. monocytogenes in Food

Contamination of foods by L. monocytogenes can occur at any point in the food chain, including on farms, in food processing plants, in retail establishments and in the home (Sauders, 2006, Nightingale, 2005, Lappi, 2004). L. monocytogenes can be detected in a wide range of foods, including both raw and processed foods. Many foods such as soft cheeses, hot dogs, and seafood have been implicated in listeriosis outbreaks, but L. monocytogenes also can be isolated from other foods such as beef, pork, fermented sausages, fresh produce and fish products (Rocourt and Cossart, 1997).

Listeria has been shown to survive within cultured buttermilk, butter and yogurt; of which these specific foods primarily depend on adequate fermentation to yield a low product pH that does not support Listeria growth. Many studies have shown that a wide variety of meats can 5 become contaminated with L. monocytogenes and most contamination is observed on meat product and poultry. For example, Bailey et al. (1990) reported that between 12- 60% of raw chicken was contaminated with L. monocytogenes and young birds were colonized by this human pathogen at a higher rate. Many studies have shown that the ability of L. monocytogenes to survive and grow on meat is dependent on temperature, pH of the meat, type of tissue, and initial miroflora already present on the meat's surface (Farber and Peterkin, 1991).

Since Listeria monocytogenes is found in soil and water, raw vegetables can become contaminated from the soil or from manure used as fertilizer (Schlech et al., 1983). Animals (i.e., wildlife and domestic livestock) can be asymptomatic carriers of L. monocytogenes and contaminate foods of animal origin such as dairy and meats through asymptomatic shedding in milk and feces. Not only can L. monocytogenes be isolated from raw foods (e.g. vegetables, uncooked meats), but it also can be detected in processed foods

such as soft cheeses and delicatessen meats (Seeliger, 1961, Fenlon et al. 1996, Fenlon, 1999).

Although Listeria can easily be inactivated by cooking and pasteurization (Petran and Zottola 1989), it remains a significant problem in ready-to-eat foods (e.g., frankfurters and delicatessen meats) that may become cross-contaminated by exposure in the food processing plant environment after cooking but before packaging (Tompkin, 2002).

1.4. Listeria in the Food Processing Plant Environment

One key reason that Listeria presents such a problem for many food manufacturers is simply because food processing conditions and the associated 6 processing environment permit the growth of Listeria. This Gram-positive, facultative anaerobe, intracellular rod is capable of growth in a broad range of temperatures including refrigeration (e.g. 1°C to 45°C), wide range of pH conditions (e.g. 4.3-9.5), relatively low water activity (> 0.90), and high salt concentrations (up to 10%), enabling survival and growth in many different food and food-associated environments (Farber and Peterkin, 1991).

Many studies have demonstrated the ability of L. monocytogenes to colonize, multiply, and persist in the food processing environment as well as on food processing equipment over extended periods, showing the environmental survival characteristics of this food borne pathogen (Lappi et al., 2004, Kabuki et al., 2004). Overall, Listeria is a very adaptable pathogen that is capable of survival even after freezing, surface dehydration, and spray chilling; however, Listeria can easily be killed with proper cooking (Seeliger and Jones 1986, Junttila et al. 1988).

2.1 Sources of Contamination in the Slaughterhouse

Antemorten inspection should remove from slaughter excessively dirty and obviously diseased animals. However, inspection cannot prevent slaughter of stock carrying human pathogens in the intestinal tract or on the hide or fleeces. During slaughter and dressing, head, hide or fleece hocks and viscera are removed. These operations are important. The object is to do this with as little contamination as possible of the exposed sterile carcass tissue and of edible offal. The rumen, lower intestinal tract and the hide and fleece all carry very large numbers of Microorganisms. The transfer of contamination through the airborne route is one of the most significant areas of high-care food production (Burfoot *et al.*, 2000*).*

Haines (1933) and Empey and Scott (1939) found that the sources of bacterial Contamination of meat are hides, hooves, soil adhering to the hide, intestinal contents, air, water supply, knives, cleavers, saw, hooks, floors and workers. The source of cross contamination exist in the slaughter process, such as processing tools and equipment, structural components of the facility, human contact, and carcass-to carcass contact (IFT, 2002). Thornton (1968) and Ingram (1972) reported that the nature and degree of initial contamination of the carcass surface mainly determined the keeping quality of meat. Prevention of Contamination during slaughtering and subsequent processing has, therefore, been identified as the most important factor in safe guarding the microbiological quality of meat.

Camel slaughter operations, such as bleeding, dressing, and evisceration, may expose sterile muscle to microbiological contaminants that are present on the skin, the digestive tract, and in the environment (Abdalla *et al.*, 2009a; Abdalla *et al.*, 2009). The risk is higher when air is contaminated with eventually foodborne pathogen microorganisms and spores. The risk of contamination derive prior to plant surfaces that includes both product contact and non- product contact surfaces. Airborne contamination should be occurred by indirect contact by

means of airborne particles which can be represented by spoilage or pathogen microorganisms(Kang and Frank, 1989).

Frazier (1967) showed that any contaminating bacteria on the knife would soon be found on meat in various parts of the carcass as it's carried by the blood. The contamination of carcasses comes from different sources including: environment and equipments with which meat comes in contact during slaughtering and processing, but hides remain as an important source of Contamination. Frazier and Westhoff (1988) reported that the healthy inner flesh of meat contained few or no microorganisms, although microorganisms had been found in lymph nodes, bone marrow and even flesh.

They also reported that the important contaminates came from external sources during bleeding, handling, and processing. They pointed out that during bleeding, skinning and cutting the main sources of microorganism's was the exterior of the animal intestinal tract, knives, air, hands and clothes of the workers. During handling, contamination came from cars, boxes and other contaminated meat in chilling storage. During processing contamination came from special equipments (grinders, sausage stuffers and casing) and ingredients in special products (fillers and spices). Main sources of contamination are the slaughtered animals themselves, the staff and the work environment (Belland Hathaway, 1996).

The contamination of equipment, material, and workers' hands can spread Pathogenic bacteria to non-contaminated carcasses. Food borne diseases often Follow the consumption of contaminated food-stuffs especially from animal Products such as meat from infected animals or carcasses contaminated with pathogenic bacteria as *Salmonella* spp., *Staphylococcus aurous, Listeria monocytogenes, Campylobacter* spp., and Escherichia *coli* O157:H7.

The majority of these germs result from contamination occurring at the where conventional veterinary inspection cannot detect the presence of these

bacteria on apparently healthy carcasses (Gill, 2000). Several studies have shown that most of the contaminants were originally of offal origin and that other microbes, originated from soil and water are involved, through the inevitable contact with handlers 'skin. These include *Staphylococci, Micrococci and Pseudomonas* (Nortije *et al.*, 1990).

Hussien (1971) isolated bacterial contaminants fresh meat from the gastro-intestinal tract and hides of the slaughtered animals and from the water, halls and air deposits. Lawrie (1979) reported that if acontaminated knife was used or organisms were in advertently introduced from the skin where the main blood vessels were severed bleeding could lead to contamination of the tissues. Decontaminating floor and other plant surfaces is most important to control Under biofilm, the potential for entrapping and protect the microorganisms against disinfectants. Thus airborne transfer of microorganisms is now seen as a significant route for contamination of food products. The shelf life of products is reduced by air borne contamination. Airborne pathogens can cause serious risk for human health.

The sources of airborne microorganisms in slaughterhouse are biological aerosols, dust and other viable and not viable particles (Kang and Frank, 1989). Unless properly cleaned, saws, steel-mesh knives and other equipment carry a high bacterial load and can be sources of contamination .Intestinal tract material (rumen and lower intestine) is most likely to be the major source of *E.coli, Salmonellae, C.jejuni, Cl. Perfringens* and other *Clostridia* for carcass and offal. The extent and nature of contamination of carcass and offal meat are reflections of the microbial status of the animal as presented for slaughter, and the care and standards of hygiene and sanitation used (ICMSF, 1998).

The inner flesh of meats of poultry and fish from healthy animals contain few or no micro-organisms, although they may be present in other parts of the carcasses.

Contamination can occur, however, during slaughtering, handling and processing (FAO, 1962).

2.2 Slaughter Processes

2.2.1 Skinning

Bacterial contamination includes the normal skin flora as well as organisms from soil and faeces which are on the skin, and includes *Yeasts, Bacilli, Micrococci, Staphylococci, Corynibacteria, Moraxella, Acinetobacter, Flavobacteria, Enterobacteriaceae, E. coli, Salmonellae* and *Listeria* species (ICMSF, 1998). Hocks are removed and incisions through the skin are made along inside of the legs, along the neck, sternum and abdomen and around the anus. Knives and operator`s fist are used to separate the skin from the underlying hock and skin become heavily contaminated, as do their knives, steels and clothes. *Salmonellae* can often be found on the hands and equipment of these workers (Smeltzer *et al*., *1980*; Stolle, 1981).

The incision through the contaminated skin carries microorganisms on-to the carcass tissue. The knife blade and handle and the hands of the operator these are used to free the skin – transfer organisms mechanically onto the carcass. Bacterial numbers are highest on region of the carcass where the initial manual removal of the skin takes place and lowest where skin is mechanically pulled away (Kelly *et al.,* 1980). Cutting the skin around the anus and freeing the anal sphincter and rectal end of the intestine are major source of carcass contamination with *E. coli* and *salmonellae*, and presumably also with *C. jejuni.*
The hide and skin around the tail are often contaminated with faeces.

Care taken during this operation is critical in limiting faecally derived contamination. During mechanical slaughter process of camel, the intestine may be occasionally squeezed through cuts in the abdomen, made from the initial knife

incision, and the intestine may rupture contaminating the abdomen and chest regions.

2.2.2 Evisceration

As part of the evisceration process, the brisket is cut, the abdomen is opened, and the organs of the thorax and abdomen are removed. Offal's are separated from the viscera and inspected. Care is needed to prevent puncture of the rumen during brisket cutting. The primary goal of effective slaughter is to protect the essentially sterile muscles of the carcass from becoming contaminated by the gastrointestinal (GI) tract. Since many pathogenic microbes originate in the GI tract and can be present on the hide. The GI tract is the major source of microbial contamination. Leakage of ingesta through the esophagus or from the feces through the anus may lead to contamination of the carcass with pathogenic bacteria. *Compylobacter* can occur in bile (Bryner *et al*., 1972).

The gallbladder and mesenteric and hepatic lymph nodes can be infected with *Salmonellae*. Normally, Salmonellae are found in less than 10% of these lymph nodes.

However in cattle and sheep held for some days in contaminated abattoir environments more than 50% of jejunal, caecal and colonic lymph nodes can harbour *salmonellae* (Samuel *et al.*, 1981*)*. Also more than 7500 *Salmonellae*/g of mesenteric, nodes (Samuel *et al.*, 1980). Incision of lymph nodes can contaminate the hands knives of veterinary inspectors and salmonellae can then spread to edible tissues. Requirements for lymph node incision have been considerably reduced in recent years. Though salmonellae are occasionally present inside livers, significant contamination of the liver surface occurs during evisceration and separation from other viscera, and from the hands and knives of veterinary inspectors livers and offals become contaminated also with *C. jejuni*. General contamination of the

heart, liver and diaphragm of camel has been shown to take place during removal from carcass cavity.

2.2.3 Washing

A usual part of the slaughter process to remove bone dust and other material from trimmed carcass, it will also remove bacteria. Raising the temperature of the wash water above 80°C tends to give a better reduction in carcass contamination, but even then the reduction may be small (Patterson, 1968). When aspray system is used to wash carcasses, there is a marked fall in temperature of the water after it leaves the nozzle.

When the temperature of sprayed water at impact on the carcass is 56-63°C, the psychrotrophic population is reduced about 10- fold. At impact temperatures of 65°C, the reduction in the mesophilic load still tends to be variable (1og x 0.2-09). Impact temperatures of 80°C and above appear to be needed to give at least a10-fold reduction in the numbers of Mesophiles on carcasses (Abdalla et al., 2009).

The addition of chlorine wash water appears to have only a small effect on reduction of contamination (Kelly *et al.,* 1981). Normally there is not more than five- fold reduction in microbial count. Low concentrations of chlorine (20-30mg/L) give some reduction which is not marked changed with increasing chlorine concentration. Populations of E. *coli* on beef were not significantly reduced by 800 ppm (Cutter and Siragusa, 2006). Both acetic and lactic acid solution, when applied to carcass surface, reduced bacterial contamination. A 1% solution of lactic acid reduced the mesophilic count on beef, veal and pork carcasses between log10 0.8 and 1.9 both acetic and lactic acid have a residual effect, reducing the rate of microbial growth on chilled meat. However, acid sprays appear to produce little reduction in E. *coli* and Salmonella on meat surface (Brackett *et al.,* 1994).

2.3 Micro-organisms which cause contamination of meat

Frazier (1967) found that meat was an ideal environment and culture medium for the growth of bacteria especially when it is minced. Mohamed (1970) suggested that in meat industry, bacteria is classified according to their temperature requirement into three groups.

2.3.1 Psychrophilic

Which grow comparatively and rapidly at temperatures below 5˚C e.g. *Listeria*, *Pseudomonas* and *Streptococci*. The growth of this type is not slowed down by refrigeration.

2.3.2 Mesophilic

Which grow at temperature between 15 and 40˚C it includes most food poisoning bacteria.

2.3.3 Thermophilic

Which grow at higher temperatures 40˚C and above. The predominant organisms on the surface of raw meat are *Brochotrix thermosphacta*, *Lactobacillus* species, *Leuconostoc* species, *Carnobacterium* species, *Pseudomonas* species and *Enterobacteriaceae* (Borch et *al.,* 1996; in`t veld 1996). Rodes and Fletcher (1966) proved that the psychrophilic and mesophilic types of bacteria were the most important ones. Banwart (1981) reported that the gaseous atmosphere surrounding the food may determine the types of organisms which become dominant. Oxygen favours the growth of aerobes while lack of oxygen will allow facultative anaerobes to dominate.

Hudson and Roberts (1979) reported that the pH of camel carcasses affected in the growth of bacterial count than those from normal pH carcasses. Nickeronand Sinskey (1974) found that Pseudomonas and Acentobacter caused spoilage of refrigerated meat as they grew at -3 ˚C – 0 ˚C. Slantez et al., (1963) suggested

that the spoilage of fresh meat was associated with the growth of Proteus, Pseudomonas and Escherichia. In addition to Gram –positive bacteria such as Bacillus and Micrococcus species, Tsubokura et al., (1973), suggested that the meat, particularly offals, contaminated with Yersinia organisms constituted an important source of infection.

Field (1948) isolated 257 strains of Salmonella dublin, *Salmonella typhimurium, Salmonella derby, Salmonella enteritidis* when he examined 554 samples of bile from slaughter camel. Hussein (1975) isolated from fresh meat samples *Staph epidermidis*, Micrococcus species, *E.coli, Proteus* species, *Aeromonas* species, *Pseudomonas species* and Achromobacter *species*. According to Dolman (1967) meat provides excellent medium for staphylococcal proliferation and if the temperature is warm enough only few hours are needed for the production of the effective amounts of enterotoxin.

2.4 Spoilage of Meat

Food spoilage usually refers to the deterioration of quality in food products due to the growth of contaminating microorganisms, although non-microbial activity, such as the activity of endogenous enzymes, can also contribute to food spoilage. The main defects of spoilage are sensory changes, such as off odors and off-flavours, slime production, texture change, discoloration and gas production.

Food spoilage processes determine the shelf life of food products, as the products can only be stored until a maximum unacceptable level of off-odour/off-flavours develops (Borch *et al.,* 1996). The properties of meat that are important in determining shelf life include water binding (or holding) capacity, color, microbial quality, lipid stability, and palatability.

Deterioration of quality may include discoloration, off-flavor and off-odor development, nutrient loss, texture changes, pathogenicity, and progression of spoilage factors (Skibsted *et al.,* 1994). Meat is a good support for bacterial growth as shown by the numerous reports dealing with the influence of microorganisms on the storage life of meat products. The main property, which explains rapid microbial growth on meats, is its composition: 75% water and many metabolites such as amino acids, peptides, nucleotides, and sugars (Gill *et al*., 1982). After slaughter, microbial contamination of carcasses is the consequence of the Processing applied from skinning to conditioning. Processing influences not only The quantity of microorganisms/cm2 but also the type of microorganisms present. Spoilage is characterized by any change in a food product that renders it Unacceptable to the consumer from a sensory point of view.

Microbial numbers are not always related to degree of spoilage, but microbial activity is considered to be of great importance for the manifestation of spoilage (Nychas *et al.*, 1998). The species and population of microorganisms on meat are influenced by animal species, state of health, and handling of live animal ;slaughter practices, plant and personnel sanitation, and carcass chilling ;fabrication sanitation, type of packaging, storage time, and storage temperature (Nottingham, 1982; Grau,1986). Discoloration, off odors, and slime production are among the deterioration factors caused by bacterial growth. Gram-positive bacteria are involved in meat spoilage. These include *Micrococcus species, Staphylococcus species Streptococcus species, Lactobacillus species, Leuconostoc, bacillus species, Clostridium species and Corynebacterium species* Gram negative bacteria genera reported in cases of meat spoilage included *Pseudomonas, Flavobacterium, Acinetobacter, Klebsiella, Salmonella, Shigella and Proteus* (Gracey and Collins,1992).

2.5 The importance of meat contamination

Fatima (1982) emphasized that pathogenic bacteria found in processed meat which she studied were *Salmonella spp, Clostridium perfringens, Staphylococcus aureus and E.coli.* Gracey (1981) reported that, the organisms responsible for food Poisoning by infection were *Salmonellae, Escherichia coli and Vibrio parahaemolyticus.* Those responsible for poisoning by toxin production included *Staphylococcus aureus, Clostridium perfringens, Clostridium botulinum, Bacillus cereus and Streptococci.* Other bacteria occasionally caused outbreaks of food poisoning, included *Streptococci, Proteus, Pseudomonas, Providencia, Citrobacter, Aeromonas hydrophilic, Yersinia enteracolitica, Compylobacter, Shigella flexneri.* Hussein (1975) isolated from fresh meat samples*; Staphylococcus epidermidis, Micrococcusspp, E.coli, Proteus spp, Aeromonas spp, Pseudomonas spp, and Achromobacterspp.* No *Salmonella or co-agulase positive staphylococci* were isolated. John *et al*., (1988) reported that *Proteus species* are important in the spoilages of meat, because they grow and spread readily on moist surface at low temperatures and produce a number of proteases.

According to Holy and Holzopfel (1988) *Pseudomonas* are susceptible to freezing and thawing. Brahmbhalt and Anjaria (1993) examined samples of raw meat obtained from shops. They isolate of *E. coli, Staphylococcus epidermidis, Staphylococcus aureus, Micrococcus luteus, Citrobacter freundii, Bacillus cerus, Streptococcus faecalis, Entrobacter aerogenes, Proteus mirabilis, Bacillus subtilis, Aeromonas liquifaciens, Proteus vulgaris, Klebsiella pneumoniac* and *Pseudomonas deruginosa.* The microbial groups that contaminated fresh beef surface are Pseudomonas *spp, Brochothrix, Thermosphacta, Moraxella spp, Lactobacillus spp, Flavobacterium spp, Vibrio spp, Aeromonas spp,* and *Arthobacter* (Gill, 1982). Gracey (1980) stated that the main types of bacteria involved in the spoilage are from the Gram-positive genera *Micrococcus, Staphylococcus, Streptococcus,*

Lactobacillus, Leuconostoc, Bacillus, Clostridium, Corynebacterium and Microbacterium.

A total of 71 strain of Gram positive, catalase positive cocci were isolated from112 abscesses observed during inspection of slaughter animals (sheep, cattle, pigs and goats). A mongst 35 co-agulase positive isolate, 30 were classified as *Staph aureus*. Of the co agulase negative isolates 5 were Staph *hominis* and 4 were *Staph xylosus.* Jay (1986) reported that sausage usually contamined more varied flora than most other processed meat due to different seasoning agents employed and *Bacillus thermosphacta* was the most predominant spoilage organisms.

Most microbial contaminants of carcasses represent commensal bacteria, some microorganisms such as *Salmonella* spp., *Escherichia coli* O157::H7, and *Listeria monocytogenes* pose a threat to consumer health (Samelis et al., 2001). The members of the genera *Pseudomonas, Acinetobacter and Moraxella* dominated the bacterial *content* of unprocessed meat exposed to air at chill temperature (International commission for microbiological specification for food – I .N.C.M.S.F, 1980). Six strains of ureolytic Staphylococci *were* isolated *from* rumen of young calves and lambs. Three of them were identified as *Staphxylosus, Staph saprophyticus* and *Staph gallinarum* (Laukova and Marounek, 1992) Matthews *et al.* (1989) isolated primarily *Staphxylosus*, Staph *hominis and Staph aureus* from bovine origin by using the API *staph*-Trac. The incidence of *Staphylococcus* species in healthy animals was investigated in young and adult individual's cattle, in pigs and in domestic fowl. The samples were taken from Slaughtered animals. *Staph aureus, Staph xylosus* and *Staph hominis* were isolated (Shalka, 1991).

A survey was made on the distribution and isolation of *Staphylococcus species* on the skin of humans and 7 kinds of animals (Pigs, horse, cows, chickens, dogs, laboratory mice and pigeons). *Staph xylosus* and *Staph hominis* were isolated

(Nagase *et al.*, 2002). Akatov *et al*. (1983), studied the species characteristics of coagulase- negative *Staphylococci*.

They isolated Staph *xylosus* from different animals (cows, sheep, hens etc.). Six strain of ureolytic *Staphylococci* were isolated from rumen of young camel. Three of them were identified as Staphxylosus, *Staph saprophyticus and Staph gallinarum* (Laukova and Marounek, 1992).

2.6 Hygienic measure adopted in slaughterhouse

Meat inspection was practiced in France as early the year 1162, in Britain in about 1319 in Germany special inspection of pigs were started in 1383, while in USA meat inspection was carried out in 1884 (Ibrahim, 1991) . Dicksone (1988) and Hennlich and Verny (1990) emphasized that hygienic measures promote the quality and safety of meat and increase its shelf life. Salih (1969) proposed that in order to improve the standards of meat hygiene should be revised the laws in the study of animals resources in order to include meat hygiene and regulation. He noted that there is lack of proper training of the various staff members working in the meat inspection services. He suggested that programmes should be formulated to improve their academic and technical abilities, and also suggested the establishment of meat research Centre where data pertaining to meat hygiene (Number of slaughtered animals, condemnations and reasons for condemnation throughout the country could be collected and analyzed). Regarding the slaughter houses he suggested that they should be run on sound economical basis and they should be able to make some financial benefits. The main objective of meat hygiene and inspection is to prevent meat spoilage and meat borne infections.

The meat hygiene, inspection and control practices are based on the concept of the transmissibility of diseases through either consumption or

handling of meat (Ibrahim, 1990). The effective operations of meat hygiene services are multidisciplinary. They involve the veterinary medicine and engineering professions. The veterinarian is the one who is trained to deal with diseases transmitted through meat (WHO, 1957).

According to Thornton (1968) the efficient meat hygiene practices, started in the farm. It should be maintained in the animal collection centers, markets, during transportation of animals for slaughter, in abattoirs, during transport of meat to butcheries and even at the consumer's home. To execute such programs necessary laws and guiding instructions should be laid out vividly and firmly .On the other hand basic knowledge about hygiene and sanitation should be disseminated among people especially those directly concerned with meat hygiene and quality control, i.e. farmers, butchers and consumers. This knowledge would contribute positively to the understanding of laid out. Policies and to establishment of proper standards .it is also necessary to study and asses the influence of social traditions and religion in the community and also the economic and environmental conditions in a particular area for achieving the goals of meat hygiene programs.

The many potential routes of contamination during processing include Contamination from human sources, vermin, or the ingredient materials. Food may be contaminated by each other and by pieces of equipment with which they come into contact. Contaminants may build up in numbers on such equipment and constantly transmit seed organisms into the foods. Disease outbreaks due to commercially processed foods are not uncommon.

2.7 Selection of animal for slaughter

The most important considerations are health, kind of animal expected meat yield, and care of the animal prior to slaughter. Fever, increased breathing rate, and diarrhea. Animals suspected of being unhealthy should be treated by a veterinarian until the animal is returned to a healthy state. It is important to exercise proper care of the animal prior to slaughter, if you expect to obtain high quality meat. Pen the animal in a clean, dry place the day before slaughtering. Restrict the animal from feed 24 hours prior to slaughter, but provide access to water at all times. The slaughter of hot, excited animals increases the risk of sickness, injury, and darker meat; therefore, do not run the animal or wrestle with it. Bruises and whip marks cause bloody spots which must be trimmed out. Prior to the day of slaughter, select the slaughter site, accumulate all equipment, prepare for waste disposal, and, if necessary, arrange with a local processor or meat market for chilling and cutting the carcass. If you plan to have the carcass chilled and make arrangements concerning the time and day on which the carcass can be accepted, the charges, and specific instructions for chilling, cutting, and wrapping.

However, to minimize the losses resulting from transportation, animals should be rested fed before slaughter to regain physiological normality (Houthis, 1957; Willsow and Payne, 1978). Ibrahim (1989) stated that ante-mortem is of a great value in detection of animals suffering from infectious diseases particularly notifiable diseases and emergency cases. It ensures that food animals released for slaughter are in good state of nutrition, cleanliness and free from signs diseases. Johnston (1990) suggested that faecal contamination of the environment can be restricted by correct disposal of animal and human waste .The use of good husbandry methods and the maintenance of high standards of animal health should be encouraged. Many food poisoning out breaks were traced to the consumption of meat from animals slaughtered while obviously ill but whose carcass and organs

showed little noticeable change on post-mortem examinations. According to Houthuis (1957) without ante-mortem inspection no adequate inspection of carcass or meat is possible especially in cases of emergency slaughter of a sick animal. The Antemorten inspection should be carried out solely by veterinarians who have had long experience of general clinical practice before talking up that type of work.

FAO (1962) suggested that if a food animals is encountered during ante-mortem inspection in a moribund state a blood smear should be taken from the animal and stained with poly-chrome methylene blue and examined for Macfadyean reaction .Such measure is to avoid public health implications.

According to the same reference the meat hygiene starts from the animal being on the farm through its journey till it reaches the consumer as fresh, wholesome, sound and safe meat. In the abattoir, ante –mortem inspection detains diseased or suspected animals for further detailed examination by the meat inspector. Ante mortem inspection is of a great value, for it aids in the detection of animals suffering from scheduled infectious disease like anthrax, rabies and glanders, which are communicable to man (Thornton, 1968).

According to Thornton (1973) there are many diseases of toxic or infectious nature which could not be detected in the carcass and organs after slaughter. Ante-mortem is of special value in cases of septic metritis and septic mastitis, sturdy in sheep and tuberculosis meningitis in young cattle, tetanus and rabies. In all these cases the post –mortem findings are of little diagnostic value but the typical symptoms could be recognized during ante-mortem. Indication of disease detected in the live animal calls for its segregation and detailed examination after slaughtering.

Ante –mortem inspection is described as the first line of defense against out breaks of food poisoning.

2.8 Sanitary in the slaughterhouse and hygienic in the meat production

It has been shown by many studies that slaughtering under strict sanitary conditions reduces the bacterial contamination of the carcasses (Dixon *et al.*, 1991).

According to Schutz (1991) the occurrence of hygienic faults and of ahigh level of microbiological contamination of carcasses in slaughterhouses are due, not to an absence of hygiene equipment or to failure to use what equipment there is, but rather to faulty slaughter techniques. The spread of pathogen can also be reduced by developing slaughter technique. Especially the technique of removing tonsils from pigs (Christensen and Luthje, 1994) and of enclosing the rectum (Andersen *et al.*, 1991) has reduced the pathogen contamination.

According to Gerats (1990), there is an association between slaughter techniques and the hygienic practice of workers. Those workers who commit many slaughter mistakes neglect hygienic practices. Grats *et al.* (1981) have found an association between the number of Enterobacteriaceae in pig carcasses and hygiene practices connected with slaughter mistakes during evisceration. The hygiene practice of slaughterhouse workers is regulated in many countries by laws (Schutz *et al*., 1991). The laws do not always distinguish between critical operation and those that have little effect on the hygiene.

There are many factorial complexity of fresh meat quality and shelf life. The microbial quality of the raw material (carcass), the maintenance of cold chain, sanitary condition of premises, equipments (like saws and mincers) and personnel hands and clothes and general management practices were but a few of factors determining the microbiological quality of the product (Nortje *et al.*, 1990). According to Gracey (1986) all building in the slaughter house must be vermin-proof and kept free from flies. The surrounding area must be well maintained so that there is no risk to the plant from vermin or insects. Also floor and walls should be of smooth impervious material and the corners must be easily and effectively cleanable. Boyle et al (1990) concluded that waste fluids in slaughter houses

can support the growth of *L-monocytogenes* .slaughter house temperature should be as low as possible and cleaning and sanitation should be frequent to minimize contamination of meat with this pathogen. The visceral organs in modern abattoirs kept without contact with the hides, skins and feet and their removal after dressing is completely under hygienic conditions (Gracey, 1985). Shuppel *et al.* (1996) suggested that the udder should be removed before skinning and it is generally judged unfit for human consumption. There are two reasons for implementing, a visual control system. It decreases cross-contamination (no handling, cutting and incision) and it reduces inspection costs. The resources released as a result may be re allocated to hygiene and surveillance programmes.

2.9 The Hazard Analysis Critical Control Point (HACCP)

Food Safety and Inspection Service, USDA, (1998) emphasized that processing operations were presently required to have sanitation standard operation procedures (SSOP`s) and Functional Hazard Analysis Critical Control Points (HACCP) system, to improve food safety through purchase requirements.

Jay (1986) explained that, HACCP was a preventive system of control that included a careful analysis of ingredients products and processes in an effort to determine those components or areas that must be maintained under very strict control to assure that the end product meet the microbiological specifications that had been developed. According to Scarafoni (1967) the dirt and skins of animals

contribute to 33% of the pollution, the abattoir atmosphere to 5% , the visceral content 3% , transport and storage elements 50%, having quartering and packing of carcasses 3%. The HACCPs can be achieved by the flowing principles (Brown, 2000).

2.9.1 Conduct a Hazard Analysis

Identify the potential hazards associated with food production at all stages up to the point of consumption, assess the likelihood of occurrence of the hazards and identify the preventive measures necessary for their control.

2.9.2 Determination of the Critical Control Points (CCP)

Identify the procedures and operational steps that can be controlled to eliminate the hazards or minimize the likelihood of their occurrence.

2.9.3 Establishment of Critical Limit(s)

Set target levels and tolerances which must be met to ensure the CCP is under Control.

2.9.4 Establishment of a System to Monitor Control of the CCPs

2.9.4.1 Establishment of the Corrective Actions

To be taken when monitoring indicates that a particular CCP is not under control.

2.9.4.2 Establishment Procedures

For verification to confirm that HACCP system is working effectively.

2.9.4.3 Establishment of a Documentation System

Establish a documentation system concerning all procedures and records appropriate to these principles and their application.

3.1. Pathogenic Listeria monocytogenes and Disease In humans

Most listeriosis cases are observed in neonates, the elderly, pregnant women, or otherwise immuno-compromised individuals such as those on chemotherapy or immuno-suppressant drugs usually transmitted through the consumption of contaminated foods (Mead et al., 1999). On very rare occasions, the pathogen also can be transmitted directly from infected animals to humans; which has been observed in veterinarians, farmers, and abattoir personnel handling contaminated tissues (Posfay-Barbe et al., 2009). Vertical transmission from mother to neonate can occur transplacentally or the infant can become infected during delivery through contact with organisms in the birth canal (Posfay-Barbe et al., 2009).

Schuchat et al (1991) described an unusual example of 7 Listeria transmission in a nosocomial outbreak involving neonates, whom became infected through contact with contaminated mineral oil that was being used to bathe the infants within a specific neonatal unit.

3.2. Symptoms of the Disease

Pregnant women are the most at-risk population for contracting a Listeria monocytogenes infection, and they are about 20 times more likely than other healthy adults to become ill with listeriosis. If pregnant women acquire listeriosis, the fetus is most heavily infected, leading to spontaneous abortion, stillbirths, or sepsis in infancy. About one-third of Listeria cases represent pregnancy-associated cases (Cossart and Bierne, 2001). In most cases, the fetus or newborn is more likely than the mother to be affected by listeriosis

associated with pregnancy (Silver, 1998); the perinatal and neonatal mortality rate is 80 percent (FDA/CFSAN, 2012). The Mayo Clinic found the following symptoms of listeriosis to be common in infants who contract this disease: loss of appetite, lethargy, jaundice, vomiting, skin rash, and/or breathing difficulties (Mayo Clinic, 2009).

Listeriosis can develop as two different forms of disease, a non-invasive form known as listerial gastroenteritis, or a severe invasive form of disease that often is accompanied by severe clinical manifestations. The non-invasive form of Listeriosis results in a wide variety of symptoms ranging from fever, muscle aches, and gastrointestinal symptoms such as nausea or diarrhea. Five days to three weeks after ingestion of the bacterium, Listeria can infect deeper tissues leading to an invasive form of Listeriosis causing a systemic infection (FDA/CFSAN, 2003). If the infection spreads to the nervous system, 8 symptoms such as headache, stiff neck, loss of balance, confusion, or convulsions can occur. With brain involvement, listeriosis may mimic a stroke, and lead to meningitis or encephalitis (Crum, 2002). Other at-risk individuals for contracting listeriosis include people with weakened or compromised immune systems, cancer patients, transplant recipients, diabetics, and persons with AIDS (Schuchat et al., 1992). Dietary precautions should be taken by those individuals most at risk of acquiring a L. monocytogenes infection in order to help decrease the chances of acquiring severe systemic disease.

3.3. Listeriosis Cases, Hospitalizations and Deaths

Annual projections in the United States indicate that approximately 2,500 cases of human listeriosis occured where nearly 500 of these cases progress to death and 300 cases will require hospitalization (Mead et al.,

1999). This projection may be underestimated by half due to asymptomatic symptoms occurring in healthy individuals who become infected, but show no clinical signs (Mead et al, 1999). .

The "Healthy People 2010" initiative was established by the federal govern ment to establish achieve a 50% reduction in the overall number of listeriosis cases by 2010 (USDA-FSIS, 2003). This national health promotion would involve national, state, local, government agencies, voluntary, nonprofit, communities, and individuals together to lead in a fight to improve the health of the Americans (USDA-FSIS, 2003). A noticeable decrease in listeriosis was observed between1996-2001, but reached a plateau after 2002 (CDC, 2009).

3.5. Distribution

Listeria are widely distributed in nature and can be found in decaying vegetation and in soils, animal feces, sewage, silage, and water (James *etal.*, 2005). *L.monocytogens* often lives in the cold and moist environment found in refrigerators and its present in all categories of food.

3.5. Transmission routes

Transmission is mainly via food (Cressey, 2007). Alternative routes include infections acquired in hospital and occupational exposure, for example through skin infections (e.g. veterinarians, farmers).

Meningitis, meningoencephalitis, or encephalitis. Cervical and generalized lymphadenopathy is associated with the adult syndrome, and thus the disease may resemble infectious mononucleosis.

Pregnant females who contract the disease (and their fetuses are often congenitally infected). Abortion, premature birth, or stillbirth is often the consequence of listeriosis in pregnant females.

The organism's ability to grow at refrigeration temperatures is significant as chilling is often used as a control measure in the food industry**.**

3.6. Control

The most effective drugs for treatment are coumermycin, rifampicin, and ampicillin, with the last plus an amino glycoside antibiotic being the best combination. Even with that regimen, antimicrobial therapy for Listeriosis is not entirely satisfactory because ill patients and immunocompromised hosts are more difficult to treat than competent hosts.

3.7. Rationale:

Listeria monocytogenes has long been acknowledged as a significant human and animal pathogen (Nightingale *et al.* 2004).

The risk of red meat contamination with *Listeria* has to be highly considered. Possible *Listeria* cross-contamination by and from employees, equipment, and surfaces, animal skin, food additives, packing material and many other sources has been highly rising in recent time.

The carcasses and their products may be contaminated during slaughtering and meat processing, thus they can be recognized as feasible transmission routes of *Listeria* to humans (Nesbakken *et al.* 1996).

Chapter Three

MATERIALS AND METHODS

3.1. Study area

The study was carried out in Tamboul Town, Aljazeera state, Central Sudan in the period from May to September 2017. Tamboul Town is the largest city of Al butana Governorate. The area is characterized by moderate rainfall, the highest percentage of rain falls during the long rainy season from August to November.

East of the Aljazeera is a patch lining the eastern Blue Nile is bordered to the north of Khartoum state local east of the Nile, on the south by a local or villages on the west-east area of the Nile and the east, the states of Kassala, Gedaref, and its

climate is located within the poor savanna, and an area of about 8449.45 square kilometers and constituted about one-third state of the Aljazeera, which is about a flat plane descends gradually to the north and west.

The most important areas are the city of Aljunied sugar industry - Wadraoh trade - Ruffaa 'education' and Tamboul market.

It’s a geographical zone which less approximately between Latitude 130, 40’ and 170, 50’ North and Longitude 320, 40’ and 360, 00’ East. It is bounded by the Main River Nile on its northwestern border, the Blue Nile on its southwestern edge, the Atbara River in the north east.

Tamboul city is located near the city of Ruffaa located east Aljunied sugar factory and is famous for its camel. Tamboul city famous for its trade, where is the Tamboul market of the largest markets in the Sudan as it is the biggest market for camel in Sudan. It follows the Tamboul 147 Village with Area 12091 (km2) and has an estimated human population of 265952.

It contains large number of animal species especially camel (*Camelus dromedaries)*.

Figure (1): Map of Tamboul.

3.1.1. Study abattoir and origin of samples

Tamboul Town Abattoir Enterprise was established before 7 years ago, and is located at the heart of Tamboul Town. The abattoir for slaughter of bovine, ovine and caprine, and camel.

The abattoir is a high output abattoir in the Albutana area providing 80% of the daily meat requirements of the city's residents. Most of the camel slaughtered at the abattoir are adult males of local camel. Other Species of animals slaughtered include bovine, ovine, caprine.

In the abattoir, regular meat inspection is being conducted by meat inspector as well as veterinarians from Ministry of Agriculture. The abattoir has not divide to clean

and dirty areas, so that after skinning and evisceration, carcass not follows the clean lines. For that reason inspection and transporting from one areas or directly after finishing the slaughter.

The swab sample was collected from camel meat from the Tamboul Town Abattoir Enterprise and butcher shops located in the city. Furthermore, swab samples were collected from equipments like knives.

3.1.2. Study population and sample size determination:

The study population represented camel meat and equipments like knives.

The approximate sample size required was determined, according to Thrusfield (2005), from expected prevalence of 50% with defined precision of 5% and level of confidence of 95%.

n=1.962 Pexp (1-Pexp)/d2

Where:

n = required sample size

Pexp = expected prevalence

d = desired absolute precision

Therefore, by using estimated prevalence of 50% in raw meat of camel and taking a confidence interval of 95% and 5% absolute precision, the minimum sample size required for this study was 800 camel meat swab samples. A total of 865 samples were taken randomly from selected carcasses (Neck, Shoulder, Thigh, and Liver) comprising of 380 Camel meat swab samples from the Tamboul Abattoir, 380 Camel meat swab samples from the butcher shops Enterprise were used for the study. In addition, 105 swab samples from equipments (knives, cutting tables and hooks), (Table 1).

The samples transported to the Microbiological laboratory of the Faculty of Veterinary Medicine, Sudan University of Science and Technology upon arrival using the ISO 11290-1 method.

Table (1): Distribution of the type and number of samples collected from camel carcasses.

Type of sample		Number of samples
Camel meat swab samples	Neck	190
	Shoulder	190
	Thigh	190

	Liver	**190**
Equipments swab samples	**knives**	**50**
	Cutting tables	**40**
	Hooks	**15**
Total		**865**

3.2. Study methodology

3.2.1. Study design

The study was conducted to determine the prevalence of *L. monocytogenes* from September 2016 to March 2017 in red camel meat slaughtered at Tamboul Town Abattoir Enterprise at the Microbiological Laboratory of the Faculty of Veterinary Medicine, Sudan University of Science, Khartoum, Sudan. On each sampling day, all the required samples (camel meat swab samples, Equipment swab samples) were taken.

3.2.2. Sampling technique:

In Tamboul Town Municipal Abattoir, the maximum numbers of animals slaughtered on Saturday and Tuesday and mainly during Marketing days. Carcasses were examined just after evisceration before washing. The meat was swabbed without distinction of race, sex or age at Tamboul Town Abattoir Enterprise and different butcher shops during several visits. The carcasses were chosen in a random sampling method and examined just after the stage of evisceration.

All samples were collected aseptically using disposable gloves to avoid contamination, and the samples were labeled with necessary information including the date of sampling, sample code and sample type. The selected meat was swabbed aseptically using the method described in ISO11290-1 (1996) by placing sterile template (10 x 10 cm) on specific sites of a carcass. A sterile cotton tipped swab (2x3 cm) fitted with shaft, was first soaked in an approximately 10 ml of buffered peptone water (Oxoid Ltd., Hampshire, England) rubbed first horizontally and then vertically several times on the carcasses. The Neck, Shoulder, Thigh, Liver and knife, which are sites exposed for contamination were chosen for sampling. On completion of the rubbing process, and leaving the cotton swab in the test tube. Finally, the carcass swabs taken was kept in a transport medium (buffered peptone water) and transported to the Microbiology Laboratory of the Faculty of Veterinary Medicine, Sudan University of Science (SUST), Khartoum, Sudan, for microbiological analysis. After arrival, the samples was stored in refrigerator at 4°C.

3.3. Method of Sterilization:

3.3.1. Dry heat

3.3.1.1. Hot air oven

The method was used for sterilization of clean glass containers which were wrapped in foil or put in stainless steel cans, at a temperature of 160 ˚C for one hour.

3.3.1.2. Flaming

This was used to sterilize the mouth of bottles, cotton plugged tubes and glass slides. It was done by exposing the object to the direct flame for about half to one second.

3.3.2. Moist Heat

3.3.2.1. Autoclaving

This method was used for sterilization of media and materials that couldn't withstand the dry heat. The temperature was 115˚C -121˚C under 10-15 pounds Pressure for 15-20 minutes.

3.4. Culture Media

Culture media were prepared according to Bridson (2006), unless otherwise specified.

3.4.1. Agar:

Can be obtained as shreds, flakes, granules or powder and is made from certain types of seaweed. The usefulness of its unusual gelling properties for bacteriological work was recognized by Frau Hesse, who suggested its use to her husband, Walther Hesse, an early colleague of Robert Koch.

When mixed with cold water, agar does not go into solution; it can therefore be washed to free it from soluble impurities. The concentration for use depends on the geographic source of species of seaweed from which the agar is made, and on the purpose for which the medium is intended (Appendix A, Table A5). In this *Manual,* the concentration of agar given in the formulae for media relates to the product derived from Japanese seaweed.

In addition to the agar concentration, other factors affect gel strength; for example, repeated melting of the medium or prolonged sterilization especially at a low pH value will decrease it.

3.4.2. Enrichment media:

Usually both selective and inhibitory, these are liquid media into which swabs or specimens are placed; after incubation for 6 and 18 hours, subcultures are made to plates of (i) selective, and (ii) non-inhibitory nutrient media (Nutrient Agar, Blood Agar). After incubation these plates are examined and selected colonies subcultured

to non-inhibitory media. This second plating is an important step in the isolation process; without it the colonies first subcultured may well yield a mixture of wanted and unwanted organisms. Whenever possible, selective media should be avoided; repeated plating on non-inhibitory media is preferable, although this is a council of perfection seldom satisfied in practice.

3.4.3. Peptone:

is a product of varying composition made by acid or enzymic hydrolysis of animal or vegetable protein, from material such as muscle, liver, blood, milk, casein, lactalbumin, gelatin and soya bean.

The exact composition depends on the raw material and the method of manufacture.No two batches of peptone are exactly alike, but commercial firms try to produce peptones in which the measurable constituents are present within certain defined limits. For many kinds of media the make or type of peptone is immaterial, but for certain tests a particular type may be specified. This does not mean that all other types are unsuitable; more often than not it means that other peptones may not have been tried. Certain batches of peptone, however, may be quite unsuitable for a particular purpose, and before general use a peptone should be tested.

In the section on media control (Appendix A3) we discuss this problem in more detail and give examples of fallacious results due to the use of unsuitable peptones.

Most peptones from reputable commercial sourcesare equally good.

3.4.4. Blood:

The choice of blood is often a matter of convenience and may depend on the animals kept by a laboratory. Horse blood from commercial sources is commonly used, but the blood of other species (man, cow, goat, rabbit, sheep) may be necessary for special purposes; they should be free from antimicrobial agents. Sheep Blood Agar can be used for detecting the different haemolysins of staphylococci and streptococci although bovine blood may give stronger reactions; haemolysis of sheep

and human blood may be used also in the identification and biotyping of some species of *Vibrio*. Sodium citrate is inhibitory to staphylococci (Rammell, 1962) as is Liquoid to some anaerobic cocci and *Streptobacillus*. In general, defibrinated horse blood is preferable; it should be relatively fresh and should not be used if haemolysed. Blood must be stored in a refrigerator but should not be allowed to freeze; all blood products must be tested for sterility as well as for inhibitory substances such as citrates.

3.4.5. Yeast extract:

Is made from bakers' or brewers' yeast and is a rich source of amino acids and vitamins of the B-complex. In culture media it is used to supplement or replace meat extracts. Meat extract (1%) can be replaced by yeast extract (0.3%) in Nutrient Broth without significant change in the growth-promoting capacity.

3.5. Isolation and identification of *listeria monocytogenes*:

The techniques recommended by the International Standards Organization (ISO 11290-1, 1996) and the French Association for Standardization were employed for the isolation and identification of *Listeria monocytogenes*.

3.5.1. Isolation of *listeria monocytogenes*:

3.5.1.1. Primary selective enrichment

Each sample unit which are kept in buffered peptone water was mixed thoroughly to ensure the homogeneity of its contents and about 0.1 ml unit was obtained aseptically in to 10ml of prepared listeria enrichment broth (LEB) followed by mixing and the sample was kept inside incubator and incubated at 30˚C for 48 hrs.

3.5.1.2. . Secondary selective enrichment

The secondary selective enrichment medium with full concentration of selective agents was employed. From the pre-enrichment culture (*Listeria* Enrichment Broth), after being well mixed 0.1 ml was transferred into 10 ml of Half Fraser broth and was incubated at 35°C for 24 hours.

3.5.2. Identification of *listeria monocytogenes*:

From Half Fraser Broth showing black color, a loopful of the culture was streaked onto PALCAM agar plates and OXA agar plates and incubated at 37˚C for 24 to 48 hours. Identification of *Listeria* species on PALCAM agar plates was based on aesculin hydrolysis and mannitol fermentation. All *Listeria* species hydrolyzed aesculin as evidenced by a blackening of the medium. Mannitol fermentation was demonstrated by a color change in the colony and/or surrounding medium from red or gray to yellow due to the production of acidic end products. The selectivity of the PALCAM medium is achieved through the presence of lithium chloride, polymixin B sulphate and acriflavine hydrochloride present in the medium base and Ceftazidime provided by PALCAM antimicrobial supplement. These agents effectively suppress growth of most commonly occurring non-*Listeria* species of bacteria present in food samples. On PALCAM agar; typical colonies were grey-green with a black sunken center and a black halo, and on Oxford agar, colonies appeared brown black or greenish black with a depressed center and a surrounding black halo.

3.5.2.1. Test of Identification:

3.5.2.1.1. Primary Identification tests:

3.5.2.1.1.1. Temperature range for growth:

The optimal growth temperature are characteristic of different groups of bacteria; of those in the medical and veterinary fields the optimal temperature is usually between 35 and 40 °C but the range for growth varies considerably.

Lesteria monocytogenes able to grow in different range of tempreture. it grow in 5°c, for this called (psycrophilic bacteria) , grow in 37°c (Rome temperture) and also able to grow in 45°c but it weak growth here .

3.5.2.1.1.2. Ability to grow under anaerobic conditions:

Is fairly widespread among bacteria but as it is not universal the knowledge that an organism cannot grow under these conditions can be diagnostically important. Some of these organisms are strict aerobes, others may need carbon dioxide for growth. In contrast the ability of *Lesteria monocytogenes* to grow anaerobically can also be diagnostically useful. And that is obseved when culture the *lesteria* monocytogenes under (UN aerobic Jar).

3.5.2.1.1.3. Motility test:

May be studied in a hanging-drop or other wet preparation. Some strains are only sluggishly motile when first isolated; motility may be speeded by using Graigie's technique (Craigie, 1931; Tulloch, 1939) in which the organism is inoculated into a central tube of sloppy agar and, after incubation, a subculture is made from those organisms that, by their motility, have migrated outside the central tube. *Lesteria monocytogenes* show positive result for motility test. Motility may be inferred by observing the spreading growth in a semisolid agar which may be seen better when a tetrazolium dye is incorporated in the medium; as the organisms grow the dye is reduced, and the medium changes color.

The temperature of incubation is important; most motile organisms are motile at lower temperatures (e.g. 15-25 °C) and may not be motile at the temperature (e.g. 37 °C) optimal for growth.

When these tests become part of the daily routine they do not take up much extra time; they are only time-consuming and upsetting of routine when they are 'special tests'. These remarks refer to the motility shown by aerobic organisms; anaerobes present special problems in that motility will be inhibited by the air present in hanging- drop preparations.

3.5.2.1.1.4. Gram reaction:

Gram did not describe a stain but a method in which he used stains and solutions devised by others; to this day its mechanism is not fully understood, but we do know that the Gram reaction is a stable characteristic of a bacterium. Gram positivity (the ability to resist decolorization with ethanol or acetone) is a feature of relatively young bacterial cells of some species; as they age, the cells lose this characteristic and apparently become Gram-negative.

It is important, therefore, to examine young cultures, preferably before the end of the logarithmic phase of growth. Genuinely Gram-negative bacteria do not retain the first stain which is easily removed by the decolorizing agent. Thus, as in many other tests, a positive finding (in this case retention of the purple stain) has much more significance than a negative result which may, in fact, be false due to (i) the age of the culture, or (ii) excessive decolorization with powerful solvents such as acetone. There are many variations of Gram's staining method (and each works well in the hands of those who practise it); the one we use under the name of Lillie's modification is simple and gives good results but, as acetone is used, the decolorization can be overdone. A modification by Preston & Morrell (1962) is claimed to be foolproof. Recently, a rapid paper-strip method has been marketed for

distinguishing between Gram-positive and Gram-negative organisms though it has doubtful practical and no taxonomic value.

Lesteria monocytogenes appears as Gram-positive rods when stained with Grams stain.

3.5.2.1.1.5. Carbohydrate breakdown:

The division of bacteria into fermenters, oxidizers, and non-utilizers by the OF test of Hugh &Leifson (1953) is one of the most heavily weighted of the primary tests used in the progressive system of identification in this *Manual*, and carbohydrate utilization also features in the secondary tests. The latter so-called 'fermentation tests' were used by early bacteriologists to distinguish one organism from another and elaborate diagnostic tables were based on them.

The introduction of the simple inverted inner tube for gas collection and the use of pH indicators enabled the production of gas and acid to be detected by inspection. Screw-capped bottles and tubes are not satisfactory for sugar tests because the CO2 evolved by the bacteria during growth is trapped and, by lowering the pH value of the medium, may change the colour of the indicator and suggest a (false) positive result. If screw-capped containers are used, the caps should therefore be loosened about an hour before the indicator colour is observed.

The failure to standardize methods has led to discrepant results in the hands of different workers, and it is only within recent years that taxonomists have given adequate thought to the significance of acid production by a bacterium growing in a medium containing a carbohydrate. *Lesteria monocytogenes* give positive result (ferment glucose , produce acid and change in color of the media) .Peptones are also present in such a medium and, during growth of the organism, these are broken down to substances that are alkaline in reaction; if, in the medium, there is a carbohydrate, alcohol, or other substance commonly called a 'sugar' that can be broken down by

the bacteria either by oxidation or by fermentation, acid will be produced, but it will be detected by a pH indicator in the medium only when the acid produced from the sugar exceeds the alkali from the peptone. The visibility of the reactions is also influenced by (i) the buffering properties of the medium, and (ii) the indicator used; for example, bromthymol blue shows acid production when the pH value falls to 6.0 or less, whereas bromcresol purple does not change colour until the pH has fallen to about 5. Peptone Water Sugars, which are commonly used in the UK, have less buffering power and yield less alkali than the broth-based sugars used extensively in the USA and elsewhere.

Some bacteria will not grow on simple media and need an enriched sugar medium.

3.5.2.1.1.6. The catalase test:

Is simple and seldom causes difficulty, but because some strains of lactobacilli, pedicococci, and a few strains of *Enterococcus (Streptococcus) faecalis* appear to form catalase, Gutekunst, Delwiche & Seeley (1957) questioned the validity of the test 'as an overriding classification feature'. False catalase reactions by some lactobacilli grown in low (0.05%) concentrations of glucose are due to an azideinsensitive, non-haem catalase (pseudocatalase) and can be avoided by using media with 1% glucose without added haematin.

Lesteria monocytogenes produce apositive reaction, which may easily be missed by those looking only for strong reactions. Gagnon, Hunting & Esselen (1959) described a simple method in which some of the growth of the organism under test was spread on discs of filter paper and dropped into 3% H2O2; when catalase was present the evolution of gas quickly brought the discs to the surface.

Alternatively, a commercial paper-strip method is available for the detection and measurement of hydrogen peroxide production (Appendix C1.17). Another method, the catalase drop test, can be used for rapid results.

3.5.2.1.2. Secondary Identification tests:

3.5.2.1.2.1. CAMP test:

A positive CAMP test**,** described by Christie, Atkins & Munch-Petersen (1944), is the production of a clear zone around a colony in an area of a blood agar plate that has been affected by staphylococcal (3-toxin; this bald statement needs amplification, for the clearing takes place only on blood agar made with sheep or ox blood, and not on media made with human, rabbit, horse, or guinea-pig blood.

The important point in carrying out this test is that the agent produced by the bacterial cells must come in contact with the sheep (or ox) red cells before the staphylococcal Beta-haemolysin. The test is almost specific for strains of *Streptococcus agalactiae* from man or animals; Christie, Atkins & Munch- Petersen (1944) failed to find any other streptococcal species that produced the clear zone, but some haemolytic strains of groups E, P, and U give positive CAMP reactions. Lesteria monocytogenes has give **+ ve** result of CAMP test with Staphylococcus aurues.

Unlike the CAMP phenomenon this observation does not seem to have led to the development of a useful specific diagnostic test.

3.5.2.1.2.2. Aesculin hydrolysis:

Is a test of value for *Listeria monocytogenes*, many anaerobic genera and some other organisms. The glycoside aesculin contains molecules of the aglycone 6,7-dihydroxycoumarin and glucose; hydrolysis of aesculin may be demonstrated in one of two ways. The usual method is to incorporate the glycoside in a nutrient base together with a ferric salt; aesculin hydrolysis is indicated by a brown coloration due to reaction of the released aglycone molecule with the iron.

In addition, hydrolysis of aesculin, which is naturally fluorescent in UV light, can be confirmed by the loss of fluorescence, thus obviating possible confusion with pigment producing organisms. Alternatively, utilization of the related glucose

portion of the aesculin molecule by the organism can be detected by acid or acid and gas production.

3.5.2.1.2.3. Haemolysin production and **hemolysis**

Are not always cause and effect; the ability to produce a soluble haemolysin is not necessarily associated with zones of haemolysis on Blood Agar plates. Streptococci produce haemolytic zones on the surface of Blood Agar made from the blood of most animal species and these organisms are rightly named haemolytic streptococci.

Brown (1919) studied the nature of the haemolytic zones around colonies in poured plates and labelled the types of haemolysis **a** (green zone, cell envelopes intact), (3 (clear, colourless zone, cellenvelopes disrupted) and *y* (no action on red cells).

The term y-haemolysis is an anachronism for 'non haemolytic' and describes a negative result. The application of the terms a and p has been extended to the haemolytic zones seen around bacterial colonies on the surface of Blood Agar.. The P-haemolysis seen on Blood Agar plates is usually due to streptolysin S: some strains of *S. pyogenes* produce only the O haemolysin and are consequently non-haemolytic on Blood Agar unless incubated anaerobically.

Lesteria monocytogenes produce large zone of hemolysis when cultured in blood agar (B-hemolysis).

3.6. Questionnaire survey

Questionnaire survey was conducted to the meat value chains in the study sites and a detailed and organized questionnaire format was designed. A structured questionnaire were prepared and pre-tested and 50 butchers and 50 abattoir workers were surveyed. The questions and answers were written in English and entered

3.7. Data management and analysis

The data were analyzed using SPSS software (Statistical Package for the Social Sciences, version 20, SSPS Inc. And Chicago, IL, USA). All bacterial counts were analysis and ANOVA was performed. Statistical significance was set at a *P-value* of ≤ 0.05.

Chapter Four

RESULTS

4.1. Prevalence of *Listeria monocytogenes* in abattoir and butcher shops

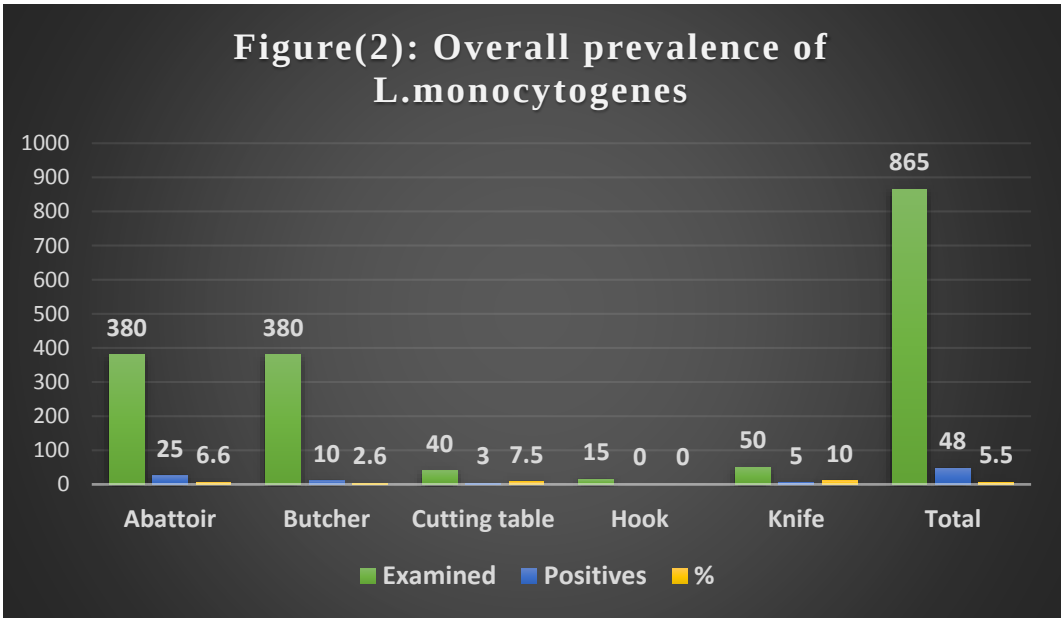
From a total of 865 samples, the overall prevalence of *L. monocytogenes* was 48 (5.5%) (Table 2). The prevalence of isolation of *L. monocytogenes* varied between sample sources. Out of each 380 samples collected from the abattoir and butcher shops, the prevalence of *L. monocytogenes* were 6.6% and 2.6% respectively. The result was higher in abattoir than butcher shops and there was significance difference in prevalence of *L. monocytogenes* from these sources of samples ($p<0.05$) (Table 3). Out of 105 equipment samples collected from both in abattoir and butcher shops, the prevalence of *L. monocytogenes* was 7.6%. There was no significance difference in prevalence of *L. monocytogenes* both in case of abattoir and butcher shops ($p>0.05$).

Table (2): Overall prevalence of *Listeria monocytogenes* from different source of samples.

Sample Type	No. examined	Prevalence (%)	95% CI
Abattoir	380	25 (6.6)	3.4-9.8
Butcher	380	10 (2.6)*	0.4-4.8
Cutting table	40	3 (7.5)*	3.4-11.6
Hook	15	0 (0.0)	-
Knife	50	5 (10)	5.7-14.3
Total	865	48 (5.5)	-

*Proportions (%) with similar letters are not statistically significant (with p-value = 0.05).
CI= confidence interval; %= percent of prevalence.

Table (2) in the above demonstrated the overall prevalence of *L. monocytogenes* in different sample sources when they were analyzed together which had the overall prevalence of 5.5. Knife was found to have the highest prevalence (10%) followed by cutting table (7.5%). Whereas the least prevalence was found to be hook (0.0%) that had statistically significant difference comparing with the others. Even though there was difference in prevalence among the others (abattoir, butcher, cutting table and knife), it was not statistically significant. This is also illustrated **in Figure (2) below.**



4.2. Prevalence of *Listeria monocytogenes* in abattoir and butcher shops

Out of the total 768 swab samples examined during the study period 25 (6.6%) and 10(2.6%) were positive for *L. monocytogenes*.

Table (3): Prevalence of *Listeria monocytogenes* from different sources of samples.

Source of sample	No. of examined	Total positive	Prevalence (%)	OR	CI of OR	χ^2	P-value
Butchers	380	10	2.6	1			
Abattoir	380	25	6.6	3.1	2.1-7.2	7.1	0.01
Total	760	35	4.6				

OR= odds ratio; CI= confidence interval; $\chi 2$= Chi square.

The total prevalence of the *L. monocytogenes* from abattoir and butcher shops was 4.6% (N=760). The prevalence in Abattoir have higher with statically significant difference (P=0.01). As the table (3) in the above indicated, the prevalence of the disease in Abattoir was almost three times (OR= 3.1, CI= 2.1-7.2) higher than the causative agent identified from Butchers. It is also indicated in the figure below (Figure 3).

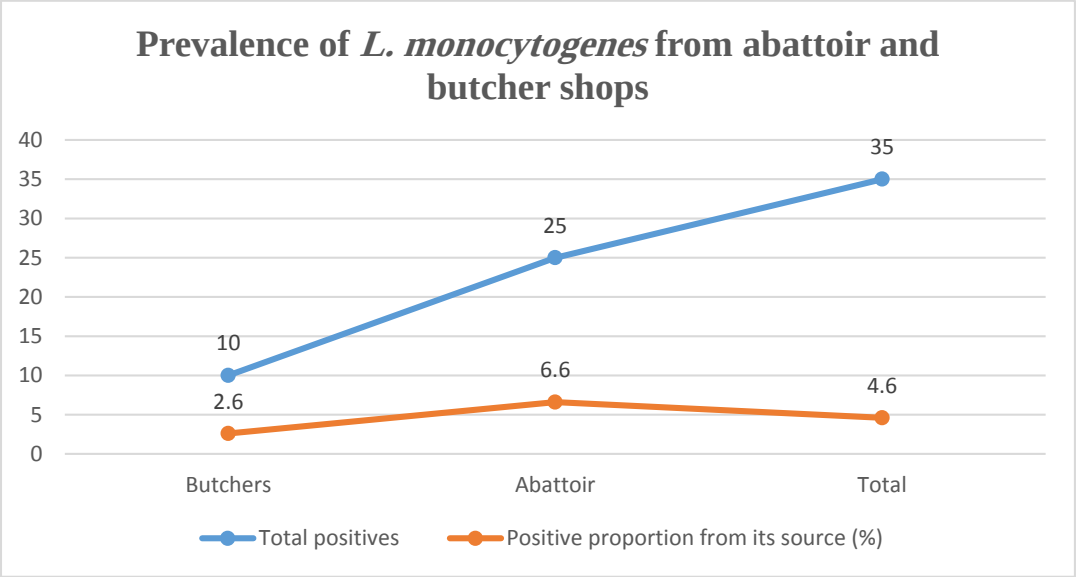


Figure (3): Proportion of positive prevalence in butcher shops and abattoir.

4.3. Contamination rate of *Listeria monocytogenes* in equipments.

Table (4): Prevalence of *listeria monocytogenes* in camel meat in contact surface materials.

Source of sample	No. of examined	Total positive	Prevalence (%)	χ^2	P-value
Hook	15	0	0.0		
Cutting table	40	3	7.5	3.6	0.1
Knife	50	5	10		
Total	105	8	7.6		

Table (4) and **figure (4)** indicated the contamination rate of *L.monocytogens* meat surface contact materials (hook, cutting table and knife). Although there was no even one sample positive for Hooks, there is no statistically significant difference among hook, knife and cutting table (P= 0.1).

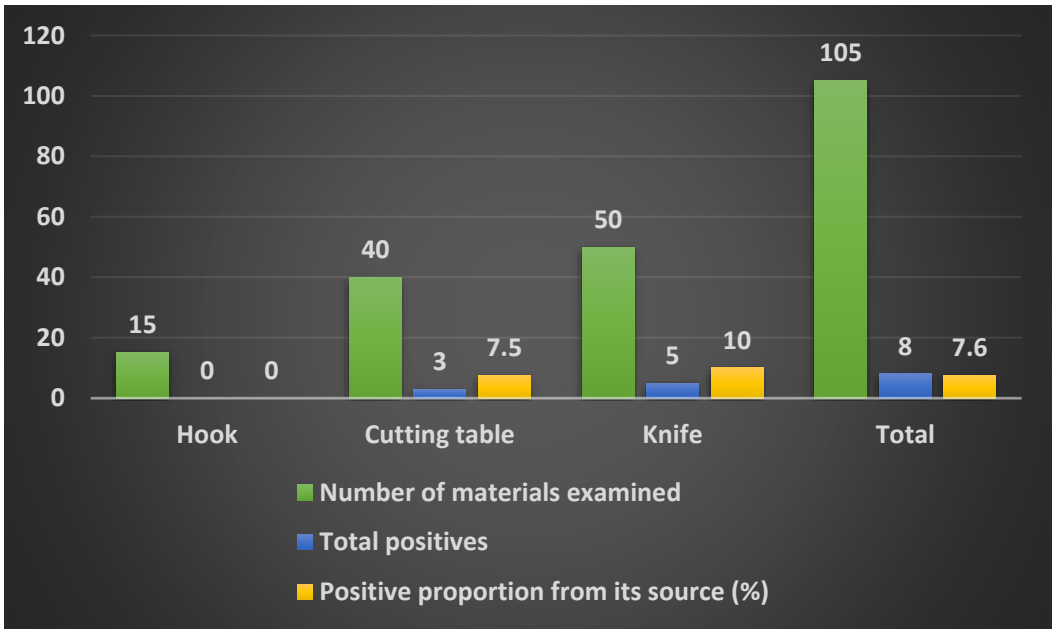


Fig (4): The positive proportion of the surface materials to *L. monocytogenes*.

4.4. Findings of questionnaire survey

4.4.1. Findings of questionnaire survey in abattoir

A total of 50 respondents were surveyed from the abattoir. About (20%) of the abattoir workers had completed high school level. Out of 50 respondents all (16%) had taken a lesson on personal hygiene. From the total of respondents (46%) and (20%) wash their hands once and twice per day during the course of working time respectively. And (44%) of the respondents reported to use detergent. Most of the respondents (84%) wash their hands after toilet. (Table 5).

From the total of respondents (54%) of the respondents cleaned the working surfaces between each process and after work. About (70%) of the respondents wash their working knives after the completion of the work and the rests (30%) wash several times during the course of working time. (Table 5).

As on observational assessment, 80% of the closets of butchers were dirty. And most of the workers (84%) in the abattoir do not wear aprons and do not wear a hair covering. 66% of them do not wear any jeweler materials. About the hygienic status of the Abattoir it is in a poor status. (Table 5).

Table (5): Summary of observational assessment and knowledge of workers on hygienic practices in abattoir

Abattoir activity	Performance	No. of respondents	Percent (%)
Educational status	1-8	35	70
	9-11	10	20
	≥ 12	5	10
Lesson on personal hygiene	Yes	8	16
	No	42	84
Time interval of washing hands	Once	23	46
	Twice	10	20
	Other	17	34
Washing of hands	With water only	28	56
	With detergent	22	44
Washing of hands after toilet	Yes	42	84
	No	8	16
Clean and disinfect working surfaces	Before work	23	46
	Between each process	27	54
Washing of knives	After work	35	70
	Between process	15	30
View of closets	Neat	10	20
	Dirty	40	80
Wearing of aprons	Yes	8	16
	No	42	84
Hair	Covered	8	16
	Not covered	42	84
Wearing of jeweler materials	Worn	17	34
	Not worn	33	66

4.4.2. Findings of questionnaire survey in butcher shops

A total of 50 respondents were surveyed from butcher shops. About (58%) of the butchers in an educational level of elementary and (22%) have completed high

school level. 14% of the respondents had taken a lesson on personal hygiene. (Table 6).

About 54% and 24% of the respondents wash their hands twice and once per day during the course of working time respectively. And (56%) of the respondents reported to use a detergent. As observed during the current study, about (86%) of the respondents wash their hands after toilet. (Table 6).

Although, about (8%) of the respondents reported the cashier is handling the money. The majority (92%) of the respondents handle the money by themselves. Some of the butchers (44%) cleaned the working surfaces and similarly washing of knives about (86%) performed after work. (Table 6).

As on observational assessment, (80%) of the closet of the butchers is dirty. And most of them (86%) didn't wear a hair covering. Wearing of jeweler materials were observed in (24%) of the butchers. About the hygienic status of the butcher shops (70%), (26%) and (4%) had poor, moderate and good status respectively. (Table 6).

Table (6): Summary of observational assessment and knowledge of workers on hygienic practice in butcher shops.

Abattoir activity	Performance	No. of respondents	Percent (%)
Educational status	1-8 9-11 ≥ 12	14 25 11	28 50 22
Lesson on personal hygiene	Yes No	7 43	14 86
Time interval of washing hands	Once Twice Other	12 27 11	24 54 22
Washing of hands	With water only With detergent	22 28	44 56
Washing of hands after toilet	Yes No	43 7	86 14
Clean and disinfect working surfaces	Before work Between each process	28 22	56 44
Washing of knives	After work Between process	43 7	86 14
Handling money	Cashier Butcher	4 46	8 92
View of closets	Neat Dirty	10 40	20 80
Hair covering	Covered Not covered	8 42	14 86
Wearing of jeweler materials	Worn Not worn	12 38	24 76
Hygienic status of the butcher House	Good Moderate Poor	2 13 35	4 26 70

Chapter Five

DISCUSSION

Production of safe food has important economic implications in an increasingly competitive global market. *Listeria* species are ubiquitous in nature and has been isolated from wide environmental sources. The organism possesses ability to survive in harsh conditions and therefore, can persist in environment. Because of such persistence *Listeria* species can easily enter in the food chain. Of the known *Listeria* species, *L. monocytogenes* is pathogenic to humans and animals (Pal, 2007; Raorane *et al*., 2014).

Raw meat and other raw food products commonly found in the retail environment may be contaminated with pathogens, including *L. monocytogenes.* Retail environments are much more open with many people coming and going. These open retail environments may allow for the introduction of *L. monocytogenes* at various points and times of the day, potentially making control of *L. monocytogenes* in the retail environment more difficult (Cutter *et al*., 2006).The detection and identification of *Listeria* species have attracted the attention of many authors.

This specific interest is related to the presence of *L. monocytogenes*, one of the most important food-borne pathogens, in the genus. It is often found in various uncooked foods, such as meat, cheese, and vegetables. It is widely diffused in the environment and this fact can cause the contamination of food during production and distribution. However, *L. monocytogenes* has been the main representative of the genus to be studied (Cocolin *et al*., 2002).

5.1. Prevalence of *L. monocytogenes* in raw camel meat

The specific prevalence of *L. monocytogenes* based on sample source was found to be statistically significant. In this study, the prevalence of *L. monocytogenes*

in camel meat was 6.6% in abattoir. This is in agreement with (Pociecha *et al.*, 1991) who noted a prevalence of 3.2% from carcass in Island and 5% from carcass in Brazil slaughter house (Ankpolat *et al*., 2004).

In the current study, the overall prevalence of *L. monocytogenes* was 4.1%. The prevalence was still higher in other country like Australia with 16% (Ibrahim and Mac Rae, 1991), and 40% (Mac Gowan *et al*., 1994).

Listeria monocytogenes has been found in different kinds of raw meat; there has been a relatively high frequency of positive findings amounting to by 20.8% by Sramova, *et al*. (2000) and 12.5% by Karpiskova (1998).

The prevalence of the pathogen (6.6%) was found to be in agreement with findings of Ankpolat *et al*. (2004) who recorded 5%. On the contrary, there was no detection of *L. monocytogenes* at abattoir from carcass in Germany (Cohen *et al*., 2006). And a prevalence of 50% by Abay *et al*. (2012) from which is very high from the current study.

The study also revealed the prevalence of *L. monocytogenes* in camel meat was 6.6% in abattoir and 2.6% in butcher shops. This is lower than Kwiatek *et al*. (1992) who observed a prevalence of 9.3% in sheep meat.

This could be attributed to the high microbial loads on raw meat entering the process and thus increase the potential for contamination of the processing environment and if separation is not adequately maintained the finished product (Gilbert *et al*., 2009).

In New Zealand, a prevalence of 30% *L. monocytogenes* was recorded by Gilbert *et al*.(2009) which was very high than the current study reported 6.6% prevalence of *L. monocytogenes* in abattoir. The reason for this was attributed to the differences in hygienic conditions of slaughter houses, storage and processing in

different countries. In Ethiopia only few researches was done, by Molla *et al*. (2004) who observed a prevalence of 5.1% in raw and ready to eat food products and one previous study revealed that a prevalence of 5.4% by Firehiwot, (2007) from raw meat, milk and milk products.

Other relative studies were done on a prevalence of 4.0% by Al Ali, *et al*. (2012) of *L. monocytogenes* from carcass in slaughter houses. And several studies confirmed that a prevalence of 4% by Ndahi *et al*. (2013) in ready to eat foods, 2.4% by Ennaja *et al*. (2008) from meat and meat products in Morocco and 4.7% by Yucel *et al,* (2005) from meat products in turkey.

The specific prevalence of *L. monocytogenes* from equipments was found to be statistically not significant. Dirty or contaminated equipments can contaminate the safe food. Improperly cleaned equipment can be a source of *L. monocytogenes* contamination. Based on FDA reports and foodborne outbreak reports provided to the CDC, three risk factors have been identified most frequently as contributing to the contamination, spread and growth of foodborne pathogens, including *L. monocytogenes*, in processing or retail environments. They are cross-contamination; improper cleaning and sanitation; or improper time and temperature control (Cutter *et al*., 2006).

In the present study, the equipments were potential source of contamination with a prevalence of 7.6% which was lowery than Lowry and Tiong (1988) and Dunja (2011) who reported 13% and 11.4% prevalence of *L. monocytogenes* in food contact surfaces, respectively.

Therefore, Control measures to reduce the carriage of these pathogens in ruminants prior to slaughter are reviewed with reference to the current regulations and guidelines relating to the primary production. This study result suggests that a

prevalence of 7.6% of *L. monocytogenes*. The prevalence was higher in other country a prevalence of 25.64% which is reported by Jankuloski *et al*, (2007).

The variation of prevalence in the two study sites may be because of environmental contaminations and poor sanitary conditions while handling of the meat before reach to the consumer. This indicates that the meat was free from *L. monocytogenes* wring distribution while slaughtering and the contamination occurs in an increasing level along the food value chain starting from slaughtering at the abattoir level, during distribution of the meat and improper handling of the meat handlers who sold it.

CONCLUSION

The consumption of improper meat is not safe from consumer point of view, as it may lead to the transmission of various diseases. In this study, the results of bacteriological assessment showed that raw meat from market and slaughter houses are a source of *L. monocytogenes*. In addition, the presence of this bacteria may be attributed to the unclean working environment, poor sanitary conditions of persons who are contacting with the meat and their equipment materials used. This may result in low meat quality and might potentially cause food poisoning especially in susceptible groups which includes pregnant women's, young, elderly and immunocompromised individuals. Due to high risk and public health concern, it may cause a high case fatality rate. The detection of this bacteria in ready to eat processed food makes it unfit for human consumption.

Listeria monocytogenes may not be seen as potential clinical threat in Sudan today, with the increasing trend of transnational spread and emerging diseases. The probable risk that it might pose in the years to come cannot be ignored. The present study demonstrated the possible risk of *L. monocytogenes* after consuming meat and RTE food stuffs available in the markets, and also highlighted the need for an effective and efficient storage process to keep such food safe, till they reached the consumers. Numerous risk factors are associated with the contamination and growth of *L. monocytogenes* in abattoir and market places. These factors need to be addressed and considered a serious hazard to identify control measures for an effective prevention and control program of the organism.

Further, sources of infection and modes of transmission should be ascertained. And addressing communication, risk perception and consumer practices to the public are mandatory.

RECOMMENDATION

1. Understanding the sources of the pathogen and factors that contribute to the risk of contamination, growth and spread of the pathogen are important building blocks to an effective control program.
2. The best approach for preventing listeriosis is reducing the exposure of susceptible populations to contaminated food.
3. The production of microbiologically safe food is fundamentally based on the implementation and application of general preventative measures, good hygienic practices and good manufacturing practices.
4. The food contamination needs to be controlled and information provided to the people who are at a greater risk.
5. Creating public awareness by disseminating the information is necessary and an extensive survey of the prevalence of *L. monocytogenes* in whole of Sudan must be undertaken.

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APPENDICES

Appendix I: Questionnaire

Date of collection _____

Code No. _____

Observational assessment and knowledge of worker's on hygienic practicesin Abattoir.

1. Worker's name _____Sub-city _____ Educational status _____

2. Have you ever received any lessons in personal hygiene? Yes _____ No ____

3. How often do you wash your hands? Once _____ Twice _____ Other ____

4. Washing hands with water only _____With water and soap ____

5. Do you wash your hands after toilet? Yes ____ No ____

6. How often do you clean and disinfect working surfaces? Before work ____ After work _____

7. Washing knives after work _____ Between each process_____

8. The view of the closets of the butchers? Neat ____ Dirty ____

9. Wearing of aprons Yes _____ No _____

10. Hair Covered _____ Not covered _____

11. Jeweller materials Worn _____ Not worn _____

Date of collection _____

Code No. _____

Observational assessment and knowledge of butchers on hygienic practicesin butcher shops.

1. Worker's name _____ Sub-city _____ Educational status _____

2. Have you ever received any lessons in personal hygiene? Yes _____ No ____

3. How often do you wash your hands? Once _____ Twice _____ Other ____

4. Washing hands with water only _____With water and soap ____

5. Do you wash your hands after toilet? Yes ____ No ____

6. Who is handling the money? Butcher _____Cashier_____

7. How often do you clean and disinfect working surfaces? Before work ____
After work _____

8. Washing knives after work _____ between each process_____

9. The view of the closets of the butchers? Neat ____ Dirty ____

10. Aprons Used _____Not used _____

11. Hair Covered _____ not covered _____

12. Jewellery materials Worn _____ not worn _____

13. Hygienic status of the butcher shops Good _____ Moderate _____
Poor _____

Appendix II: Laboratory Data Collecting Sheet

		Isolation and primary identification				Confirmation		
S. N	**Sample code**	**Gram stain**	**Motility test**	**Catalase**	**Oxidase**	**Hemolysis**	**CAMP test**	**Carbohydrate utilization tests**

								D-mannitol	Rahmnose	Xylose

Appendix III: Procedure of Gram's staining

1. Prepare the smear and heat fix.
2. Stain with crystal violet for 60 seconds and rinse with tap water and drain.
3. Flood the slides with iodine and allow remaining 60 seconds and rinsing with tap water and drain.

4. Decolorize with 95% ethanol until 15 seconds and rinse with tap water and drain.
5. Counter stain with safranin for 60 seconds and rinse with tap water and drain.
6. Examine microscopically under oil immersion.

Appendix IV: Procedure for catalase test

1. Place a drop of 3% H2O2 on a glass slide.
2. Touch a sterile loop to a culture of the organism to be tested and pick up a visible mass of cells (colony).
3. Mix the organism in the drop of hydrogen peroxide.
4. Observe for immediate and vigorous bubbling.

Interpretation: Bubbling indicates a positive test and no bubbling indicates a negative test.

Appendix V: Procedure for oxidase test

1. Prepare a solution of 1% tetramethyl-p-phenylenediaminedihydrochloride.
2. Piece of filter paper is moistened in a petridish with fresh reagent.
3. The test bacterium is streaked firmly across the filter paper with a glass rod.
4. A dark purple color along the streak line with in 10 seconds indicates a positive reaction.

Appendix VI: Procedure for haemolysis test

1. Isolates colony was taken with an inoculating needle from a typical colony on TSYEA (Tryptone Soya Yeast Extract Agar).
2. Streak the sample in to 7% Sheep Blood Agar Base.
3. It was incubated at 37°C for 24 hours.

4. After incubation positive test cultures show narrow, clear and light zones (β-haemolysis).

Appendix VII: Procedure for CAMP test

1. Take a colony culture with an inoculating needle from a typical colony on TSYEA
2. *Staphylococcus aureus* was taken (CIP: Collection of Institute of Pasteur, 5710).
3. It was streaked vertically in a single line across a sheep blood agar plate and *Listeria* isolates horizontally to *S. aureus* streak and
4. The plates were incubated at 37oC for 18 to 24 hours.
5. An enhanced zone of beta hemolysis between the test strain and culture of *S. aureus* was considered a positive reaction. *L.monocytogenes* showed an enhanced zone of hemolysis, forming anarrow head towards the *S. aureus* culture.

Appendix IX: Procedure for carbohydrate utilization test

1. Isolated colonies from TSYEA was transferred into test tubes containing xylose, rhamnose and mannitol and
2. It was incubated at 37oC for up to 5 days.
3. Positive reactions were indicated by yellowcolor (acid formation).

Appendix X: Composition and preparation of culture media used for the study.

 Pre-enrichment - *Listeria* enrichment broth

Specifications; KM 10505

Composition (gm/l)

Peptone mixture …….. 20

Yeast extracts ……...6.0

Sodium chloride ……...5.0

Potassium dihydrogen phosphate …....2.5

Glucose ……2.5

Nalidixic Acid ……0.04

AcriflavinHCl ……..…0.015

Cyclohexamide…….....0.05

pH 7.3+/- 0.2

Preparation;

- ✓ 36.1 gm of powder was weighed and added to 1lt of deionized water (conductivity <10ms).
- ✓ Then warmed until complete dissolution.
- ✓ It was mixed well and 225 ml was distributed into each of 250ml erlenymer flasks and sterilized by autoclaving at 121oC for 15 minutes.

 Secondary selective enrichment media (*Listeria* Fraser broth)

Specification KM 10335

Use: For isolation and enumeration of *Listeria* species.

Composition (gm/l)

Peptone mixture ……..10

Yeast extract ……5

Sodium chloride ……..20

KH2PO ……...….1.35

Na2HPO ……..……9.5

Beef extract …….5.0

Nalidixic Acid ……0.010

AcriflavinHCl …….……0.0125

Aesculine ……..….1.0

Lithium chloride ……....3.0

PH= 7.2+/- 0.2

Preparation

- ✓ 27.4 gm of the powder was weighed and added to 500ml of the deionized water (conductivity<10ms).
- ✓ Then it was well mixed and sterilized by autoclaving at 121o C for 15 minutes.
- ✓ After sterilization, it was allowed to cool to around 47oC and previously prepared and filtered (sterilized), 5 ml of 5% Ferric ammonium citrate* supplement was added to this broth.
- ✓ Then it was well mixed and 10ml of the broth was aseptically dispensed in to sterile tubes.

*Ferric ammonium citrate (17% Fe)-MERCK.

 ***Listeria* isolation agar**; two selective media were used for this purpose

A. Oxford Agar

Specification: KM1049

Use: A selective medium for the isolation of *Listeria monocytogenes* from food and clinical materials.

Composition (gm/l)

Coloumbia agar ……..42.0

Aesculine…… 1.0

Ferric Ammonium citrate ….. 0.5

Lithium chloride ……....15.0

PH= 7.2 +/- 0.2

Preparation

- ✓ 5 g of the powder was weighed and added to 1lt of deionized water and then it was allowed to soak for 10 minute and
- ✓ Swirled to mix and sterilized by autoclaving at 121oC for 15 minutes.
- ✓ It was kept at room temperature until it cooled to around 47o C and 2 vials of dry powder of the selective supplement KM ‘SO49 was suspended with 1 ml distilled water and added into this medium.
- ✓ At last this prepared medium was thoroughly mixed by agitating and it was pour plated into sterile petri dish.

KM SO49 supplement contains;

CCNAF selective supplement (MICRO TRADE)

Formula;

CEFOTITAN …… 1 mg

COLISTIN …….10 mg

FOSOMYCIN ……5 mg

ACRIFLAVINE……. 2.5 mg

NATAMYCIN ……..12.5 mg

B. PALCAM (Polymixin acriflavin lithium chloride ceftazidime, aesculin and mannitol) agar base

Specification; KM S079

Use: An important selective differential medium for the isolation of *Listeria monocytogenes* from food, clinical and environmental specimens.

Composition (gm/l)

Columbia peptone mix …...….23.0

Aesculine ……...….0.8

Ferric ammonium citrate …..... 0.5

Lithium chloride …… 15.0

Corn starch ……1.0

Yeast extract ……. 3.0

Mannitol ……...10.0

Sodium chloride …….5.0

Glucose ……..0.5

Phenol red ……. 0.08

Agar ……. 12.0

PH=7.2 +/- 0.2

Preparation

- ✓ 1liter of PALCAM medium was prepared by weighing and adding of 70.8 g of the powder into one liter of deionized water.
- ✓ Followed by mixing and sterilizing of the medium at 1210C for 15 minutes.
- ✓ Then, it was allowed to cool to around 47 0C and 2 vials of the selective supplement KMSO79 was added (as in OXA case), mixed and pour plated.

At this level the PALCAM medium was used after keeping for some time to allow drying of the medium.

KMSO79 selective supplement- PAC (MICRO TRADE)

Formula; Polymixin B ……. 6.25 mg

Cetrazidine ……. 10 mg

Acriflavine……... 2.5 mg

D. Tryptose yeast extract agar

Specification; KM 1116

Use: an agar for performing total viable count by the pour plate method.

Composition (gm/l)

Tryptone ……5.0

Yeast extract ……. 2.5

Glucose ……. 1.0

Agar …….. 15.0

pH 7.0+/-0.2

Preparation

- ✓ 23.5 g of the ingredients was suspended in1 lit of the deionized water and boiled with frequent stirring.
- ✓ Then, it was dispensed into screw-capped tubes and autoclaved at 1210C for 15 minutes and the rack was kept in slant position in order to prepare slants.

E. Tryptone Soya Yeast Extract Agar (TSYEA)

Composition (gm/l)

Tryptone.....17.0

Soya peptone…… 3

Sodium Chloride ……. 5.0

Di potassium phosphate ….. 2.5

Yeast extract ……..... 6

Glucose …… 2.5

Agar ……. 15.0

Preparation

- ✓ 1liter of deionized water dissolved in 40gm powder and mix well.
- ✓ Heat with frequent agitation and boil for one measure to completely dissolve the powder.
- ✓ Autoclave at 121o C for15minutes.
- ✓ Dispense in to petri dishes.

F. Blood Agar Base

Composition (gm/l)

Heart infusion from (solids) ……..…2.0

Pancreatic digest of casein……....13.0

Yeast extract ……..…5

Agar……...15.0

Sodium chloride ……5.0

Preparation

- ✓ 1liter of deionised water dissolved in 40gm powder and mix well.

- ✓ Heat with frequent agitation and boil for one measure to completely dissolve the powder.
- ✓ Autoclave at 121o C for15minutes.
- ✓ Cool the base to 45 to 50oC and add 5% sterile, defibrinated sheep blood.
- ✓ Dispense in to petri dishes.

G. Carbohydrate utilization broths (rhamanose, xylose and mannitol)

i. Purple broth base

Composition (gm/l)

Peptone from meat …….. 5

Peptone from casein ……5

Purple base …….. 0.018

Sodium chloride

Preparation

- ✓ Dissolve 15gm of powder in 1lit of purified water.
- ✓ Autoclave at 121o C for 15 minutes and cool to about 60oC.

ii. Carbohydrate solution

Rhamanose

Xylose

Mannitol

Preparation

- ✓ Dissolve 5gm of each carbohydrate in 100ml of water separately.
- ✓ Sterilization by filtration.

iii. Complete medium

Preparation

- ✓ For each carbohydrate, add aseptically 0.5 ml of filter sterilized carbohydrate solution to 4.5 ml of Phenol red solution prepared.

H: Buffered peptone water (Oxoid, England)

Composition (g/l)

Peptone ……10.0

Sodium chloride …5.0

Final pH 7.5 ± 0.2 (at 25 o C)

Preparation

- ✓ 15 g of the powder was dissolved in 1 liter of distilled water. Stirred and dissolved completely. Then, sterilized by autoclaving at 121o C for 15 minutes after dispensing into the test tubes.