

**Sudan University of Science & Technology  
College of Graduate Studies**

**Bacteriological Quality of Export Sheep Meat  
in Khartoum Slaughtered House**

الجودة الباكترية للحوم الضان المذبوحة للتصدير بمسلخ بولاية الخرطوم

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# **DEDICATION**

To my husband and my daughters

To Soul of my father

To my Sincerely mother,

To my sisters and brothers

To my friends

For their tremendous support, encouragement and patience.

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First of all thanks and praise to Almighty Allah for giving me strength and health to do this work.

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## LIST OF CONTENTS

Title	Page
DEDICATION	I
ACKNOWLEDGEMENTS	II
LIST OF CONTENTS	III
LIST OF TABLES	VII
ABSTRACT	VIII
المستخلص	x
INTRODUCTION	1
<b>CHAPTER ONE:LITERATURE REVIEW</b>	
1.1 Sources of Meat Contamination	3
1.2Meat hygiene and inspection	7
1.3 Low Temperature Food Preservation	8
1.4The effect of freezing on microorganisms	9
1.5 Important bacteria causing contamination	10
1.5.1 <i>Escherichia- coli</i>	10
1.5.2 Genus :Salmonella	11
1.5.3 <i>Staphylococcus aureus</i>	12
1.6 Public health risk	14
1.7 Dispatching Meat for freight	15
1.8Hazard analysis and critical control points (HACCP)	15
1.8.1 Principles of the HACCP system	15
1.9 Applied range	16
<b>CHAPTER TOW :MATERIALS AND METHODS</b>	
2.1.1 Study area	17

2.1.2.Type of study	17
2.1.3 Sampling precedence	17
2.1.4 Size of the samples	17
2.2.Normal saline	17
2.3 Culture media	17
2.3.1 Solid media	17
2.3.1.1 Nutrient agar (Oxoid CM0003)	17
2.3.1.2 Blood agar (Oxoid CM0055)	18
2.3.1.3 MacConkey's agar (Oxoid CM0007)	18
2.3.1.4 Serum agar	18
2.3.1.5 Urea agar (Oxoid code CM0053)	18
2.3.1.6 Simmons citrate agar (Oxoid Code CM135a)	18
2.3.1.7 Semi –solid Media	19
2.4.2.1 Hugh and leifson s (OF)medium	19
2.3.3 Liquid media	19
2.3.3.1 Nutrient broth (Oxoid CM0001)	19
2.3.3.2 Peptone water sugars	20
2.3.3.3 Glucose phosphate broth(V.P medium )	20
2.3.3.4 Nitrate broth	20
2.4 Reagents and chemicals	20
2.4.1 Hydrogen peroxide	20
2.4.2 Oxidase test reagent	20
2.4.3 Nitrate test reagent	21
2.4.4 Kovac's reagent (oxoid MB0209)	21
2.4.5 Voges Proskauer test reagent	21

<u>2.4.6 Methyl red solution</u>	21
<u>2.4.7 Lead acetate paper...</u>	21
<u>2.4.8 Andrade's indicator</u>	22
<u>2.4.9 Gram's stain solutions</u>	22
<u>2.4.9.1 Crystal violet</u>	22
<u>2.4.9.2 Lugol's iodine</u>	22
<u>2.4.9.3 Decolourizing stain</u>	22
<u>2.4.9.4 Counter stain</u>	22
<u>2.5. Types of samples</u>	22
<u>2.6. Collection of samples.....</u>	22
<u>2.6.1 Microscopic examination...</u>	23
<u>2.6.2 Isolation procedure</u>	23
<u>2.7. Examination of culture ...</u>	23
<u>2.7.1 Purification of culture...</u>	23
<u>2.8. Identification of isolated bacteria</u>	23
<u>2.8.1 Primary identification</u>	24
<u>2.8.1.1 Preparation of smears</u>	24
<u>2.8.1.2 Gram's technique</u>	24
<u>2.8.1.3 Catalase test</u>	24
<u>2.8.1.4 Oxidase test</u>	24
<u>2.8.1.5 Oxidation-fermentation test (O.F)...</u>	24
<u>2.8.1.6 Motility test</u>	25
<u>2.8.1.7 Sugar fermentation test</u>	25
<u>2.8.2 Secondary Identification .....</u>	25
<u>2.8.2.1 Indole test</u>	25
<u>2.8.2.2 Voges-Proskauer test (V.P)</u>	25
	26

<u>2.8.2.3 Methyl red test</u>	26
<u>2.8.2.4 Urease test</u>	26
<u>2.8.2.5 Citrate utilization test</u>	27
<u>2.8.2.6 Nitrate reduction test</u>	27
<u>2.8.2.7 Sugar fermentation...</u>	27
<u>2.8.2.8 Aesculin hydrolysis</u>	27
<u>2.8.2.9 Gelatin Hydrolysis (or liquefaction)</u>	27
<u>2.8.2.10 Hydrogen sulphide production</u>	27
<u>2.8.2. Pigment production</u>	28
<u>2.8.2.12 Coagulase test</u>	28
<u>2.8.2.13 Phosphatase test</u>	28
<u>2.8.2.14 Growth in media with increased NaCl concentration test</u>	28
<u>2.8.2.15 Growth at 45°C</u>	28
<u>2.9 Methods of sterilization</u>	28
<u>2.9.1. Dry Heat</u>	28
<u>2.9.1.1. Hot air oven</u>	28
<u>2.9.1.2 Red Heat flame</u>	28
<u>2.9.1.3 Flaming</u>	29
2.9.2. Moist Heat	29
2.9.2.1 Autoclaving _____	
<u>2.9.2.2 Momentary autoclaving</u>	
2.9.3 Disinfection of media preparation room	31
	33

<u>CHAPTERTHREE: RESULTS</u>	34
3.1 Bacteria isolated from different samples	37
3.1.2 <i>Escherichia- coli</i>	37
.3.1.2. Staphylococcus aureus	42
<u>CHAPTERFOUR: DISCUSSION</u>	44
Discussion	
Conclusion and recommendations	
<u>REFERENCES</u>	



## LIST OF TABLES

Table NO		Page
1.	Type and number of samples	23
2	Bacteria isolated from different meat samples	31
3	Comparison of the mean Total Viable Count of Bacteria ( $\log_{10}\text{cfu}/\text{cm}^2$ ) $\pm$ Std at Different operational Points of Investigation at different sites of sheep carcasses	32
4	Characters used for identification of <i>E. coli</i> isolated from samples	33
5	Characters used for identification of <i>Staph aureus</i> isolated from swab samples of sheep	34
6	Number and Percentage of <i>Escherichia- coli</i> Isolated from Different Operational Points from different sites at Sheep carcasses	35
7	Number and Percentage of <i>Staphylococcus aureus</i> Isolated from Different Operational Points from different sites at Sheep carcasses	37

## ABSTRACT

This study was conducted in bacteriological quality of mutton slaughtered in the Karari National slaughterhouse, The aim was to isolate *Escherichia coli* and *Staphylococcus aureus* that cause contamination of meat during five stages of slaughterhouse (skinning stage through the washing, chilling, Transportation and Container). The samples from slaughtered lambs were collected from April 2013 to July 2013. Two hundred twenty-five samples (swabs) from (neck, shoulder, back) from carcasses at every stage of were collected slaughter process. Also 15 Samples were taken from hand's worker and 15 samples from Knives that were used in slaughtered and skinning stages. The samples were cultured in MacConkey agar, nutrient agar, blood agar the Incubated aerobically 37°C after isolates were been prepare samples were examined microscopically and classified into species level depending on the primary and biochemical characteristics.

Total Viable Count (TVC) of contaminating bacteria was done besides isolations and identification of bacteria. The results revealed that there was significant difference in the TVC after skinning, washing stages ( $p \leq 0.05$ ). The highest TVC  $3.04 \pm 0.28$  Log<sub>10</sub>CFU/cm<sup>2</sup> was recorded at shoulder site in transportation. The lowest TVCs  $2.9 \pm 0.10$  Log<sub>10</sub>CFU/cm<sup>2</sup> were recorded at neck site in three points which include skinning, washing and container respectively.

The hands of workers during the process of slaughtering the contamination levels recorded  $2.74 \pm 0.20$  Log<sub>10</sub>CFU/cm<sup>2</sup>. The bacteriological examination on Knives used during skinning and evisceration stages respectively revealed that contamination levels recorded  $2.89 \pm 0.16$  Log<sub>10</sub>CFU/cm<sup>2</sup>. Contaminating bacteria isolated were *Escherichia coli* and *Staphylococcus aureus*

The study revealed that *Escherichia coli* were predominant the most isolated from the muttons contaminations in slaughterhouse, followed by *Staphylococcus*

*auras*, which constituted a relatively approach mutton contamination affects uality, as well as to public health, leading to significant economic losses.

## الملخص

أجريت هذه الدراسة في الجودة البكتريولوجية للحم الضأن ذبحت في مسلخ كرري الوطني، وكان الهدف هو عزل اشريشيا كولاي والمكورات العنقودية الذهبية التي تسبب تلوث اللحوم خلال خمس مراحل من (السلخ، الغسيل التبريد، الترحيل و صناديق الشحن). تم جمع عينات من الحملان التي ذبحت في الفترة من أبريل ٢٠١٣ إلى يوليو ٢٠١٣ مئتان خمسة وعشرين عينة (مسحة) من (الرقبة والكتف والظهر) من الذبيحة الواحدة في كل مرحلة جمعت أثناء عملية الذبح. كما ١٥ عينة من ايدى العمال الذبيح، و ١٥ عينات من السكاكين التي كانت تستخدم في مراحل الذبح والسلخ. وكانت العينات المعزولة في أجار ماكونكي، الأجار المغذي والدم أجار والمحتضنة هوائيا ٣٧ ° C بعد أن كانت العزلات إعداد تم فحص عينات مجهريا وتصنيفها إلى مستوى الأنواع اعتمادا على الخصائص الأساسية والحيوية.

وقد تم العدا الإجمالي للبكتريا (TVC) من البكتيريا الملوثة إلى جانب العزلة والتعرف على البكتيريا. وكشفت النتائج أن هناك فرق كبير في TVC بعد السلخ، مراحل الغسيل ( $p \leq 0.05$ ). وسجلت أعلى  $\text{Log}_{10}\text{CFU} / \text{TVC} = 3.04 \pm 0.28$  / سم مربع في موقع الكتف في النقل. وأدنى مستوى من التلوث سجل  $2.9 \pm 0.10 \text{ Log}_{10}\text{CFU} / \text{cm}^2$  were سجلت في موقع العنق في ثلاث نقاط والتي تشمل السلخ والغسيل والحاوية على التوالي.

أيدي العمال أثناء عملية ذبح مستويات التلوث سجلت  $2.74 \pm 0.20 \text{ Log}_{10}\text{CFU} / \text{cm}^2$ . The الفحص البكتريولوجي على السكاكين المستخدمة أثناء السلخ ومرحلة تفريغ الأحشاء كشف على التوالي أن مستويات التلوث سجلت كانت  $2.89 \pm 0.16 \text{ Log}_{10}\text{CFU} / \text{cm}^2$ . البكتيريا المعزولة الاشريكيا القولونية والمكورات العنقودية الذهبية.

وكشفت الدراسة أن الإشريكية القولونية كانت سائدة في معظم معزولة عن التلوث الخراف في مسلخ،  
تليها المكورات العنقودية، التي تعد نسبيا تلوث لحم الضأن تؤثر ، على الصحة العامة، مما يؤدي إلى  
خسائر اقتصادية كبيرة.

## INTRODUCTION

Sudan is rich in animal resources, sheep exportation contributes greatly to the growth at national income. Number of exported sheep was estimated to be about 1.5 million heads in 2009 and about 1.8 million heads in 2010. Exported sheep carcasses during the same period was estimated to be about 64 million in 2009 and 41 million in 2010 (Statistical Bulletin for Animal Resources, 2011).

Food can be unsafe for human consumption due to change in its biological, chemical or physical properties (Food safety in section service, 1997).meat is major source of protein in human diet which is highly susceptible to microbial contaminations and can cause its spoilage and food borne infections in human, resulting in economic and health losses (Komba et al., 2012).Although muscles of healthy animals do not contain microorganisms, meat tissues get contamination during the various stages of slaughter and transportation (Ercolini et al., 2006). A great diversity of microbes inhabit fresh meat generally, but different types may become dominant depending on pH, composition, textures, storage temperature, storage temperature, and transportation means of raw meat (Ercolini et al., 2006; Li *et al.*, 2006; AduGyamfi *et al.*, 2012).

Normal sheep have microflora that established in their early life. Beside this microflora they tend to harbor different types of organisms found in their environment, since they were contaminated by soil, air and feed excreta Banwar( 1981). Wholesome meat, which is hygienically produced, is pathogen free, retains its natural state and nutritive value, insures to maintenance a degree of microbial contamination control and is unconditionally acceptable to the consumer, (Govinadajan, 1990; Gill, 2004). Evisceration processes contamination of sheep carcasses was studied and the increase of microorganisms in the abattoirs compared with their post level was also noticed (Borse et al., 1998; Gill and Baker,

1998). A significant increase in total bacterial counts at skinning points than those at washing points are recorded (Gilmour et al., 2004)

The different stages of the conversion from live animals into meat make the microbial quality of carcasses an unavoidable and undesirable result. During the slaughtering process, main sources of contamination are the slaughtered animals themselves, the staff and the work environment (Bell and Hathaway, 1996). The contamination of equipment, materials, and worker's hands and knives can spread pathogenic to non-contaminated carcasses.

Accordingly, washing and sanitizing agents are effective in reducing bacterial population and the presence of pathogenic bacteria on carcasses (Thornton and Gracey, 1976; Gill, 2004).

**Objectives:**

- To evaluate bacteriological quality of sheep meat for export in slaughterhouse Khartoum state.
- To assess meat hygiene in slaughterhouse in Khartoum state.

## CHAPTER ONE

### LITERATURE REVIEW

According to Jay (2000) it is generally agreed that internal tissues of healthy slaughtered animals are free from bacteria at the time of slaughter, provided that the animal was not in a state of exhaustion when slaughtered.

#### **1. 1 Sources of Meat Contamination:**

There are several genera of bacteria specially associated with the hands and nasal cavities and mouth, important of which are *Micrococcus* and *Staphylococcus*, (Bryan, 1978; Jay, 1986). The most important genera of bacteria known to occur in foods were given by Jay(1986). Their primary sources were; soil , water , plant and plant products .food utensils ,intestinal tract of man and animals ,food handler's feet, animal hides ,air and dust. Bryan (1986) and Jay (1986) considered food handler to be the important source of contamination. The microflora on their hands and outer garments generally reflect the environment and the habits of individuals in addition to those from water, dust, and soil. 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, cleaves, saws, hook and workers. Gracey (1985) reported that bacteria associated with meat depend on bacteriology of the soil on which the animals were kept prior to slaughter. The bacteria were transferred to the hides and then to the exposed meat. Frazier (1978) showed that any bacteria on the knife would soon be found on meat in various parts of carcasses as it was carried by the blood. The contamination of carcass come from different sources including environment and equipments with which meat comes in contract during slaughtering and processing, but hides remain as an important source of contamination. Hussein (1971) isolated bacterial contaminants of fresh meat from the gastro-intestinal tract and hides of the slaughtered animals



and from the water, walls and air deposits. Jepsen (1967) noticed that bacteria were carried to the abattoir on skin, hooves and body cavities of animals. Lawrie (1979) reported that if a contaminated knife was used or organisms were inadvertently introduced from the skin where the main blood vessels were severed bleeding could lead to contamination of the tissues. Gracey (1980) reported that there are different sources of meat contamination for example, invasion of blood vessels by bacteria from the intestines of weakened ill animals just prior slaughter. The animal's digestive tract was claimed to carry dangerous loads of bacteria. The hide, legs and hooves contain varying amounts of soil bacteria. Actual contagion with dirty hands, clothing's and equipment are important factors in the presence of bacteria in meat. Frazier and Westhof (1988), emphasized the importance of contamination from external sources during bleeding, skinning and cutting. The intestinal contents, knives, air, hands and clothes of the workers are important external sources. They also reported that during handling, contamination came from carts, boxes and other contaminated.

Meat is considered to be spoiled when it is unfit for human consumption. Meat is subjected to changes by its own enzyme, by microbial action and its fat may be oxidized chemically. Microorganisms grow on meat causing visual, textural and organoleptic changes when Jackson and McGowan (2001). Among the factors that affect microbial growth in meat are the intrinsic and extrinsic factors (Rombout and Nout, 1994), however the factors having the great influence on the growth of microorganisms in meat and meat products are the storage temperatures, moisture and oxygen availability (Forest et al., 1985; Frazier and Westhoff, 2004). Meat, the flesh of animals suitable for food has a very high nutritional value and moisture content with pH value of 5.4, could serve, as an excellent medium for microbial contamination growth and spoilage Lawrie (1985).

Meat is considered to be spoiled when it is unsuitable for human consumption. Spoilage can be caused by a wide variety of factors, such as improper handling, exposure to air and high temperature, or conditions that trigger chemical reactions or microbial contamination, although the most common cause is the presence of microorganisms together with metabolite production. Spoiled meats and meat products are inedible mainly due to off-odor and flavor, but consumer rejection is also due to discoloration, blown packages, souring, surface slime, and other alterations of meat quality. However, meat may also contain pathogens without showing signs of deterioration Zamudio( 2006). The organisms spoiling meat may infect the animal either while still alive ("endogenous disease") or may contaminate the meat after its slaughter ("exogenous disease"). There are numerous diseases that humans may contract from endogenously infected meat, such as anthrax, bovine tuberculosis, brucellosis, salmonellosis, listeriosis, trichinosis or taeniasis. Contaminated meat, however, should be eliminated through systematic meat inspection in production, and consequently, consumers will more often encounter meat exogenously spoiled by bacteria or fungi after the death of the animal. One source of infectious organisms is bacteremia, the presence of bacteria in the blood of slaughtered animals. The large intestine of animals contains some  $3.3 \times 10^{13}$  viable bacteria, which may contaminate the flesh after death if the carcass is improperly dressed. Contamination can also occur at the slaughterhouse through the use of improperly cleaned slaughter or dressing implements, such as powered knives, on which bacteria persist. A captive bolt pistol's bolt alone may carry about 400,000 bacteria per square centimeter. After slaughter, care must be taken not to infect the meat through contact with any of the various sources of infection in the abattoir, notably the hides and soil adhering to them; Water is used for washing and cleaning, the dressing implements and the slaughterhouse personnel. Bacterial genera commonly contaminate meat while it is

being processed, cut, packaged, transported, sold and handled include: *Salmonella spp.*, *Shigella spp.*, *E. coli*, *proteus*, *Staph. Albus* and *Staph. aureus*, *Cl. welchii*, *B. cereus* and faecal streptococci. These bacteria are all commonly carried by humans. Infectious bacteria from the soil include *Cl. botulinum*. As these microorganisms colonize a piece of meat, they begin to break it down, leaving behind toxins that can cause enteritis or food poisoning, potentially lethal in the rare cases of botulism. The microorganisms do not survive a thorough cooking of the meat, but several of their toxins and microbial spores do. The microbes may also infect the person eating the meat, although against this the microflora of the human gut is normally an effective barrier (Lawrie and Ledward, 2006). Fast and accurate detection of spoilage, even before evident signs appear, is necessary to prevent losses during production, distribution, and storage of meat products. Microbial analysis by traditional methods evaluates freshness, spoilage, and safety of meat and meat products; these are precise but time-consuming methods. A similar situation occurs with the usually lengthy sensory analysis methods. Various authors report the advantages of analyzing the chemical compounds related to spoilage, mainly of microbial origin (Borch, et al., 1996). Methods such as the electronic nose, biosensors, and fluorescence spectroscopy provide accurate and fast tools for spoilage detection. Finally, molecular techniques present a new opportunity to determine the type and load of spoilage microorganisms (Bjorkroth and Korkeala, 1996).

Even though, people are aware that food-borne diseases could occur due to consumption of street foods, the majority disregards these health hazards Bryan( 1998). Human food-poisoning is commonly associated with bacteria originating from animal sources; in most cases, infection is contracted indirectly by eating contaminated meat and meat products Report( 1970). Such contamination may

occur within the slaughterhouse Walton( 1970) or in processing and handling before sale (Foster, 1972; Casman, et al 1963; Timoney 1970 Watson, 1971). The high incidence of bacterial food-poisoning in man indicates that it is necessary to prevent contamination of meat and meat products in the food. Fatima( 1985) the most frequent coliform bacteria present in meat were *Escherichia coli*, *Klebsiella spp*, *Citrobacter spp*, *Enterobacter cloacae* and *Arizona spp*.

## **1.2 Meat hygiene and inspection**

Meat inspection practice is one of the important activities of the veterinary services .Its aim is to insure that meat is free from diseases, wholesome and fit for human consumption (Mitchell.1980). Alonge (1991). defined meat hygiene as a system of principles designed to ensure meat and meat products are safe, wholesome and processed in a hygienic manner and are fit for human consumption. According to Thornton (1968). the efficient meat hygiene practices begin in the farm and maintained throughout the chain i.e. in the animal collection centers, markets, during transportation of animals in abattoirs during transport of meat to butcheries and even at the consumer home meat hygiene is essentially a public health function, the primary role of which is to safeguard against infectious diseases by preventing their transmission to humans thereby providing safe wholesome meat and meat products for human consumption (Ibrahim and Salih, 1970). To insure that meat quality standards are maintained, slaughter of animals for human consumption should be done in abattoir. An abattoir has been defined as a premise approved and registered by the controlling authority for hygienic slaughtering and inspection of animals processing and effective preservation and storage of meat products for human consumption is also practiced in abattoirs Alonge( 1991).Meat hygiene programmer should have as their primary goal the protection of public health. This should be based on a scientific evaluation of meat

–borne risks to human health and also takes into account all relevant food safety hazards, as identified by research, monitoring and other relevant activities (FAO/WHO, 2005). In developed countries, the role of the veterinary profession in addressing needs of urban communities has long been focused on public health and hygiene (Bllani et al., 1978 ,WHO, 1981). The main objective of meat hygiene and inspection is to prevent food –borne infection and meat spoilage. The meat hygiene inspection and control practices are based on the transmissibility of diseases through either consumption or handling of meat Ibrahim( 1990).

The effective operation of meat hygiene services is 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 series, 1957).

Salih (1969) proposed that in order to improve the standards of meat hygiene laws of animal health should include meat hygiene regulations. 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 a meat resources center where data pertaining to meat hygiene throughout the country could be collected and analyzed. Regarding the slaughterhouses, and suggested that they should be run on sound economical bases and they should be able to make some financial benefits.

### **1.3 Low Temperature Food Preservation**

According to Jay (2000) preservation of food at low temperature used the fact that the activities of food microorganisms can be slowed at temperature above freezing and generally stopped at freezing temperature .The reason is that all metabolic reactions of microorganisms are catalyzed and the rate of enzyme

reactions is dependant on temperature. The term psychrophile was coined by Schmaltz-Nielsen (1902) for microorganisms that grow at 0°C. This term is now applied to organisms that grow over the range of subzero to 20°C, with an optimum range of 10 - 15°C. The Psychrotrophs are organisms able to grow at 5°C or below. It is now widely accepted among food microbiologist that psychrotroph is an organism that can grow at temperature between 0-7°C and produce visible colonies (or turbidity) within 7-10 days. Because some psychrotrophs are organisms that can grow at temperature at least as high as 43°C they are in fact, Mesophiles. By these definition psychrophiles would be expected to occur only on products from extremely cold climate. The organism that causes the spoilage of meat in the 0-5°C range would be expected to be psychrotrophs.

There are three destined temperature ranges for low temperature stored food: Chilling temperature: are those between the usual refrigerator 5-7 °C and Ambient temperature usual about 10-15 °C. Freezer temperature are those at or below -18°C Under normal circumstance growth of all microorganisms is prevented at freezing temperature, never less some can grow within the freezer range but at extremely slow rate.

#### **1.4 The effect of freezing on microorganisms**

In considering the effect of freezing on those microorganisms that are unable to grow at freezing temperature, it is well known that freezing means of preserving microbial cultures with freezing, drying being perhaps the best method known. However freezing temperature have been known to of certain microorganisms of importance in food.

Ingram summarized the salient fact of what happens to certain microorganisms upon freezing. There is sudden mortality immediately on freezing, varying with species, the proportion of cell surviving immediately after freezing die gradually when stored in frozen state. This decline in number is relatively in rapid at temperature just below the freezing point especially about( -2°C)but less so at lower temperature ,and it is usually slow below( -2°C). Bacteria differ in their capacity to survive during freezing, with cocci being generally more resistant than Gram-negative rods of the food poisoning bacteria, salmonella are less resistant than staphylococcus aureus or vegetative cell of clostridia, whereas endospores and food poisoning toxins are apparently unaffected by low temperature.

## **1.5 Important bacteria causing contamination**

### **1.5.1 *Escherichia coli***

*Escherichia coli* form pink, smooth, large colonies on MacConkey's agar and white to yellowish white, moist colonies in blood agar. *E. coli* appears Gram negative coccoid to short bipolar and is motile. The Organism ferments dextrose, lactose, maltose, dulcitol, mannitol and sucrose. Methyl red positive, Voges – Proskauer negative and produces indole. (Jones et al, 1997; Ali et al, 1998; Haider et al, 2004). Most *E. coli* strains are harmless, but some serotypes can cause serious food poisoning in human, and are occasionally responsible for product recalls due to food contamination. Dippold (2005). The harmless strains are part of the normal flora of the gut, and can benefit their hosts by producing vitamin K<sub>2</sub>, (Bentley and Meganathan 1982) and by preventing the establishment of pathogenic bacteria within the intestine (Hudault et al 2001 and Reid et al 2001). *Escherichia coli* and related bacteria constitute about 0.1% of gut flora (Eckburg, et al 2005). And fecal-oral transmission is the major route through which pathogenic strains of the bacterium cause disease. Cells are able to survive outside the body for a limited

amount of time, which makes them ideal indicator organisms to test environmental samples for fecal contamination.(Feng et al ,2002 and Thompson, 2007). There is, however, a growing body of research that has examined environmentally persistent *Escherichia coli* which can survive for extended periods of time outside of the host. (Ishii and Sadowsky, 2008).

In May 2011, *Escherichia coli* has been the subject of a bacterial outbreak that began in Germany. Certain strains of *Escherichia coli* are a major cause of foodborne illness. The outbreak started when several people in Germany were infected with enter hemorrhagic *Escherichia coli* (EHEC) bacteria , leading to hemolytic-uremic syndrome (HUS),a medical emergency that requires urgent treatment .The outbreak did not only concern Germany, but 11 other countries ,including regions in North America (Mellmann et al., 2011).

### **1.5.2 Genus: Salmonella**

This genus is of rod-shaped, Gram-negative, non-spore-forming, predominantly motile enteric bacteria with diameters around 0.7 to 1.5  $\mu\text{m}$ , lengths from 2 to 5  $\mu\text{m}$ , and flagella, which grade in all directions (i.e.peritrichous). They are chemoorganotrophs, obtaining their energy from oxidation and reduction reactions using organic sources, and are facultative anaerobes. Most species produce hydrogen sulfide (Clark and Barret 1987). Which can readily detected by growing them on media containing ferrous sulfate, such as TSI. Most isolates exist in two phases: motile phase 1 and non-motile phase. Cultures that are non-motile upon primary culture may be switched to the motile phase using a Craigie tube. *Salmonella* infections are zoonotic and can be transferred between human and animals. Many infections are due to ingestion of contaminated food .Distinctions are made between enteritis *Salmonella* and salmonella typhoid, paratyphoid *Salmonella* ,where the latter because of a special virulence factor and a capsule



protein (virulence antigen) can cause serious illness, such as *Salmonella enterica* subsp. *Enterica* serovar Typhi. *Salmonella typhi* is adapted to human and does not occur in other animals. *Salmonella* can survive for weeks outside living body. *Salmonella* are not destroyed by freezing. (Sorrells et al 1970; Beuchat and Heaton 1975). Ultraviolet radiation and heat accelerate their demise; they perish after being heated to 55°C (131 F°) for 90 min, or to 60°C (140F°) for 12 min (Fate of *Salmonella* 1978). To protect against *Salmonella* infection, it is recommended that food be heated for at least ten minutes at 75°C (167 F°) so that the centre of the food reaches this temperature. (*Partnership for Food Safety Education PFSE*).

### 1.5.3 *Staphylococcus*

All strains were identified as *Staphylococci*. The organism is non-motile, non-spore forming and non-acid fast. Colonies are small, smooth, and yellowish to golden in colour. All tested strains are catalase and coagulase positive, reduced nitrate to nitrite and all isolates are oxidase negative. All the isolates produce acid without gas from glucose, maltose, sucrose, ribose, mannose and glycerol. Some strains produced acid from fructose, lactose, and galactose. All strains attack salicin, xylose, arabinose or dulcitol. On the other hand the isolates of *staph. Epidermidis* are Gram-positive cocci and usually arranged in grape-like clusters and some times as short chains and in pairs. They are non-motile, non-spore forming and non-acid fast. The colonies are round, smooth, opaque, with entire edge, creamy or white in colour. They are coagulase and oxidase negative, urease and catalase positive. Variable results are obtained for nitrate reduction and hydrolysis of gelatine. The organism attacked glucose, maltose, sucrose, lactose and fructose with production of acid and no gas. Some strains fermented galactose and mannose. All tested strains not attack xylose, dulcitol mannitol or salicin. *Staphylococci* are wide spread in nature, although they are mainly found living on

the skin and mucous membranes. The coagulase – positive species *Staph. aureus* is well known as a human pathogen. Serious infections produced by *Staph. aureus* include bacteraemia, pneumonia, osteomyelitis, acute endocarditis, myocarditis, pericarditis, encephalitis, meningitis, choriamnionitis, mastitis, scalded skin syndrome and abscesses of the muscle, urinogenital tract and various intra - abdominal organs. Microorganisms can easily be introduced either in the pre or post processing stages of meat processing Johansson( 1983).The high coliform count observed from goat meat is assumed to be an indicator of fecal contamination. It is likely that the observed increase of fecal bacteria is due to problem associated with removal of the fleece and its coming into contact with the surface of carcass Ozlem( 2005). (Chaubey et. al, 2004). enumerated the coliform in the majority of the meat samples and suggested that raw meat and meat products should be handled under strict hygienic condition and stored in cool places to avoid contamination and safe guard the health of consumer. The high microbial load could be from the fleece of goat to the carcass surfaces during hide removal (Bell *et al*, 1993).The area of highest contamination was those sites where cuts were made through the skin (Bell and Hathaway, 1996). The finding of present study is a reflection of the unhygienic practices of meat processing vomiting (Singleton, 1995; Frazier and Westhoff, 2004). in the developing countries (Bhandare *et al*, 2007). It has been observed that the inner tissues of health animals are sterile, however, contamination comes from external sources during bleeding, handling and processing. During bleeding, skinning and cutting, the main sources of microorganisms are the exterior of the animal which includes the hide, hooves and hair and the numbers and many kinds of microorganisms from the soil, wash water, feed and manure, as well as its natural surface flora and the intestinal contents contain the intestinal organisms. Knives, cloths, air environment of the abattoir, slaughter-slabs, hands and clothing of the workers and the physical

facilities can serve as intermediate sources of contaminants. It has also been shown that during handling, contamination comes from carts, boxes or other containers, other contaminated meat, air and personnel. These resulted in the increase in the microbial load of the fresh goat samples (Lawrie, 1984; Rombout and Nout, 1994; Westhoff (2004)). Retail cut could also result in greater microbial load because of the large exposed surface area, more readily available water, nutrient and greater oxygen penetration available Forest *et al.*, (1985). Hence smaller retail cuts displayed are conducive for microbial growth and proliferation which leads to spoilage of the meat (Agnes, 1995). The presence of these meat is indicative of public health hazard and gives a signal of the possible occurrence of food borne intoxication and infection. This also implies that these meats are viable source of various diseases. Some of these diseases could spread and acquire epidemic status which poses serious health hazards. *Staphylococcus aureus*, which is a normal flora of the body, indicates contamination from handlers. The organism can pass onto food during harvesting, processing or even storage. It is the major cause of food poisoning known as staphylococcal food poisoning. The poisoning is caused by the ingestion of an enterotoxin produced, which is characterized by (Eze et al, 2008).

#### **1.5.4 *Listeria monocytogenes***

*Listeria monocytogenes* is a Gram –positive rod-shaped bacterium that forms single short chains (Theivagt, and Friesen 2006.), and can be resistant to the effects of freezing, drying, and heat (Sallami, et al 2006). *Listeria* are mainly found in the soil, though *Listeriae*, a pathogen, may specifically be found in raw foods, such as unpasteurized fluid milk (Fleming et al, 1985). Raw vegetable, raw and cooked poultry Dykes (2003). It has the ability to grow at low temperatures; thus, allowing it to grow in refrigerated foods. *Listeria monocytogenes* was thought to

be exclusively associated as infections in animals, but recently, this pathogenic species has also been isolated, in its dormant form, in the intestinal tract of small percentage of the human population (Rouquette and Berche, 1996). Because *Listeria monocytogenes* is an agent of listeriosis, a serious disease where the overt form has a severe mortality greater than 25 percent (Rouquette and Berche, 1996).

### **1.6 Public health risk**

There is considerable evidence of foodborne pathogens. Mainly of microbial origin which constitute major health hazards. Among all the microbial, *Salmonella* and *Campylobacter* are the most serious foodborne pathogens. There are two pathogens causing as many as 4 million illness and 4000 death per year in USA (Berry (1987)). Other important pathogenic bacteria associated with food safety issue is *Listeria* and coagulase positive *Staphylococcus*. Listeriosis can occur in healthy adults and children, however, the most vulnerable groups include pregnant women, infants, elderly and immune compromised persons (Jaradat et al., 2002).

### **1.7 Dispatching Meat for freight :**

- Cargo pallets, load securing devices, and loading equipment should be kept clean and free of potential food contaminants and be regularly washed and sanitized –Equipment used in transferring meat and meat product, such as hand trucks, containers, conveyors and Fork lifts, should be well maintained and kept in a sanitary condition.
- Loading time should be kept as short as possible to prevent temperature changes (increases or decreases) that could threaten the safety or quality of the meat.

## **1.8 Hazard analysis and critical control points (HACCP)**

### **1.8.1 PRINCIPLES OF THE HACCP SYSTEM**

The HACCP system consists of the following seven principles :

- Conduct a hazard analysis.
- Determine the Critical Control points (CCPs).
- Establish critical limits(s).
- Establish a system to monitor control of the CCP.
- Establish the corrective action to be taken when monitoring indicates that a particular CCP is not under control.
- Establish procedures for verification to confirm that the HACCP system is working effectively.
- Establish documentation concerning all procedures and records appropriate to these principles and their application. (FAO Corporate Document Repository ,1997).

### **1.9 Applied range**

It can be applied to several food categories, sea food, bulk milk production line. Bulk cream and Butter production Line, animal meat industry, Organic Chemical Contaminations in Food, Corn Curl Manufacturing Plant, etc. (FAO Corporate Document Repository ,1997).

## **CHAPTER TWO**

### **MATERIALS AND METHODS**

#### **2.1.1 Study area**

The study was conducted in Karari National slaughterhouse. The aim was to isolate *Escherichia coli* and *Staphylococcus* for bacteriological quality of sheep meat slaughtered for export in abattoir in Khartoum State .

#### **2.1.2 Type of study**

#### **2.1.3 Sampling precedence**

The samples from slaughtered lambs was collected from April 2013 to July 2013. Two hundred twenty-five samples (swabs) from (neck, shoulder, back) from carcasses at every stage of were collected slaughter process. Also 15 Samples were taken from hand's worker and 15 samples from Knives that were used in slaughtered and skinning stages.

#### **2.1.4 Size of sampling**

All swabs 255 were labeled and placed in a Thermos flask that containing ice and transported to the laboratory. Samples were processed immediately and cultured later

#### **2.2 Normal saline**

Amount of 8.5g sodium chloride was added to 1 liter of distilled water, mixed, dissolved and then sterilized by autoclaving at 121o C for 15 min (Gruickshank et al., 1975).

#### **2.3 Culture media**

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

### **2.3.1. Solid media**

#### **2.3.1 Collection of blood**

Blood for enriched media was collected with sterile syringe containing an anticoagulant (sodium dicitrate) by puncture of the jugular vein of healthy donor sheep kept at the Department of Microbiology. Blood for coagulase test was obtunded from Humans and centrifuged after collection to get fresh and sterile plasma.

##### **2.3.1.1. Nutrient agar (Oxoid CM0003)**

It consisted of lab – lemco powder (1.0g), yeast extract (2.0g), peptone (5.0g), sodium chloride (5.0g) and agar (15g). Twenty – eight grams of dehydrated medium were dissolved in one liter of distilled water, the pH was adjusted to 7.4, then was sterilized by autoclaving (121°C for 15 minutes), cooled to 50-55oC and then poured into sterile Petri dishes, 20ml each.

##### **2.3.1.2. Blood agar (Oxoid CM0055)**

Hundred 100 ml of fresh, sterile, defibrinated blood was added aseptically to 900 ml of melted sterile nutrient agar at 55°C, mixed and distributed into sterile Petri dishes 20ml in each dish.

##### **2.3.1.3. MacConkey's agar (Oxoid CM0007)**

The medium consists of peptone (20.0g), lactose (10.0g), bile salt (5.0g), sodium chloride (5.0g), natural red (0.075g) and agar (12.0g). It was prepared by adding the medium to 1000 ml distilled water, dissolved completely by boiling and sterilized by autoclaving at 121°c for 15 minutes, cooled to 50-55°C and then poured into sterile Petri dishes, 20ml each.

##### **2.3.1.4. Serum agar**

The medium was prepared according to Barrow and feltham (2003) by the addition of 10% sterile serum to melted nutrient agar.

#### **2.3.1.5. Urea agar (Oxoid code CM0053)**

The medium was prepared by dissolving 2.4 grams of the medium in 95 ml distilled water by boiling. The pH was adjusted to 6.8. After sterilization by autoclaving at 115°C for 20 minutes the base medium was cooled to 50°C and aseptically 5ml of sterile 40 % urea solution were added. and distributed in to screw –capped bottles 10 ml each and then was allowed to set in the slope position.

#### **2.3.1.6. Simmon’s citrate agar (Oxoid code CM0155)**

Twenty-three grams of the medium were dissolved in 1000 ml distilled water by boiling. The pH was adjusted to 7.0, and the medium was sterilized by autoclaving at 121°C for 15 minute, distributed into sterile screw-caped bottles and allowed to solidify in a slope position.

#### **2.3.1.7. Nutrient gelatin (Oxoid Code-CM135a)**

A total amount of 128g which consisted of 3g of lab-lemco powder, 5g peptone and 120g gelatin was suspended in 1 liter of distilled water, boiled to dissolve, steriled by autoclaving at 115° C for 15 min after adjusting the pH to 6.8 and poured into sterile bijou bottle.

### **2.3.2 Semi-solid Media**

#### **2.3.2.1 Hugh and leifson,s (O.F) medium**

The medium was prepared by dissolving 10.3 grams of medium in 1 liter of distilled water by heating, and the pH was adjusted to 7.1.filtered bromothymol blue (0.2% aqueous solutions) was added and then sterilized at 115o C for 20



minutes. Sterile solution of glucose was added aseptically to give final concentration 1%, mixed and distributed aseptically into sterile tubes.

#### **2.3.2.2 Motility medium:**

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

#### **2.3.3 Liquid media**

##### **2.3.3.1 Nutrient broth (oxid CM0001)**

This medium was prepared by dissolving 13g of the medium in 1 liter of distilled water. The pH was adjusted to 7.4, distributed into screw-capped bottles 5 ml each and sterilized at 121°C for 15 minutes.

##### **2.3.3.2 Peptone water sugars**

Nine hundred ml of peptone water was prepared and pH was adjusted to 7.1-7.3 before 10 ml of Andrade's indicator was added. Ten grams of the appropriate sugar was added to the mixture, distributed into tubes 5 ml in each one. The peptone water was sterilized by autoclaving at 110°C for 10 minutes.

##### **2.3.3.3 Glucose phosphate broth (V.P medium)**

Five grams of peptone and 5g of potassium phosphate were dissolved in 1 liter distilled water by steaming. The pH was adjusted to 7.5 and 5g of glucose was added and mixed. The medium was distributed into test tubes 5 ml each and sterilized by autoclaving at 110°C for 10 minutes.

#### **2.3.3.4 Nitrate broth**

One grams of nitrate was dissolved in 1 liter of nutrient broth, distributed into tubes and sterilized by autoclaving at 115°C for 15 minutes.

### **2.4 Reagents and chemicals**

#### **2.4.1 Hydrogen peroxide**

This is produced by British Drug House, London; a 30% solution was diluted to give 3% hydrogen peroxide solution and was used for catalase test.

#### **2.4.2 Oxidase test reagent**

This is manufactured by British Drug House, A 1% tetramethyl – p phenylindiamine aqueous solution was added to 1% ascorbic acid as fresh solution, before a 50 x 50mm of filter paper was impregnated with the solution and it, then dried at 50°C.

#### **2.4.3 Nitrate test reagent**

The consisted of two solutions: Solution A: 0.8% sulphanic acid in 5 N-acetic acid, prepared by dissolving by gentle heating. Solution B: 0.6% dimethyl-alpha-naphthylamine in 5 N-acetic acid.

#### **2.4.4 Kovac's reagent (Oxoid MB0209)**

The consisted of 5g p-dimethyl amino-benzaldehyde, 75ml of amyl alcohol, and 25ml concentrated hydrochloric acid. The aldehyde was dissolved in the alcohol by gentle warming in water bath (50-55°C), cooled, and then the acid was added. The reagent was protected from light and stored at 4°C.

#### **2.4.5 Voges Proskauer test reagent**

They consisted of two solutions:

- 1) Alpha-naphthal solution consisted of 5% alpha-naphthol in ethanol.
- 2) 40% of KOH solution.

#### **2.4.6 Methyl red solution**

This solution was prepared by dissolving 0.04g of methyl red powder in 40 ml of ethanol and the volume was diluted with distilled water to 100 ml (Barrow and Feltham, 2003).

#### **2.4.7 Lead acetate paper**

This was prepared from filter paper, cut into strips of 5-10 mm wide and 50-60 mm long, which impregnated with the hot saturated lead acetate solution, dried at 50-60°C and stored in screw-capped containers.

#### **2.4.8 Andrade's indicator**

This was prepared by dissolving 5g of acid fuchsin in 1 liter of distilled water, and then 150ml of alkali solution (NaOH) was added. It was used in peptone sugar medium.

#### **2.4.9 Gram's stain solutions**

##### **2.4.9.1 Crystal violet**

This reagent was prepared by dissolving crystal violet powder in distilled water to give 1% concentration.

##### **2.4.9.2 Lugol's iodine**

This was prepared by dissolving 20 grams of potassium iodide in 50 ml of distilled water and then 10 grams of iodine were added by shaking and the volume was adjusted to 100 ml.

#### **2.4.9.3 Decolourizing stain**

This was prepared by mixing 475 ml of absolute ethanol, 25 ml of distilled water in 500 ml of acetone.

#### **2.4.9.4 Counter stain**

This stain was prepared by dissolving 10 grams basic fuchsin, 50 grams phenol and 100 ml absolute ethanol, in one liter of distilled water, then the stain was diluted (1:10) in distilled water to use as counter stain.

### **2.5 Types of samples**

Swabs were used to collect samples from different organs of sheep slaughtered in karari Slaughter House during the period from March 2013 to June 2014. A total of 255 samples were used in this study

**Table (2-1): Type and number of samples collected from sheep**

<b>Organs</b>	<b>No of samples ( )%</b>
Neck	75(29.41)%
Shoulder	75(29.42)%
Back	75(29.41)%
Hands	15(5.88)%
Knives	15(5.88)%
Total	255(100)%

### **2.6 Collection of samples**

Swabs<sup>255</sup> were labeled and placed in a Thermos flask that containing ice and transported to the laboratory. Samples were processed immediately and cultured later.

## **2.7 Examination of cultures**

Cultures on solid media were observed for type of growth, pigmentation, colonial morphology, haemolysis as well as changes in the media. Plates that showed visible growth were subjected to further bacteriological tests.

### **2.7.1 Purification of cultures**

Different colonies from primary cultures were subcultured on blood agar and nutrient agar plates. Subculture was repeated several times until pure colonies were obtained.

#### **Total viable count:**

## **2.8 Identification of isolated bacteria**

Identification was carried out according to the procedures described by Barrow and Felthman (1993).

### **2.8.1 Primary identification**

#### **2.8.1.1 Preparation of smears**

Smears were prepared by emulsifying small portions of different bacterial colonies in drops of sterile normal saline on clean slides and spread. The smears were allowed to dry and then fixed by gentle heating.

#### **2.8.1.2 Gram's technique**

This was done as described by Barrow and Feltham (2003). Smears of isolated bacteria were subjected to microscopic examination under oil immersion lens and the shape, arrangement and Gram's reaction were recorded.

#### **2.8.1.3 Catalase test**

This test was used to identify bacteria which produced the enzyme catalase (Cheesbrough, 1987). A portion of the test colony was placed on a drop of 3% hydrogen peroxide on a clean slide using a wooden stick. Production of air bubbles indicated a positive result.

#### **2.8.1.4 Oxidase test**

Portion of the test colony was picked using sterile bent glass rod and rubbed on a filter paper saturated with oxidase reagent. The development of dark purple color within 10 seconds indicated a positive result.

#### **2.8.1.5 Oxidation-fermentation test (O.F)**

Two tubes of Hugh and Leifsons medium were inoculated with each test bacteria and one of them was covered with a layer of sterile paraffin oil. The tubes were incubated at 37°C and examined daily for 7 days. Fermentative organisms produced a yellow color on both tubes while oxidative organisms produced a yellow color only in tubes without oil.

#### **2.8.1.6 Motility test**

Motility was tested by stabbing each isolated bacterium with a straight wire loop in semi solid medium (Craigie tube method). The medium was then incubated for up to 3 days at 37° C together with an uninoculated media as control.

#### **2.8.1.7 Sugar fermentation test**

Sugar media were inoculated with 24 hours broth culture of the test organism. They were incubated at 37°C and examined daily for up to 7 days. Acid production was indicated by the development of a pink color in the medium and gas production was indicated by trapped air in the Durham's tube.

## **2.8.2 Secondary Identification**

### **2.8.2.1 Indole test**

The test organism was inoculated in peptone water and incubated at 37°C for 48 hours. Two to three drops of Kovac's reagent were added to culture and shaken well. Production of pink color on the upper layer of the reagent was considered positive for indole production.

### **2.8.2.2 Voges- Proskauer test (V.P)**

This test was performed to detect the production of acetyl methyl carbinol. Glucose phosphate broth was inoculated with the test organism and incubated at 37°C for 48 hours. Then 0.6ml of alpha - naphthol solution followed by 0.2ml of 40% potassium hydroxide solution were added to 1 ml of the culture, mixed well and examined after 15 min. Development of a bright red color indicates a positive result.

### **2.8.2.3 Methyl red test**

Glucose phosphate medium was inoculated with the test organism and inoculated tubes were incubated at 37°C for 48 hours. Two drops of methyl red solution was added and a positive reaction of the test was indicated by a red colour at the surface.

#### **2.8.2.4 Urease test**

The test organism was inoculated on a slope of urea agar medium and incubated at 37°C for up to 5 days. The change of color of the medium to red or pink indicated a positive result.

#### **2.8.2.5 Citrate utilization test**

Simmon's citrate medium was inoculated with the test organism, incubated at 37°C and examined daily for up to seven days. The development of a blue color in the medium was considered as a positive result.

#### **2.8.2.6 Nitrate reduction test**

The test organism was grown in nitrate broth and incubated at 37°C for 5 days. One ml of nitrate reagent A was added followed by 1 ml of reagent B. Development of a deep red color was considered as a positive reaction. Zinc powder was added to tubes which did not show red color and development of a red color in these tubes indicated that nitrate was present and the test organism did not reduce it.

#### **2.8.2.7 Sugar fermentation test**

Sugar media were inoculated with the tested bacterium grown in peptone water and incubated at 37°C. The tubes were examined daily for up to 7 days. Acid production was indicated by the development of pink color in the media.

#### **2.8.2.8 Aesculin hydrolysis**

Aesculin broth was inoculated with the colonies of the test organism and then examined daily up to 5 days. Aesculin hydrolysis was indicated by blackening of the medium.

#### **2.8.2.9 Gelatin hydrolysis (or liquefaction)**



Nutrient gelatin was inoculated with a straight wire containing tested colonies and incubated at 37°C for up to 14 days. Every 2-3 days the tubes were placed in a refrigerator for 2hr. and then examined for liquefaction.

#### **2.8.2.10 Hydrogen sulphide production**

Colonies of tested organism were inoculated in nutrient broth or peptone water and a lead acetate paper was inserted between the plug or cap and the tube cultures were examined daily for 7days for blackening of the paper.

#### **2.8.2.11 Pigment production**

Organisms were inoculated on nutrient agar plates, incubated for 24 hr at room temperature and observed for up to 5 days for color production.

#### **2.8.2.12 Coagulase test**

To 0.5ml of 1/10 dilution of plasma an equal volume of broth culture of organism was added. The mixture was incubated at 37oC for 4 hr and examined after 1 and 4 hr. for coagulation.

#### **2.8.2.13 Phosphatase test**

Organisms were inoculated in phenolphthalein phosphate agar, and incubated for 18 hr before 0.1 ml of ammonia solution (sp, gr.0.880) was placed in the lid of the Petri dish. Phosphate-positive colonies became bright pink.

#### **2.8.2.14 Growth in media with increased NaCL concentration test**

Organisms were inoculated on nutrient agar containing 6.5% NaCL and incubated at 37oC for 2 days for growth.

### **2.9 Methods of sterilization**

## **2.9.1 Dry Heat**

### **2.9.1.1. Hot air oven**

This method was used for sterilization of clean glassware, such as Petri-dishes, pipettes, tubes, flasks, bottles, sand, mortars and pestles. The temperature and time of exposure was 160o C for one hour (Oxoid, 2006).

### **2.9.1.2 Red Heat flame**

This was used for sterilization of wire loops, straight wires and forceps. It was done by holding the object as near as possible to the flame until it become red hot (Gruickshank et al., 1975).

### **2.9.1.3 Flaming**

This was done to sterilize the mouth of cotton-plugged tubes and for glass slides and was done by exposing the object to the direct flame for about half to one sec (Gruickshank et al., 1975).

## **2.9.2 Moist Heat**

### **2.9.2.1 Autoclaving**

This technique was used for sterilization of media, solutions, plastic wares such as rubber stoppers, which could not with stand the dry heat. The temperature was 121°C for 15min, under pressure of 15 pounds/sq. inch (Barrow and feltham, 2003).

### **2.9.2.2 Momentary autoclaving**

This technique was turned off as soon as it reached 121°C. The valve of the autoclave was opened when the temperature reached 100°C and the autoclave is unloaded below the 80°C (Barrow and feltham, 2003).

## **2.9.3 Disinfection of media preparation room**

For aseptic preparation of media and pouring onto plates, phenol disinfectant and absolute alcohol were used for disinfecting the floor and benches of media preparation; the room was also irradiated with ultraviolet light for complete sterilization.

### **Collection of swab samples**

A total of 255 swab samples were collected from 15 carcasses of sheep from El Karari Slaughterhouse. Khartoum State. The samples were taken from 3 different sites viz neck, shoulder, and back. In addition, 15 samples were taken from the workers' knives, and also 15 samples from their hands.

The operational points were, skinning, washing, chilling, during transporting and from containers. Sterile swabs (3 x 1 cm) moistened in 0.1% Peptone

Water were used an area was marked by sterile frame (10 x 10 cm) for each collection site of the carcasses. The swab was rubbed on the marked-site for 30 seconds and transferred to a screw-clipped bottle containing 10 ml sterile maintenance medium (0.85% NaCl and 0.1 % peptone). The bottles were put in ice container and sent to laboratory for bacteriological examination.

### **Bacteriological analysis**

All samples were cultured in Nutrient Broth and onto Blood and MacConkey's agars, for the growth of microorganisms. Biochemical tests were performed for Identification of the isolates (Barrow and Feltham, 1993). The total viable count (TVC) of the isolated microorganisms was carried out according to the method of Miles and Misra (1938).

### **Statistical analysis**

All TVCS bacteria were converted to log<sub>10</sub> CFU /cm<sup>2</sup> for analysis and ANOVA was performed using SPSS. Significant differences were determined at the 5% level (P<0. 05).

## CHAPTER THREE

### RESULTS

Two hundred and fifty five samples of the tested specimens gave bacterial growth. The result from three groups one was *Staphylococcus aureus* only and group two it was *Escherichia coli* anther group was mixed culture from (*Staphylococcus aureus*, *Escherichia coli*)

#### 3.1. Bacteria isolated from different samples collected from sheep in slaughterhouse

Type of organisms	Number of The isolates from (carcasses,Knives,Hands worker)	Relative frequency of isolates%
Staphylococcus aureus	106	41.57%
Escherichia coli	138	54.11%
(Staphylococcus aureus +Escherichia coli )	11	4.32%
Total	255	100%

The TVCs in all 225 swab samples were recorded as mean±Std. Log<sub>10</sub> CFU/ cm<sup>2</sup>. The highest TVCS 3.04±0.28 Log<sub>10</sub> CFU/ cm<sup>2</sup> was recorded at shoulder site in transportation. The lowest TVC s 2.9±0.10 Log<sub>10</sub> C FU/ cm<sup>2</sup> were recorded at neck site in three points which include skinning, washing and container respectively.

**Table 3. 2. Comparison of the mean Total Viable Count of Bacteria log<sub>10</sub>cfu/cm<sup>2</sup>) ± Std at Different operational Points of Investigation at some sites of sheep carcasses**

Sites	Operational Points					Significance Difference
	Skinning	Washing	Chilling	Transportation	Container	
	Log <sub>10</sub> cfu/Cm <sup>2</sup>					
<b>Shoulder</b>	2.93±0.91	2.94±0.08	2.95±0.09	3.04±0.28	2.94±0.08	NS
<b>Neck</b>	2.91±0.10	2.91±0.10	2.93±0.11	2.97±0.07	2.91±0.10	NS
<b>Back</b>	2.92±0.10	2.96±0.04	2.94±0.98	2.98±0.05	2.96±0.04	NS
<b>Workers Hands</b>	2.74±0.20	ND	ND	ND	ND	NS
<b>Knives</b>	2.89±0.16	ND	ND	ND	ND	NS

NS no significant different at (P < 0.05), ND not done

There were no significant differences between these processes (P>0.05). The mean TVCs on knives 2.89±0.16 log<sub>10</sub> CFU/cm<sup>2</sup> at skinning with no significant differences among them (P>0.05). Moreover, the TVCS of the workers hands at skinning was 2.74±0.20 log<sub>10</sub>CFU/cm<sup>2</sup>, with no significant differences among them (P>0.05) (Table 1).

**Table 2.3** shows that *Escherichia coli* was isolated from the different sites of the carcasses in different operational points. the highest isolated number was in back site 50

(34.01%) isolates whereas the lowest isolated number of *Escherichia coli* was at workers

hands at skinning 2 (1.36) isolates .+

**Table 3.3 Number and Percentage of *Escherichia- coli* Isolated from Different Operational Points and Sites on Sheep carcasses**

Sites	Operational Points					Total
	Skinning	Washing	Chilling	Transportation	Container	
	Escherichia. coli NO(%)					
Shoulder	6(4.08)	8 (5.44)	9(6.12)	10(6.80)	10(6.80)	43(29.25)
Neck	8 (5.44)	10(6.80)	11(7.48)	6(4.08)	9(6.12)	44(29.93)
Back	9(6.12)	11(7.48)	10(6.80)	9(6.12)	11(7.48)	50(34.01)
Workers hands	2 (1.36)	ND	ND	ND	ND	2 (1.36)
Knives	8(5.45)	ND	ND	ND	ND	8(5.45)
Total	(33(22.45)	29(19.73)	30(20.41)	25 (17.00)	30(20.41)	147(100)

ND not done

### 3.1.2. *Escherichia coli*

Isolates of *Escherichia coli* were Gram-negative rods, occurring singly. They were smooth, shiny, large colonies and pink color on macConkey's agar and white, glistening and circular with entire edge on blood agar. On nutrient agar were large, round pale yellow in color and smooth.

Also Table 4 shows that *Staphylococcus aureus* was isolated from the different sites of the carcasses in different operational points, the highest isolated number of it in shoulder site 35 isolates (31.5%) whereas the lowest isolated number of *Staphylococcus aureus* was recorded at workers knives 7 (6.25%) isolates.

### 3.1.2. *Staphylococcus aureus*

In stained smears the organism was Gram positive, spherical to oval cocci arranged in irregular clusters. On blood agar the colonies were smooth, shiny, round,convex, yellowish to golden in colour.*Staph.aureus* produced round, smooth, low convex,glistening,opaque and yellow to white colonies.

**Table 4. Number and Percentage of *Staphylococcus aureus* Isolated from Different Operational Points and Sites on Sheep carcasses**

Sites	Operational Points					Total
	Skinning	Washing	Chilling	Transportation	Container	
	Staph. Aureus NO (%)					
<b>Shoulder</b>	9 (8.04)	8(7.14)	7(6.25)	6(5.36)	5(4.46)	35(31.25)
<b>Neck</b>	7(6.25)	5(4.46)	4(3.57)	10(8.92)	6(5.36)	32(28.57)
<b>Back</b>	5(4.46)	5(4.46)	5(4.46)	6(5.36)	4(3.57)	25(22.32)
<b>Workers hands</b>	13(11.61)	ND	ND	ND	ND	13(11.61)
<b>Knives</b>	7(6.25)	ND	ND	ND	ND	7(6.25)
<b>Total</b>	41(36.61)	18(16.07)	16(14.29)	22(19.64)	15(13.39)	112(100)

N D not done

**Biochemical properties of the bacterial species isolated from the different samples**

**Table (5): characters used for identification of *E. coli* isolated from samples**

<b>Character</b>	<b><i>Escherichia coli</i></b>
Motility	+
Oxidation fermentation test	F
Gas from glucose	+
Catalase	+
Oxidase	-
Simmon's citrate	-
Growth on MacConkey's agar	+
Urease	-
MR test	+
VP test	-
Indole test	+
H <sub>2</sub> S(pbas paper)	-
Gelatine hydrolysis	-
Adonitol	-
Dulcitol	+
Inositol	-
Lactose	+
Starch	-
Maltose	+
Mannitol	+
Raffinose	+



Salicin	+
Sorbitol	+
Sucrose	+
Trehalose	+
Xylose	+

F= fermentative

+ = positive

- = negative

**Table (6):** characters used for identification of *Staph aureus* isolated from swab samples of sheep

<b>Character</b>	<b><i>Staphylococcus aureus</i></b>
Glucose	+
Catalase	+
Oxidase	-
Motility	-
Oxidation fermentation test	F
Coagulase	+
Nitrate reduction	+
VP test	+
Urease	+
Phosphatase	+
Lactose	+
Trehalose	+
Sucrose	+
Xylose	-
Raffinose	-
Maltose	+
Mannitol	+

F = fermentative

- = negative

+ = Positive

## Chapter Four

### Discussion

Most of the meat contamination is caused by aerobes. These organisms may gain access to meat from the digestive system of living animal or as a result of slaughter contamination Lawrie( 1979). Meat contamination is of economic importance because it inverse the meat quality. Poor meat hygiene practices in the slaughterhouses before and after slaughter would lead to meat contamination. FAO/WHO (1962) and Thornton (1968), emphasized that meat hygiene should be observed at all stage of meat production till it reaches the consumer as fresh, sound, wholesome and safe meat. The aerobic bacterial isolated in the present study were *Staphylococcus aureus* and *Escherichia coli*. These finding are in agreement with finding of Brahmhalt and Anjaria (1993). Who isolated *Escherichia coli*, *Bacillus cereus*, *Staphylococcus aureus* and *Micrococcus spp.* from raw meat. Meanwhile Brahmhalt and Anjaria (1993), isolated *staphylococcus epidermidis*, *Citrobacter freundii*, *streptococci faecalis*, *Enterobacter aerogenes*, *Proteus mirabilis*, *Bacillus subtilis*, *Aeromonas Liquifaciens*, *Proteus vulgaris*, *Klebsiella pneumonias* and *pseudomonas aeruginosa*. The present is finding are also in agreement with Amanie (2000) who isolated *Micrococcus spp.* *Staphylococcus leutus*, *staphylococcus auricularis* and *Escherichia coli* from meat at stages of processing she also isolated *Bacillus firmus*, *Bacillus pantotheni-eus*, *Bacillus thuringiensis*, *Bacillus anyaligufaciens*, *Aerococcus spp.* *Proteus mirabilis* *Pseudomonas pseudocaligenes* ,*Shewan-ella putrefaciens*, *Acinetobacter lowff* and *Acinefobacter calcoaeetus*. The present studies revealed that, the Gram-negative aerobes are the most frequently isolated bacteria. This observation disagrees with Khalid (2004), who reported that Gram-positive were most frequently isolated from different intervals of time. But this

observation was in agreed with Lmwidihaya et.al. (1987), who found that the fresh meat samples were contaminated mainly by Gram-negative bacteria. Ajit *et. al.* (1989) isolates from muscle included *Escherichia coli*, *Pseudomonas*, *Klebsiella* and *Citrobacter*. This agree with my isolate specially *Escherichia coli*, Also the present studies agree with Salih (1971), who reported heavy contamination of fresh meat in Khartoum State with spoilage bacteria genera like *Micrococcus*, *Streptococci*, *Bacilli*, *Pseudomonas* and *Aerogenes*, *Bacilli*, *Pseudomonas* and *Aerogenes*. Thronton (1952) reported that the types of bacteria expected in the slaughterhouse were *staphylococcus*, *Micrococcus*, *Baceil-hus*, *Pseudomonas*, *Achramobacterium*, *Aerobacter* and *Coli-forms*. The most frequently isolated bacteria in this study from both fresh and chilled mutton were *Escherichia coli*. Which were isolated, an important in public health and their isolation from meat is a normal phenomenon. These bacteria may originate from environment where exposure of meat to more handling by the workers. The higher bacterial counts obtained during this work may be due to surface contamination of meat which came from different sources, mainly hides, hoofs, air, water, equipments, intestinal contents and slaughtering floor as reported by Haines (1993); Empey and Scott, (1939); and String, Bilskie and Nauman, (1969). *Staphylococcus aureus*, which is a normal flora of the carcasses, usually indicates contamination from handlers. The organism can pass onto food during harvesting, processing or even storage. It is the major cause of food poisoning known as Staphylococcal food poisoning. The poisoning is caused by the ingestion of an enterotoxin produced, which is characterized diarrhea ( Eze et al, 2008).

In this study, the surface region of shoulder had the highest rate of contamination compared to all parts of the carcass. This may be due to the handling and contamination by intestinal contents. That are significantly different

( $P < 0.05$ ), this agrees with Fadlalla (2004), who recorded that the highest count appears in the middle of the work, while the lowest count were obtained in the beginning of the work. Also in this study, the hands of worker had high contamination by Gram-positive bacteria compared with Gram-negative bacteria. The behavior of worker is an important thing in the contamination as reported; by Elamine (2002) and Jeffery et al (2003), their result indicated that the sources of meat contamination included the hands and arms of meat handlers, equipment and contact surfaces. This may be due to of the processing of the carcass in slaughterhouses, As reported in the study that the stages of processing of the carcass in slaughterhouse (skinning, washing, chilling, transportation, and container) had high contamination by Gram-negative bacteria, and this may be due to contamination by intestinal contents. In all different operation points at different sites on carcasses and worker hands, the highest contamination levels was recorded before the point of chilling stage. These findings are similar to those of Biss and Hathaway (1995) and Gill et.al. (2000) who recorded high bacterial type after washing of lamb carcasses in the abattoirs. Moreover, Emey and Scott (1939) had reported that the surface bacterial count ranges between 1000 and 100000 CFU per  $\text{cm}^2$ .

The present findings are in contrast to those of Brose et al. (1998) and Gill and Baker (1998) who observed a highly significant reduction in bacterial count (1.8, 0.31 log units) after washing of sheep carcasses. And also is contrast to those of Abdalla et.al (2009), who found the average TVCs after skinning, evisceration and after washing in the abattoir were  $5.5 \pm 0.89$ ,  $6.0 \pm 0.33$  and  $5.1 \pm 0.41$  Log CFU/ $\text{cm}^2$ , respectively.

The present results recorded a rate of total viable count between  $3.04 \pm 0.28$  Log<sub>10</sub> CFU/  $\text{cm}^2$  was recorded at shoulder site in transportation and the lowest

TVC s  $2.9 \pm 0.10$  Log<sub>10</sub> CFU/cm<sup>2</sup> were recorded at neck site in three points which included skinning, washing and container respectively. This is similar to the result of Nouichi and Taha, (2009) who found a mean log TVC of 3.11 cfu/cm<sup>2</sup> also in accord to Phillips et.al. (2001b) and Zweifel and Stephan (2003) who recorded the finding of 3.33 log cfu/cm<sup>2</sup> and 3log cfu/cm<sup>2</sup> on sheep carcasses at slaughter-house. The present studies one lower than the result of El-Hadef et al (2005), who recorded a rate of 5.42log cfu/cm<sup>2</sup>, Bhandare et al (2007) who noted an average of 6.06 log cfu/cm<sup>2</sup> on sheep and goat carcasses, and Elamin (2002), who found uncountable levels of contamination varying from less than 10<sup>7</sup> and more than 30x10<sup>7</sup> cfu/cm<sup>2</sup> on the surface. In this study the prevalence of *E. coli* (33%) of sheep carcasses but Abdalla, et al (2009) found (16%). Phillips et al (2001) detected *Escherichia coli* on 29.2% of sheep carcasses. Sumner et al (2002) found the percentage of 36.2% of *E.coli* in South Australia. In USA, Siragusa et al (1998) found that 44% of carcasses were positive for *E.coli* for sheep carcasses. Doyle and Schoeni, (1987) in surveys of retail fresh meat products in North America, they detected *E. coli* O157:H7 in 2% of the 205 lamb samples tested. Duffy et. al. (2001) surveyed 2522 chilled lamb carcasses at six USA plants finding (in spring and winter) overall prevalence of *E.coli* of 66.2%. In the present study *Salmonella* was not isolated, this result is similar to some authors who have reported the absence of *Salmonella* on sheep carcasses, included Phillips et al. (2006b) who did not isolate *Salmonella* on any of 1117 sheep carcasses tested, Bhandare et al (2007) from 144 carcasses, Sudhakar et al, (2006) in Deonar abattoir in (India) could not isolate *Salmonella spp.* from any of the carcass site. In Brazil Martineli et al (2009) could not isolate *Salmonella* from 60 sheep carcasses. However, some authors reported high rate of this *Salmonella* including Sierra et al (1995) and Small et al (2006) who recorded rates of 10% and 9.6% respectively.

Also the latest National Microbiological Database summary (1998) for ovine showed that *Salmonella* was not detected in 322 carcasses and 1268 samples of primal cuts and bulk products (Armitage, 1995). But, on the other side some authors reported low percentage of this bacteria, Nouichi and Taha, (2009) isolated *Salmonella* from one ovine (1.11%) out of 90 animal and microbiology of Australia meat result for *Salmonella* isolation rate was 0.1% for sheep carcasses. (Millard and Simon, 1998).

## **Conclusion**

### **Conclusion**

- It was found that the contaminated bacteria were Gram-positive and Gram-negative bacteria.
- Gram-negative bacteria were the most dominate than Gram-positive bacteria.
- The contamination was high in the skinning and washing stages more than the other stages (chilling, transportation and container).
- The high-contaminated site of the carcass was the neck, and the shoulder was also high contaminated but less than back.
- Worker's hands the highest contamination levels by Gram-positive bacteria.
- Knife the highest contamination levels by Gram-negative bacteria.

The sources of contamination of mutton intended for export were water, air, intestinal contents and the workers whom handled the meat during the processing of meat



**Recommendation:**

- The system of working in slaughterhouses should contain the sanitation and training for workers to use clean clothes and gloves.
- The system of washing in the slaughterhouse must be used clean and healthy.
- Cleaning and sterilization of knives and machines must be used in slaughterhouse so as to reduce the contamination.
- Hazard Analysis and Critical Control Point (HACCP) system should be applied in slaughterhouses.
- The meat handlers should be subjected to continuous education on food safety and hygiene.

## Chapter Five

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