

## INTRODUCTION

Meat is defined as those animal tissues which are suitable for use as food. The majority of meat consumed is derived from domesticated mammals and birds like cattle, sheep, camels, buffaloes, goats and chickens. In Sudan the meat consumed is mainly derived from cattle (Gracey, 1981).

Meat and meat products are considered an important source of protein, essential amino acid, B complex vitamins and minerals. (Gill, 1998; Heinz and Hautzinger, 2007 ; Abdalla *et al.*, 2010). Due to this richness in composition, meat is also considered a good medium for the growth and propagation of many pathogenic bacteria such as *Salmonella* species, *Staphylococcus aureus*, *Listeria monocytogenes*, *Campylobacter* species, *Escherichia coli* O157:H7 and numerous other bacteria (Gill, 1998; Abdalla *et al.*, 2009a; Nouichi and Hamdi, 2009; Amine *et al.*, 2013). The contamination of meats has been reported to be due to presence of micro-organisms or their toxins in an amount that render the meat unacceptable or potentially harmful to consumers (Gracey, 1998). Food borne diseases often follow the consumption of contaminated food-stuffs especially from animal products. Where conventional veterinary inspection Cannot detect the presence of these bacteria on apparently healthy carcasses (Brown *et al.*, 2000; Gill and Jones, 2000).

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

Meat borne diseases to human is a big problem nowadays particularly caused by bacteria (Anon, 1983; Mc Capes *et al.*, 1991; Danielson and Tam, 1996). Food can be unsafe for human consumption due to change in its biological, chemical or physical properties (Food safety inspection service, 1997).

(Brownlie, 1966) reported that the keeping quality of meat and meat products depends on the numbers and types of the contaminating bacteria and their metabolism and rate of growth It also depends on the physical or chemical environment. The microbiological contamination of carcasses occurs mainly during processing and manipulation, such as skinning, evisceration, storage and distribution at slaughter houses and retail establishments (Gill, 1998; Abdalla *et al.*, 2009b).

. The most prevalence bacteria were *Staphylococcus aureus* 10.54%, *Klebsiella* spp. 10.12% and *Escherichia coli* 8.86%. High contamination level on flank sites and lower contamination level on rump sites during skinning was recorded by Ali (2007). Salih (1971) isolated *Salmonella* spp., *Staphylococcus* spp., *Micrococcus* spp., *Streptococcus* spp., *Bacillus* spp., *Clostridium* spp, *Pseudomonas* spp. and *Coli aerogenes* from fresh meat of ovine and bovine Obtained from Omdurman Central Abattoir.

Developed countries have adopted mechanized or automated slaughtering techniques that ensure the reduction of the amount of exposure of carcasses to atmospheric contaminants and to manipulation. In addition, the implementation of the Hazard Analysis Critical Control Point (HACCP) system allows them to regulate the control of the general hygiene of the chains of production( .Zweifel and Stephan, 2003). But in Sudan like many developing countries, hygienic measure to control microbial contamination of meat is unsatisfactory applied. And slaughterhouse more problems included poor waste disposal systems, environmental sanitation, lack of workers training, water was not heated and No disinfectants and traditional conventional methods of meat hygiene do not match the international standard.

The HACCP system is systemic approach to identification, assessment and control of food microbiological hazard (Bryan, 1992). A hazard is an unacceptable bacterial contamination in food, chemicals or substance such as toxins, enzymes or products of microbial metabolism that may adversely affect food safety or quality and its keeping qualities (Bryan, 1992). Meat hazards include such elements as biological, chemical and physical contamination (Gracey and Collions, 1992). Hazard analysis in reference to meat hygiene involves identification of hazard, which is qualitative indication that a condition or substance in meat may severely affect human health, and a risk assessment, which is the likelihood of adverse effects from exposure to such specific hazard or absence of beneficial influence (Hathaway *et al.*, 1988).

### **Rational:**

The state of ELGadaraf is one of the largest states in Sudan .It is inhabited by large number of people of different races and cultures. the local slaughter house of ELGadaraf is the largest slaughterhouse in the state and the only source of healthy meat exposed by the veterinary authorities There for ,this study was conducted in order to assess the general health situation in the slaughter house to reduce the risk

of meat borne disease that are concern for whole world for human death and economic losses .the finding of this study may give source of information based on correcting the general health situation in slaughterhouse .

**Objectives:**

1. To identify the main points of contamination of cattle carcasses during slaughtering operations .
2. -To determine bacterial number (total viable count) on cattle meat.
3. -To identify the type of contaminated bacterial

## **CHAPTR ONE**

### **LITERATURE REVIEW**

#### **1.1 Sources of contamination:**

##### **1.1.1 Animal hides ,feces , water, air and dust**

In fact the muscles of live healthy animals are sterile while lymph nodes some organs and especially surfaces exposed to the environment such as hide “pelts or fleeces the mouth and the gastrointestinal tracts carry extensive contamination (Sofos *et al.*, 1999).

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). On the other hand (Bell and Hathaway, 1996; Gihan 2004) found that the sources of bacterial contamination of meat are hides, hooves, soil adhering to the hide, intestinal contents, air, water supply, knives, cleavers, saws, hooks, floor and worker

It was reported that bacteria associated with meat depend on bacteriology of the soil on which the animals were kept prior to slaughter. And the bacteria were transferred to the hides and then to the exposed meat. The bacteria were carried to the abattoir on skin, hoofs and body cavities of meat animals. (Jepsen, 1967; Gracey, 1985)

Every effort should be made to prevent accumulation of excess mud and dung on the animals because it may introduce bacterial pathogens into the plant environment (Sofos *et al.*, 1999)

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.

The initial microbial population on meat depends on the physiological state of the animal at the moment it is slaughtered and on the level of environment contamination

in the slaughter house and area in which subsequent handling of the carcasses is performed. Including the level of hygiene of employees and the tools and equipment used (Josef, 2013).

The risk is higher when air is contaminated with eventually food borne pathogen microorganisms and spores. The risk of contamination derives prior to plant surfaces that include both products 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)

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.

The exterior hide and hair of beef are highly contaminated with bacteria, including pathogens and may serve as source of contamination for the plant environment and the resulting carcasses and meat products (Sofos, 2002).

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).

During harvesting processing, distribution, and preparation, food is contaminated with soil, air, and water-borne microorganisms. Hence high numbers of microorganisms are found in animal intestinal traces, and some of these microorganisms find their way to the carcass surfaces during evisceration. When Carcasses and cuts are subsequently handled through the food distribution channels, where they are reduced to retail cuts; they are subjected to an increasing number of microorganisms from the cut surfaces (Forrest *et al* 1975; Marriott and Gravani., 2006).

### **1.1.2 Equipment 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).

Wahib (2004) 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 animal's digestive tract was claimed to carry dangerous load of bacteria. Actual contagion with dirty hands, clothing and equipments are important factors in the presence of bacteria in frozen meat in chilling storage that reported by( Gracey , 1980 ; Cockburn *et al.*, 1962; Riemann, 1969).

Dirty workers hands, clothes and equipment of the slaughterhouse act as intermediate sources of contamination of meat (Glimour *et al.*, 2004; Abdelsadig, 2006).

Workers can carry pathogens internally and on their hands skin and hair. It is imperative that they follow and understand basic food protection practices and maintain a high degree of personal cleanliness and good sanitation practices to prevent food product contamination (Luceyn, 2006).

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 what the important contaminants 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). According to Jay (1970) and Dempster (1973) Meat grinders were contaminated with millions of bacteria.

## **1.2 Slaughter Processes**

Cattle 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 (Gill and Jones, 1999; Bacon *et al.*, 2000; Abdalla *et al.*, 2009a; Abdalla *et al.*, 2009b).

Fresh meat becomes contaminated with microorganisms during various processing stages up to consumer uptake. Contaminated raw meat is one of the main sources of food-borne illness (Bhandare *et al.*, 2007).

### **1.2.1 Skinning**

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).

Bacterial contamination includes the normal skin flora as well as organisms from soil and feces which are on the skin, and includes *Yeasts*, *Bacilli*, *Micrococci*, *Staphylococci*, *Corynebacteria*, *Moraxella*, *Acinetobacter*, *Flavobacteria*, *Enterobacteriaceae*, *E. coli*, *Salmonellae* and *Listeria* species (ICMSF, 1998).

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 feces.

### **1.2.2 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 ingest 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 gall bladder 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 harbor *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.

### **1.2.3 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 water above 80°C tends to give a better reduction in carcass contamination, but even then the reduction may be small (Patterson, 1968). When a spray 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 (10 x 0.2-0.9). Impact temperatures of 80°C and above appear to be needed to give at least a 10-fold reduction in the numbers of Mesophiles on carcasses (Kelly *et al.*, 1981; 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 markedly changed with increasing chlorine concentration. Populations of *E. coli* on beef were not significantly reduced by 800 ppm (Cutter and Siragusa, 1995). 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 log<sub>10</sub> 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 spray appear to produce little reduction in *E. coli* and *Salmonella* on meat surface (Brackett *et al.*, 1994)

### **1.3 The types of microorganisms which cause contamination of meat:**

The significance of bacteria in meat was recognized during *Pasteur era* 1980. It was then evident that meat favors multiplication of many kinds of bacteria which may reach it from various sources beside the air (Miller, 1951).

Jay (2000) suggested that the microorganism individually and as a group ,grow over very wide range of temperature ,therefore ,it is well to consider at this point the temperatures growth ranges for organism of importance in food as an aid in selecting the proper temperature for the storage of different types of food . The lowest temperature at which microorganism has been reported grow is -34°C somewhere in



excess of 100 °C, it is customary to place microorganisms into three groups based on their temperature requirement for growth in the following:

- Those organisms that grow well at or below 7°C and have their optimum temperature between 20°C and 30°C are referred to as Psychrophiles.
- Those that grow well between 20°C and 45°C with optima between 30°C and 40°C are referred to as mesophiles.
- Where those that grow well at and above 45°C with optima between 50°C and 65°C are referred to as thermophiles.

Rodgers and Fletcher (1966) noted that psychrophilic and mesophilic types of bacteria are the most important.

Hudson and Roberts (1979) reported that the pH of beef carcasses affected the growth of bacteria. Samples from high pH beef carcasses showed faster spoilage and higher bacterial counts than those from normal pH carcasses. 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 Spp* Slantzev *et al* (1963)

Salih (1971) isolated from fresh meat samples spoilage bacteria of the genera *Micrococcus*, *Streptococcus*, *Bacillus*, *Clostridium*, *Pseudomonas* and *Coli*-aerogenes. He also isolated haemolytic and coagulase positive *Staphylococci* from ovine and bovine liver and rumen samples obtained from Omdurman Central Abattoir, and isolated *Micrococci* and *Salmonella doblin* from ovine and bovine offal. Tsubokura *et al* (1973) suggested that the meat, particularly offal's, contaminated with *Yersinia* organisms constituted an important source of infection. While Zahra (1985) found that *E coli* were the most predominant bacterium in fresh beef.

Among the bacteria present in the air and dust are *Bacillus* and *Micrococcus spp.*, which were able to tolerate dryness to varying degrees Jay (1986)

Meat and its products were known to be potential sources of food poisoning by *Salmonella* (Hubbert *et al.*, 1975).

According to Dolman (1967) meat provides an excellent medium for *Staphylococcal* proliferation and if the temperature is warm enough only a few hours are needed for the production of the effective amounts of enterotoxin.

#### **1.4 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-flavors, 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 dour/ off-flavors 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 (Renner and Labadie *et al.*, 1994). 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/cm<sup>2</sup> 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 (Butler *et al.*, 1953). 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).

### **1.5 The importance of meat contamination:**

Carter (1975) found that the most frequent pus forming organism in cattle and sheep is *Corynebacterium Pyogenes*.

Fatima (1982) emphasized that pathogenic bacteria found in processed meat which she studied were *Salmonella spp*, *Clostridium perfringens*, *Staphylococcus aureus* and *E.coli*. According to Holy and Holzopfel (1988) *Pseudomonas* are susceptible to freezing and thawing . The members of the genera *Pseudomonas*, *Acinetobacter* and *Moraxella* dominated the bacterial content of unprocessed meat exposed to air at chill temperature (Inter National Commission for Microbiological Specification for Food I. N.C.M.S.F, 1980). 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 pneumoniae* and *Pseudomonas deruginosa* . The microbial gups 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 *Microbactrium* . Meat spoilage may also be caused by bacteria from the genera *Pseudomonas* , *Flavobacterium* , *Actinobacter* , *Achromobacter* , *Alcaligenes* , *Halobacterium* , *Moraxella* , *Escherichia* and *Kelbsiella* . A total of 71 strains of Gram positive, catalase positive cocci were isolated from 112 abscesses observed during inspection of slaughter animals (sheep, cattle, pigs and goats) .Amongst 35 coagulase positive isolates,s 30 were classified as *Staph aureus*. Of the coagulase negative isolates 5 were *Staph hominis* and 4 were *Staph xylosus* (Menes *et al.*, 1984)

The Food Safety and Inspection Services in the USA (1997) reported the following pathogenic bacteria in meat and meat products (*Bacillus cereus*, *Clostridium botulinum* , *Clostridium perfringes* , *Escherichia coli* , *Salmonellae* , *Staphylococcus aureus* and *Yersinia enterocolitica* ) .

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).

. Hussein (1975) isolated *Bacillus* , *Staphylococcus epidermidis* , *Micococcus* , *Streptococcus faecalis* , *Lactobacillus* , *Escherichia coli* , *Citrobacter freundii* , *Proteus mourgani* , *Alcaligenes* , *Aeromonas* and *Pseudomonas* from fresh and refrigerated beef.

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 (Gustavsson and Borch, 1993; Samelis *et al.*, 2001).

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.

Matthews *et al* (1989) isolated primarily *Staph xylosus*, *Staph hominis* and *Staph aureus* from bovine origin by using the API Staph-Trac.

Akatov *et al* (1983) studied the species characteristics of coagulase negative staphylococci. They isolated *Staph xylosus* from different animals (cows, sheep, hens etc).

Adegoke (1985) found *Staph lentus* in strains isolated from goat and sheep. 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).

Laukofa (1997) isolated *Staph xylosus*, *Staph lentus*, *Staph aureus* ,*Staph hominis* and *Staph auricularis* from the rumen content of deer.

Of 136 strains of coagulase-negative staphylococci isolated from healthy and sick human beings, goats, sheep, antelope and other animals, 88(64.7) were *Staph lentus* (Adegoke , 1968).

Akiyama *et al* (1998) studied the coagulase-negative staphylococci isolated from various skin lesions. Some of them were *Staph hominis* and *Staph auricularis* six strains of ureolytic staphylococci were isolated from rumen of young calves and lambs. Three of them were identified as *Staph xylosus* , *Staph saprophyticus* and *Staph gallinarum* (Laukova and Marounek, 1992 ).

Intisar (1998) isolated *Staph auricularis* and *Staph lentus* from beef samples. While Amanie (2000) studied aerobic bacteria which were found in meat at different stages of processing. She isolated *Staph auricularis* , *Staph lentus* , *Escherichia coli* and *Micrococcus spp*

Lawrie (1991) found that the organisms derived from infected personal or healthy carriers include *Salmonella SPP.*, *Shigella Spp.*, *Escherichia coli*, *Bacillus*, *Proteus*, *Staphylococcus albus* and *Staphylococcus aureus*, *Colistidium welchii*, *Bacillus cereus*, *Bacillus faecal* and *Streptococcus spp*

Dolman (1967) reviewed that *Streptococci* as a cause of food poisoning and reported that meat can serve as a vehicle.

Bersani *et al* (1991) identified the spoilage bacteria *Pseudomonas putida* and *Actinobacter wolfi* in dark meat of elevated PH.

Khalid (2004) reported that the contamination of beef in Khartoum slaughterhouse by the aerobic bacteria from the Gram-positive genera were *Micrococcus sp.*, *Staphylococcus sp.* and *Bacillus cereus*, while the Gram-negative species was *E. coli* from working tools, hands of the workers and various parts of bovine carcasses.

Abdalla *et al* (2009b) evaluated the bacteriological contamination in indigenous cattle in slaughterhouse, Khartoum State, and isolated twelve species of bacteria on the carcasses, the highest average prevalence was *Staphylococcus aureus*, *Pseudomonas spp.*, *Klebsella spp.* and *Escherichia coli*, *Bacillus spp.*, *Micrococcus spp.*, *Proteus spp.*, *Staphylococcus spp.*, *Salmonella spp.*, *Staphylococcus epidermidis*, *Streptococcus spp.* Yusuf *et al.* (2012) isolated *B. cereus*, *Staph. aureus*, *E. coli*, *B. lentus*, *B. alvis*, *Proteus vulgaris*, *Proteus mirabilis*, *Salmonella spp.*, *Shigella spp.*, *Citrobacter freundii*, *Strept. faecalis*, *K. pneumoniae*, *Enterobacter aerogenes* and *Micrococcus spp.* from meat products.

Adil *et al* (2014) isolated *Bacillus spp.*, *Staphylococcus epidermidis*, *Diphtheroids*, *Micrococcus spp.*, *Streptococcus fecalis*, *Lactobacillus spp.* from fresh and refrigerated meat of beef samples while Gram-negative isolates were *Escherichia coli*, *Citrobacter freundii*, *Edwardisella tarda*, *Acinetobacter spp.*, *Pseudomonas spp.*, *Aeromonas spp.*, *Alcaligenes spp.*, *Proteus spp.* and *Moroxella spp.*

Shuaib *et al* (2015) evaluated the safety status of beef during slaughtering and carcass processing at three slaughterhouses in Omdurman, Sudan and isolated *E. coli*, *Salmonella species*, *Pseudomonas species*, *Shigella species*, *Staphylococcus species* and *Streptococcus species* on the surface carcasses.

## **1.6 Hygienic measure adopted in slaughterhouse**

The control of quality standards for meat production has been demanded since man first began to eat meat. Meat inspection was practiced in France as early as 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,

1990) . Dickson (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 animal 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.

### **1.7 Selection of animal for slaughter:**

To minimize the losses resulting from transportation, animals should be rested and fed before slaughter to regain physiological normality (Houthis, 1957; Willson and Payne, 1978). Ibrahim (1989) stated that ante-mortem is of a great value in detection of animals suffering from infectious diseases particularly noticeable diseases and emergency cases. It ensures that food animals released for slaughter are in good state of nutrition, cleanliness and free from signs of diseases. Johnston (1990) suggested that fecal 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. Much food poisoning outbreaks 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

Ante mortem inspection is of great value for it aid in the detection of animal disease (Houthuis , 1957 Thornton, 1968). Beside some other examination should be done like staining by poly chrome methylene blue for Mcfadyean reaction (FAO, 1962)

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.

### **1.8 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 (Hess and Lott 1970; Smulders and Woolthuis 1983; Chandran *et al.*, 1986; Dixon et al., 1991).). According to Schutz (1991) the occurrence of hygienic faults and of a high 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, 1991). The laws do not always distinguish between critical operation and those that have little effect on the hygiene (Huis in`t Veld ;*et al.*,1994). 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 minces) 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*).

(Gracey, (1985); Gracey, (1986); Boyle *et al* (1990) stated health condition of slaughterhouse building and surrounding areas.

Shuppel *et al.* (1996) suggested that the udder should be removed before skinning and it is generally judged unfit for human consumption. Mousing *et al.* (1997) suggested that 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 allocate

### **1.9 History of HACCP**

IN 1961, Codex commission (1961) formed general principles of food hygiene and followed the food chain from primary production through to the consumer, highlighting the key hygiene controls at each stage and recommending an HACCP approach wherever possible to enhance food safety. These controls are internationally recognized as essential to ensuring the safety and suitability of food for human consumption and international trade.

The HACCP concept developed by the national aeronautics and space administration (NASA) and Natick Laboratories for use in the aerospace manufacturing. This national approach to process control for food products was developed jointly by the Pillsbury Company, NASA and the U.S army Natick laboratories in 1971 as an attempt to apply zero defect program to the food processing industry. HACCP was incorporated to guarantee that food use in the US space program would be 100% free of microbial pathogens because it is designed to prevent rather than detect food hazards. HACCP has been identified by the US department of agriculture, food safety and inspection service (FSIS) as a tool to prevent or control food safety hazards during meat and poultry production the developments.

### **1.10 Conception of HACCP system:**

On 1996, the food safety and inspection service (FSIS) of the United States department of agriculture (USDA) published a final rule on pathogen reduction hazard analysis and critical control point (HACCP) system (PR/HACCP). The (PR/HACCP) rule requires meat and poultry plants under federal inspection to take the responsibility for other things reducing the contamination of meat and poultry



products with disease causing (pathogenic) bacteria and reducing the number of deaths and illness linked to meat and poultry products. The preamble to the final rule describes an overall system in which preventive and corrective measures. The HACCP system, which is a science, based and systematic identifies specific hazard and measures for their control to ensure the safety of food HACCP is a tool to assess hazards and establish control system that focus on prevention rather than relying mainly on end products testing. HACCP system is capable of accommodation change such as advance in equipment design processing. HACCP can be applied throughout the food chain from primary production to final consumption. The HACCP system program is a preventive approach to safe food production. It was based on the two important concepts of safe food production, which are prevention and documentation. The major thrusts of HACCP are to determine how and where safety hazards may exist and their prevention. The documentation concept is essential to verify that potential hazards have been controlled. The primary objective of HACCP is to insure that effective sanitation and hygiene and other optional consideration be conducted to produce safe product to provide proof that safe practices have been followed.

(HACCP) is a food safety management system, which concentrates prevention strategies on known hazards and the risks of this of them occurring at specific points in the food chain (Shmoury, 2000). On the other side also he reported that (HACCP) comes from two key phrases, “Hazard Analysis” and “Critical Control Points”. Hazard Analysis involves investigation intended to disclose (through examination), identify, estimate, and calculate the risks of all factors associated with the processing and marketing of a given product. In simple terms , it is necessary to assess all possible hazards and the likelihood of their occurrence ( by analysis ) . Control Points are steps, operations or stages in the manufacturing or marketing process that needs to be controlled and monitored as they have a great effect on the quality of the product.

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 following principles (Brown, 2000)

### **1.11 Application of the HACCP**

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

confidence. Any HACCP system should be capable of accommodating change, such as advances in equipment design, changes in processing procedures or technological developments. While the application of HACCP to all segments and sectors of the food chain is possible, it is assumed that all sectors should be Operating according to good manufacturing practices (GMPs) and the codex General principles of food hygiene . The ability of an industry segment to support or implement the HACCP system depends on the degree of its adherence to these practices. The successful application of HACCP requires the full commitment and involvement of management and the workforce. It requires a multidisciplinary approach which should include, as appropriate, expertise in agronomy, veterinary health, microbiology, public health, food technology, environmental health, chemistry, engineering, extension...etc. according to the particular situation. The application of HACCP system is compatible with the implementation of T Q M systems such as the ISO 9000 series. However, HACCP is the system of choice in management of food safety within such systems.

#### **1.11.1 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.

#### **1.11.2 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.

#### **1.11.3 Establishment of Critical Limit(s)**

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

#### **1.11.4 Establishment of a System to Monitor Control of the CCPs**

##### **1.11.4.1 Establishment of the Corrective Actions**

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

##### **1.11.4.2 Establishment Procedures**

For verification to confirm that HACCP system is working effectively.

##### **1.11.4.3 Establishment of a Documentation System**

Establish a documentation system concerning all procedures and records

Appropriate to these principles and their application.

## **CHAPTER TWO MATERIALS AND METHODS**

### **2.1 Study area:**

The study was carried out in EL Gadaraf state which is lays between 16.4°-14.4° latitude and 33.35° -35° longitudes. It is boarded by Sinaar state, Kassala, Khartoum and Elgaziara states and by Ethiopia (Fig1).

ELGadaraf population is about 2208385 Heads according to the Central Bureau of Statistics- ELGadaraf State, their livelihood depends mainly on agriculture, livestock and other sources such as public employment.



Fig1: Map of Sudan

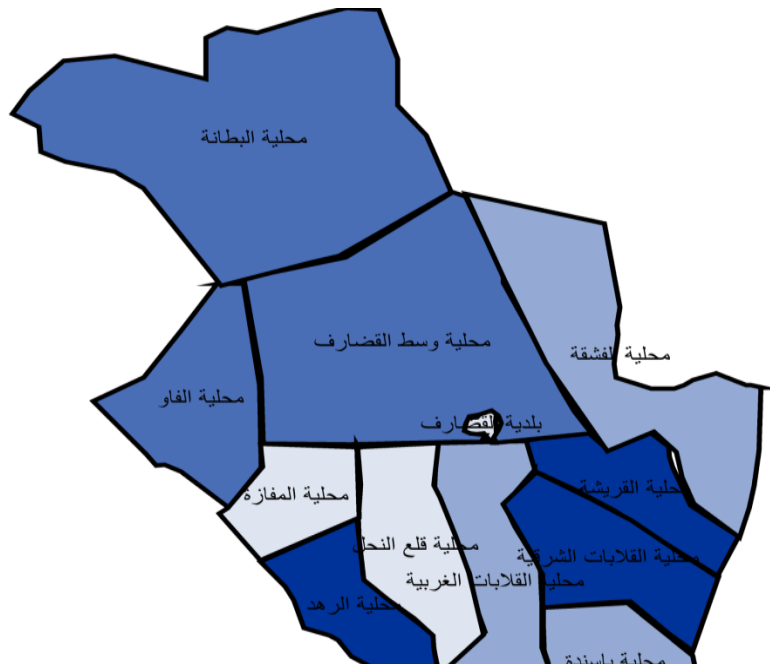


Fig2: Map of EL Gadaraf State

The dominant climate is semi arid to poor savannah climate and the rainfall ranges between 300 to 800 mm annually. In the autumn season, all animals are oriented through different animal path from ELGadaraf different localities and neighboring state to Sahal Elbuttana .In the 1940<sup>th</sup> the space of pasture was 86% of the total land but now due to extension of the mechanized agriculture, it was decreased to 6%. The pastures covered by Elseha, Elhantot, Eltabar, elghobash and Elsafari grasses. ELGadaraf state depends on seasonal rivers, pools and shallow wells as the water sources. Livestock kept under the pastoralist system, include Camels, Cattle, sheep and goats. The famous tribes which are breeding cattle are Alshukria – Bani Amer –Hadandawa -Umbararou -Kenana and Rufaa and the predominant cattle breeds are El- butane and EL-kenana – Gash- Um bararou- Crossbreeds.

The total Animal population in EL Gadaraf state is illustrated in table 1:

Table 1: No. of different species Animal in EL Gadaraf state

	Cattle	Camel	Goat	Sheep	Total
ELGadaraf	1890332	625055	953627	5336313	8805327

( source - Federal Ministry of Animal Resources & Fisheries.2017)

## 2.2Method of collection of samples

Three hundred swab samples were collected by using sterile swabs from four sites of carcass, namely shoulder, rump, neck and brisket regions at the point of skinning, evisceration and washing and the hand of workers at the moment of skinning, evisceration and washing also the knife of workers at skinning and evisceration. .

The study was conducted to determine bacterial contamination of cattle carcasses at slaughterhouses in ElGadaraf town in the period from March to June 2016. A total of 300 swab samples were collected for total viable counts (TVCs) from 25 cattle carcasses which were randomly selected and sampled from different site. The samples were collected from carcasses after manual slaughter of animals. Skinning was done manually and then the animals were eviscerated after that they were washed thoroughly with bottle water transferred from tanks and, then left to dry, and sent to market. The 25 cattle carcasses were randomly selected. From each carcass, 4 swab samples were collected from the brisket, shoulder, neck and rump after skinning, after evisceration and after washing respectively. In addition 30 swab samples were collected from the hands of workers after skinning, after evisceration and after washing and 30 swab samples which were collected from the knives surface. The samples were stored in a cooling box and transported to the laboratory, where the microbiological analysis was performed at the same day.

### **2.2.1 Samples preparation**

Samples were inoculated onto liquid nutrient broth and incubated over night then diluted with normal saline then, Small portion was streaked with a sterile loop on solid media (MacConkey's agar, Mannitol salt agar Eosin methylene blue agar and Deoxy chocolate agar) and incubated at 37°C for 24 h. for isolation and identification of Gram-Negative bacteria and *staphylococcus aureas* bacteria .

### **2.2.2 Total Viable Count:-**

Total viable count of bacteria was done as described by Miles and Misera method (1938) and Abdalla *et al.*, (2009a). Sterile normal saline was used as diluents to make tenfold dilution from each sample. And then 1 ml was transferred from both 10<sup>-4</sup> and 10<sup>-5</sup> dilutes. then were spread over the sterile nutrient agar plates for aerobic bacterial count, and cultured in duplicates, then plates were incubated at 37°C for 24-48 hours. Plates with 20-300 colonies were counted, then the average number of colonies was multiplied by the dilution factor to give the number of colony forming

units (CFU) per ml and divided by 10 to give the number of colonies forming unit per cm<sup>2</sup>.

## **2.3 Method of Sterilization**

### **2.3.1 Dry heat**

#### **2.3.1.1 Hot air oven**

This method was used for sterilization of clean glass containers which were wrapped in foil; temperature of 160°C was applied for one hour as described by Barrow and Felthman (1993).

#### **2.3.1.2 Red heat**

This method was used for sterilization of wire loops and point of tissue forceps by holding the object over flame as near and as vertical as possible until it becomes red hot (Barrow and Felthman, 1993).

#### **2.3.1.3 Flaming**

It was used to the sterilization the mouth of the bottles, cotton plugged tubes, and glass slides .it was done by exposing the object to the direct for one second.

### **2.3.2 Moist heat**

#### **2.3.2.1 Autoclave**

This method was used for sterilization of culture media, solutions, plastic wares such as rubber stoppers, which could not withstand the dry heat .the holding temperature 115°C- 121°C under10-15 pound pressure for 15-20 minute (Barrow and felthman, 1993).

## **2.4 Cultural media**

These media were prepared accord to Barrow and Felthman (1993).

### **2.4.1Sold media**

#### **2.4.1.1 Nutrient agar (NA)**

25grams of the powder were added to one liter of distilled water and brought to boil to dissolve the powder completely . Sterilized by autoclaving for 15 minutes at 121° c and 15 pounds per square inch. then poured aseptically as 18-20 ml in Petri-dishes.

#### **2.4.1.2 Mac Conkey's agar medium**

52 grams of Mac Conkey's agar powder) were added to one liter of distilled water and brought to boiling until dissolved completely. The PH was or- adjusted to 7.4

then sterilized by autoclaving at 121°C for 15 minutes. Then it was aseptically distributed in sterile Petri –dishes as 15-20ml portion and left to solidify

#### **2.4.1.3 Deoxy Chocolate Citrate Agar (DCA)**

It prepared by 70.52 grams in one liter of distilled water then heat to boiling to dissolve the medium completely .

#### **2.4.1.4 Simmons citrate agar**

32 grams of medium were dissolved by boiling in one liter distilled water .Then dispensed in sterile Mc-Cartneys bottles in 10ml volumes or 3 ml volumes into bijoux bottles and sterilized by autoclave at 121°C for 15 minutes. After removal from the autoclaving, the bottles were set in slope position until cooling.

#### **2.4.1.5 Urea agar base medium**

A mount of 2.4 gram of urea agar base powder (*oxid*) was suspended in 95ml of distilled water and dissolved by boiling, sterilized by autoclave at 121°C for 15 minutes and cooled to 50°C .Then 5 ml of sterile urea solution were aseptically added and mixed well, poured in 3 ml volumes into bijoux bottles, then put in the slope position until cooled.

#### **2.4.1.6 Mannitol salt agar**

111 gram in one liter of distilled water and bring to the boil to dissolve completely .sterile by autoclaving at 121°C for 15 minutes.

#### **2.4.1.7 Eosin m ethylene blue agar**

37,5 gram of medium in one liter distilled water heat with frequent agitation and boil to one minute to completely dissolve the medium sterile by autoclaving at 121°C for 15 minutes.

### **2.4.2 Semi sold media**

#### **2.4.2.1 Motility medium**

The medium was described by *Cruickshank et al. (1975)*. 0.2% was dissolved in nutrient broth and distributed sin sterile test tubes containing Craigie tubes and then the media was autoclaved at 121°C and 15 pounds per square inch.

#### **2.4.2.2 Hugh and Liefson's (LO/FT)medium**

This medium was prepared as described by Barrow and felthman (*1993*). 2 grams of peptone water, 5g Nacl, 0,3g potassium hydrogen phosphate (K<sub>2</sub>HPO<sub>4</sub>) and



3g agar were added in one liter of distilled water. The suspension was heated in water bath at 55°C to dissolve the pH was adjusted to 7.1, indicator bromothymol blue 0.2% aqueous solution was added. The mixture was sterilized by autoclaving at 115°C for 15 minutes. Filtered sterile glucose solution was added aseptically to give a final concentration of 1%, mixed well and distributed aseptically in 10 ml volumes into sterile test tubes..

### **2.4.3 Liquid cultural media**

#### **2.4.3.1 Peptone water**

50 grams of peptone water (*Oxoid*) were added to one liter of distilled water and mixed well .Then distributed into sterile test tubes and sterilized autoclaving at 121°C for 15 minutes.

#### **2.4.3.2 Nutrient broth**

Nutrient broth (*Oxoid Lab*) contained lab-lemco powder (1g) yeast extract (2g), peptone (5g) and sodium chloride (5g). PH was adjusted to 7.4 approximately. An amount of 13g of the dehydrated medium was added to one liter of distilled water .the reconstituted medium was mixed well then distributed in 5ml amounts and sterilized by autoclaving at 121 c for 15 minutes under pressure of 15lb per square inch.

### **2.5 Reagent**

#### **2. 5.1 Kovac's reagent**

This reagent was prepared as described by Barrow and Felthman (1993) five grams of P-dimethylaminobenzaldehyde were dissolved in 75 ml of isoamy lalcohol by warming in water bath. After the mixture was cooled, 25ml of concentrated hydrochloride acid were added. It is used for indol test.

#### **2.5.2 Hydrogen peroxide**

Hydrogen peroxide produced by B.D.H (British Drug House) was diluted to 3% aqueous solution for catalase test.

#### **2.5.3 Oxidase test reagent**

Tetra methyl-pheynlene-diamine dihydro chloride was prepared as 1% aqueous solution.. Filter paper of 50 x 50 millimeter size were impregnated in the reagent before and dried at 50°C. (Barrow and Felthman, 1993).

#### **2.5.4 Lugol's iodine**

It consisted of 5 g iodine and 10g potassium iodide which were dissolved in 100ml of distilled water it was used for Gram stain.

### **2.6 DILUENTS:**

#### **2.6.1 Normal saline**

Normal saline 9 % for dilution.

#### **2.6.2 Distilled water.**

For preparation of all media

### **2.7 Purification of cultures**

The primary isolates were sub cultured on nutrient agar. The subculture was repeated several times until pure colonies were obtained.

### **2.8 Examination of cultures**

#### **2.8.1 Visual examination of bacterial growth**

Examination of all cultures on solid media was performed for detection of growth, pigmentation, colonial morphology as well as changes in the media. Plates that showed visible growth were subjected to further bacteriological tests while those that did not show visible growth were incubated for further 48hours and discarded if no growth was detected.

#### **2.8.2 Microscopic examination**

##### **2.8.2.1 Preparation of smear culture**

Smears were prepared by emulsifying part of typical isolated colony in drop of sterile normal saline and spread on clean slide. The smears were allowed to dry in air then fixed by gentle flaming and placed in rack. All smears were examined by Gram stain.

##### **2.8.2.2 Gram stain**

Gram stain was done according to Barrow and Felthman (1993).

1. Crystal violet solution was added to a fixed smear for one minutes.
2. Washed by water
3. Lugol's iodine was added for one minute
4. Washed by water
5. De colorized with actone for no time.
6. Washed by water

7. Then added dilute carbol fuchsin was added for one minute.
8. Washed by water.
9. Then it was dried by air and examined microscopically under oil immersion.

## **2.9 Biochemical testing**

### **2.9.1 Primary Biochemical tests**

All of the Biochemical tests were done as described by (Barrow and Feltham 1993).

#### **2.9.1.1 Catalase test**

A drop of 3% aqueous solution of hydrogen peroxide was placed on clean slide. Then a colony of the test culture on nutrient agar was picked and mixed with the hydrogen peroxide. A positive reaction was indicated by production of gas bubbles.

#### **2.9.1.2 Oxidase test**

The test was performed by placing the oxidase reagent –soaked dried filter paper strip on a clean slide. A small amount of fresh culture was smeared on the strip. A positive reaction gave deep purple color within seconds.

#### **2.9.1.3 Sugar test**

Sugars were weighed (0.5 gram) and dissolved. Trypticase (1 gram), Sodium chloride (0.5 gram), and Phenol red (0.0189 mg) in 100 ml distilled water and transferred into conical flasks. Addition of 0.5% to 1% of desired carbohydrate into all flasks. Insertion of inverted Durham tubes into all tubes. Sterilization at 115°C for 15 minutes. Inoculation of the broth with bacterial culture and incubation of the tubes at 18-24 hours at 37°C. Yellow color means acid production, while yellow color and presence of small bubbles in the inverted Durham tubes means acid and gas production. (Hugh and Leifson, 1953).

#### **2.9.1.4 Oxidation fermentation test**

Two tubes of Hugh and Leifson's medium were inoculated with the test culture. One of them was covered with a layer of sterile paraffin oil to a depth of 1-2 cm and the two tubes were incubated at 37°C and examined daily. Then oxidative bacteria gave yellow color in open tube only while the fermentative bacteria gave yellow color in both tubes.

#### **2.9.1.5 Motility test**

The tube of motility medium was stabbed by the inoculums stabbed straight with a wire loop, to a depth about 15mm .the culture was incubated at 37°C for 24 hours. Motility of bacteria indicated by the migration of the organism through medium which became turbid, no growth migration could be seen in non motile bacteria as growth was restricted to the path of the inoculums

## **2.9.2 Secondary biochemical tests**

### **2.9.2.1 Citrate test**

This was inoculated as single streak over the surface of a slope of simmons citrate medium and examine daily for up to 7 days for growth and color change. Positive reaction is blue color and streak of growth citrate utilized and original green color citrate not utilized.

### **2.9.2.2 Urease activity**

The test organism streaked on to a slope of urea agar medium and incubated at 37°C for two days. Pink color indicated positive reaction.

### **2.9.2.3 Indole test**

Inoculate peptone water and incubate for 48h .added 0.5 ml Kovacs reagent for indole production. Shake well and examined after about one minute. A positive reaction as a red ring colour in the reagent layer indicates positive indole production.

### **2.9.2.4 Kligler Iron agar**

Fourty nine grams of powder were mixed with 1 liter of distilled water. Bring to the boiling with frequent stirring to dissolving completely. Then the agar dispense in to tubes and sterilized for15 minutes at 121°C .Cooling in aslant position over deep butts approx.3cm in depth pH 7.4-0.2.

### **2.9.2.5 Slide coagulase test**

Using a sterile wire loop one colony was picked and emulsified in physiological saline on clean glass slide and then a drop of plasma was added and the mixture was rotated. A positive reaction is indicated by clumping within 5 seconds.

## **2.10 Data analyses**

The data were analyzed using the software Statistical Package for the Social Sciences version 18.0 (SSPS Inc. and Chicago, IL, USA). All bacterial counts were converted to log<sub>10</sub> CFU/cm<sup>2</sup> for analysis. Analysis of Variance (ANOVA) was performed to evaluate the differences in the levels of TVCs between the different

operational points/critical control points. Moreover, the statistical significance was set at a *p-value* of  $\leq 0.05$

## CHAPTER THREE

### RESULT

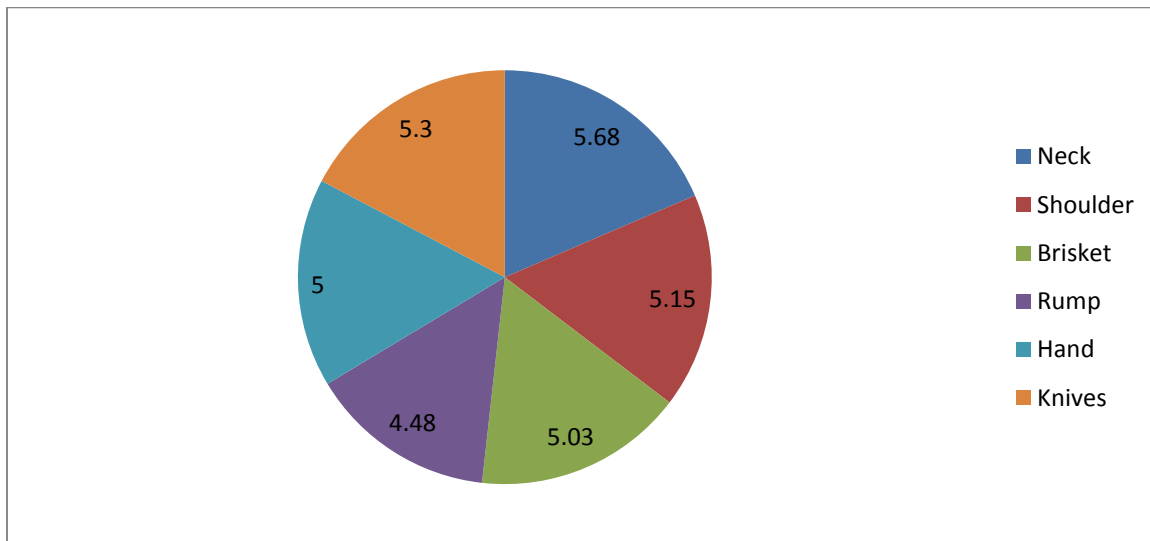
This study was conducted to determine bacterial contamination of cattle carcasses in ElGadaraf slaughterhouses. By using sterile swabs from four sites of carcass, namely shoulder, rump, neck and brisket region at the point of skinning, evisceration and washing and also from the hands of workers at the moment of skinning, evisceration and washing, in addition to the knives of workers at skinning and evisceration.

Table (2) shows The mean total viable count at neck site was ( $5.68 \pm .40$ ,  $5.17 \pm 0.46$  and  $5.79 \pm 0.39$  log<sub>10</sub> CFU/cm<sup>2</sup>) at the three points of operation (at post skinning , post evisceration and post washing) respectively with statistically significant difference ( $P < 0.05$ ). In shoulder site, TVCs were ( $5.15 \pm 0.41$ ,  $4.99 \pm 0.34$  and  $5.26 \pm 0.31$  log<sub>10</sub> CFU/cm<sup>2</sup>, at the three points of operation (at post skinning, post evisceration and post washing) respectively with statistically significant difference ( $P < 0.05$ ). In brisket site was  $5.03 \pm 0.35$ ,  $5.50 \pm 0.35$  and  $5.28 \pm 0.42$  log CFU/cm<sup>2</sup>, at the three points of operation (at post skinning, post evisceration and post washing) respectively with statistically significant difference ( $P < 0.05$ ). In rump site TVCs were  $4.48 \pm 0.41$ ,  $4.97 \pm 0.30$  and  $5.11 \pm 0.40$  CFU/cm<sup>2</sup> at the three points of operation (at post skinning, post evisceration and post washing) respectively with statistically significant difference .The TVCs of the hands of the workers at post skinning, post evisceration and post washing were,  $5.00 \pm 0.27$ ,  $5.52 \pm 0.23$  and  $5.00 \pm 0.20$  log<sub>10</sub> CFU/cm<sup>2</sup>, respectively without statistically significant differences ( $P > 0.05$ ) between them. TVCs in knives after skinning and evisceration were  $5.30 \pm 0.50$  and  $5.04 \pm 0.49$  log<sub>10</sub> CFU /cm<sup>2</sup>, respectively without statistically significant difference ( $P > 0.05$ ) (F3,F4,F5 )

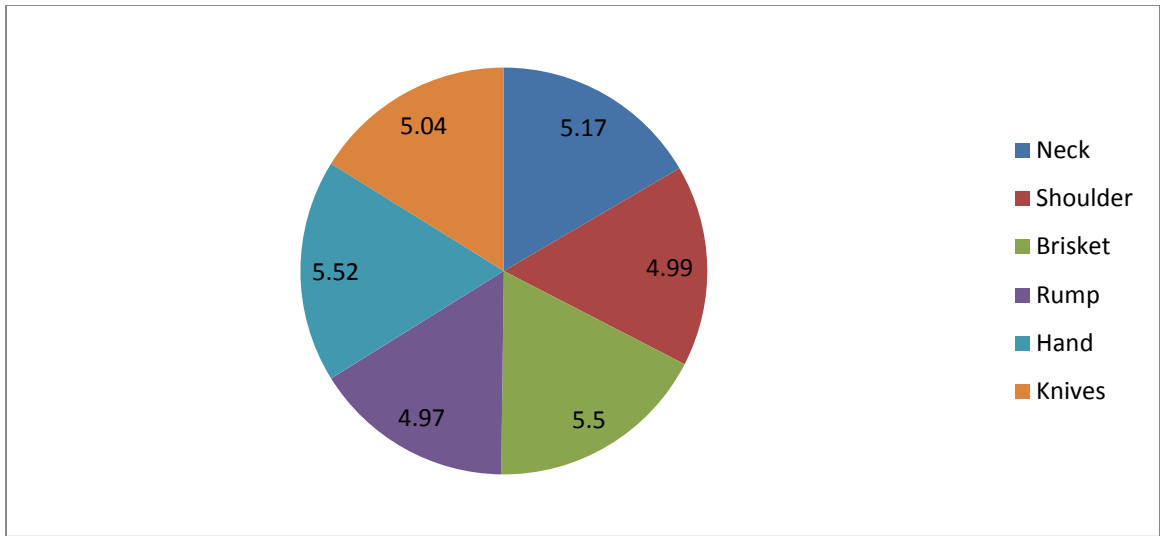
**Table 2: the mean total viable counts on different sites of bovine carcasses and operational points in Al Gadaraf Slaughterhouse**

Sites of swabbing	Operational Points			P value
	After skinning	After Evisceration	After wash	
Neck	5.68± .40	5.17±0.46	5.79±0.39	*
Shoulder	5.15±0.41	4.99±0.34	5.26±0.31	*
Brisket	5.03±0.35	5.50±0.35	5.28±0.42	*
Rump	4.48±0.41	4.97±0.30	5.11±0.40	*
Hand	5.00±0.27	5.52±0.23	5.00±0.20	NS
Knives	5.30±0.50	5.04±0.49	-	NS

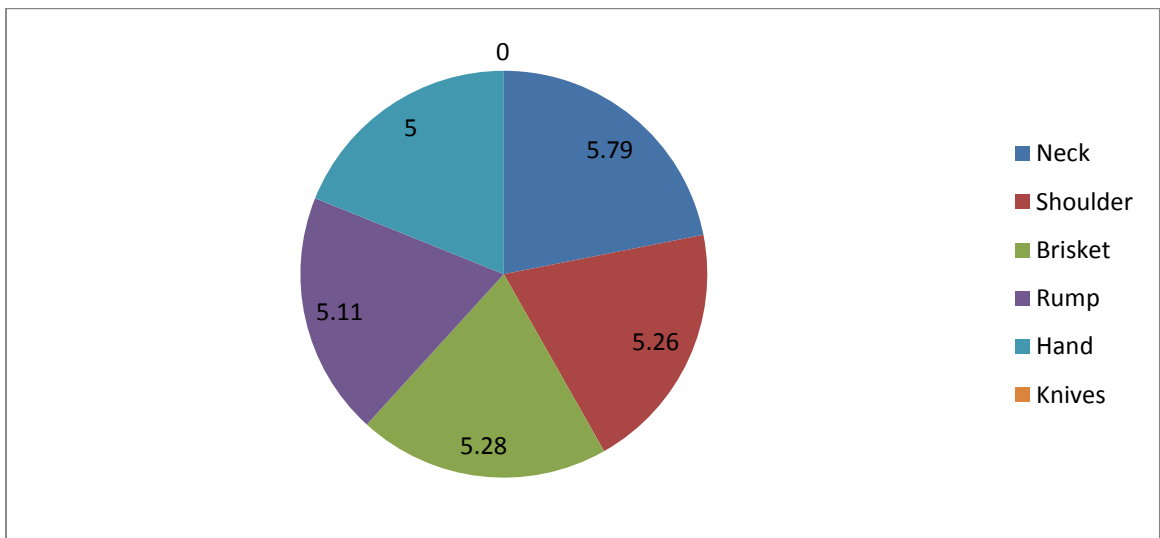
\* = Statistically significant, NS = Not statistically significant



**Fig. 3: The mean total viable counts after skinning of different sites of bovine carcasses.**



**Fig. 4: The mean total viable counts after evisceration of different sites of bovine carcasses.**



**Fig. 5: The mean total viable counts after wash of different sites of bovine carcasses.**

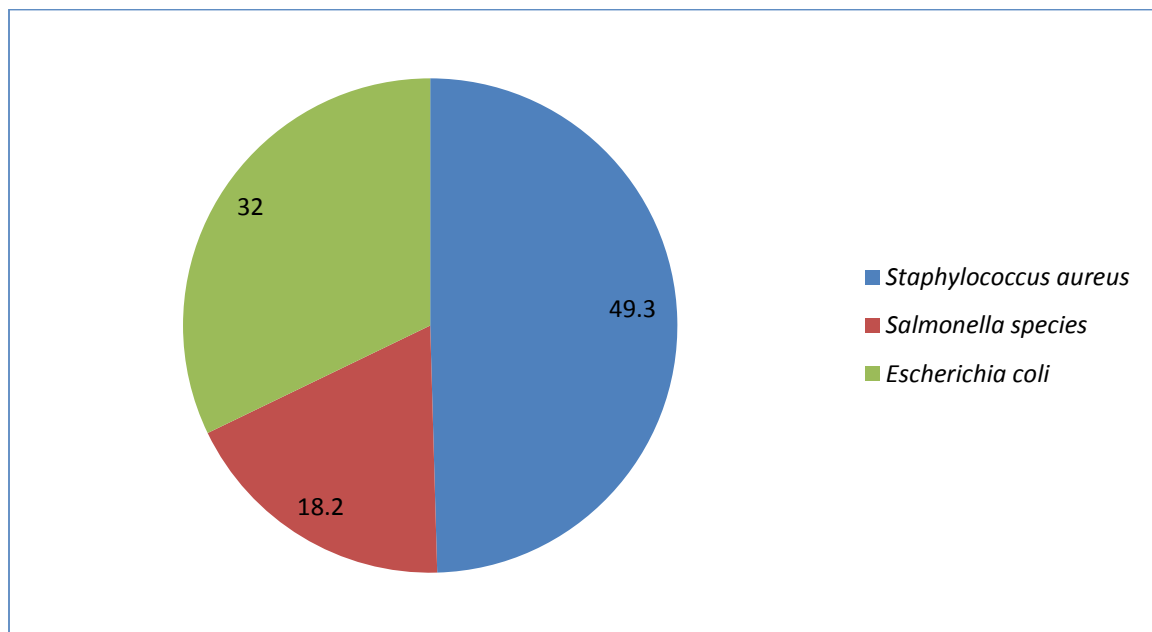
The study also revealed the contamination of the carcasses resulted from three types of bacteria as shown in Table (3) Fig. (6). The relative frequency of isolates



was *Staphylococcus aureus* (49.3%), *Escherichia coli* (32.0%) and *Salmonella spp* (18.2%).

**Table 3: Bacterial contamination of the carcasses in Al Gadaraf Slaughterhouse.**

Bacteria	No. of positive carcasses	% of positive carcasses	95% C I	
			Lower	Upper
<i>Staphylococcus aureus</i>	148	49.3	191.71	206.80
<i>Salmonella species</i>	56	18.2	188.46	214.93
<i>Escherichia coli</i>	96	32.0	190.28	209.30

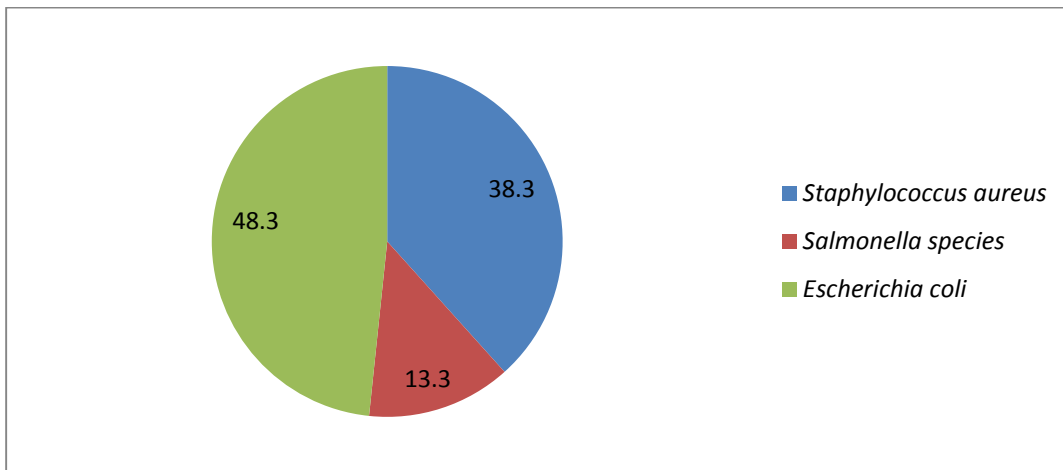


**Fig. 6: Bacterial contamination of the carcasses in Al Gadaraf Slaughterhouse**

Table (4) and Figure (7) show the contamination resulted from three types of bacteria on the labors' hands and knives. The high frequencies of isolated bacteria were *Escherichia coli* (48.30%), *Staphylococcus aureus* (38.30%) and *Salmonella* (13.30 %), respectively.

**Table 4: Bacterial Contamination of the labors' hands and knives in Al Gadaraf Slaughterhouse.**

Bacteria	N 0 of positive labors' hands and knives	% of positive labors' hands and knives	95% C I	
			Lower	Upper
<i>Staphylococcus aureus</i>	23	38.30	180.88	214.33
<i>Salmonella species</i>	8	13.30	194.76	215.49
<i>Escherichia coli</i>	29	48.30	187.86	217.65



**Fig. 7: Bacterial Contamination of the labors' hands and knives in Al Gadaraf Slaughterhouse**

## CHAPTER FOUR

### DISCUSSION

Most of the meat contamination is caused by aerobes. These organisms may gain access to meat from the system of living animal or as a result of slaughter contamination (Lawrie, 1979). Meat contamination is 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 level of the TVC was set and agreed to be a criterion for assessing and evaluating the microbial contamination of carcasses and a useful mean to know the hygienic and safety states of meat (Zweifel and Stephan, 2003). In this study, the TVC ranged from ( $4.48 \pm 0.41$  to  $5.79 \pm 0.39 \log_{10}$  CFU /cm<sup>2</sup>) at slaughterhouse. Slaughterhouse had showed TVC above the acceptable value of (2.0 log CFU/cm<sup>2</sup>) set by Decision 2001/471/EC of the EU Commission (Anonymous, 2001). Some of the levels of the TVCs recorded in the present study are similar to what have been concluded by Nouichi and Hamdi (2009) who found the superficial bacterial contamination levels of ( $4.48 \pm 0.63 \log$  CFU /cm<sup>2</sup>). And agree with El-Hadef *et al* (2005) who found a mean log TAVCs of (5.34 CFU /cm<sup>2</sup>) at Constantine slaughterhouse. And this result is similar Dennai *et al* (2001) who have obtained a contamination level of (5.15 log CFU/cm<sup>2</sup>) at Kenitra slaughterhouse

The results are higher than those recorded by Abdalla *et al.* (2009b), who reported TVCs that ranged from ( $2.73 \pm 0.04$  to  $3.74 \pm 0.02 \log_{10}$  CFU/cm<sup>2</sup>) from indigenous bovine carcasses, in Khartoum and in Switzerland by Zweifel and

Stephan (2003) who reported a mean log TVCs that ranged from (2.5 to 3.8 log CFU /cm<sup>2</sup>) and in Australia by Sumner *et al* (2003) and Phillips *et al* (2006) who have respectively reported rates of (2.59. and 2.28 log CFU/ cm<sup>2</sup>)

While on the other hand, some studied recorded higher TVCs like study conducted by shuaib *et al* (2015) who evaluate the safety status of beef during slaughtering and carcass processing at three slaughterhouses in Omdurman, the TVCs levels Ranged from (3.19±0.11 to 6.90±0.99 log<sub>10</sub> CFU/cm<sup>2</sup>) At Al-Huda slaughterhouse, from (3.15±0.49 to 6.43±0.25 log<sub>10</sub> CFU /cm<sup>2</sup>) at Al-Sabalogah slaughterhouse, from (3.23±0.11 to 8.33±0.82 log<sub>10</sub> CFU /cm<sup>2</sup>) at Al-Salam slaughterhouse. Abdalla *et al*(2009a)who reported from sheep carcasses at El-Kadero slaughterhouse the TVCs (3.0±0.59 to 6.0±0.33 log<sub>10</sub> CFU /cm<sup>2</sup>) and Elamin(2002) who estimated surface bacterial contamination of mutton carcasses at the production and retail levels Omdurman and found TVC from (1.0 × 10<sup>7</sup> CFU/cm<sup>2</sup>). El-Hassan *et al*.(2011) who assessed the microbiological validity of mutton purported for export to international markets from El-Kadero export slaughterhouse and recorded TVCs of(1×10<sup>3</sup> - 6×10<sup>6</sup> CFU/cm<sup>2</sup>). In this study the types of bacteria isolated indicated that the sources of contamination of the bovine carcasses were diverse. Some of these species are in the soil, normal flora of skin, gastrointestinal tract; some of them may be from slaughter halls, workers and condemned parts which may harbour pathogenic organisms. The worker personnel did not wear the recommended protective clothes and they moved freely between clean and dirty areas. Knives were cleaned in the same water more than once and in addition the water was not heated and no disinfectants were used. Knives were used for processing more than one animal before being washed and they are sharpened by the same device. As well as carcasses are cut by the same device without cleaning and the ambient temperature in the processing halls was not adjusted to the recommended degrees. This confirms the conclusions of *Abdalla et al. (2010)*; *Shuaib et al. (2015)* and *Magada et al. (2014)* that the main sources of contamination during the processes of converting live animals to consumable meat are the slaughtered animals themselves, the environment and the working personnel.

In this study the TVC revealed the highest contamination level recorded after skinning which was from the neck (5.68± .40 log<sub>10</sub> CFU/cm<sup>2</sup>) (P > 0.05). This result agrees with *Shuaib et al (2015)* and *Abdalat et al (2009b)*. This could probably be due to the fact that the neck is the first part of the animal to be exposed to the

ambient environment. While the highest contamination level after evisceration was from hand ( $5.52 \pm 0.23 \log_{10} \text{CFU/cm}^2$ ) But the highest contamination level after washing was from the neck ( $5.79 \pm 0.39 \log_{10} \text{CFU/cm}^2$ ). This result agrees with Shuaib *et al* (2015) and Abdalla *et al* (2009). This could be explained by the fact that the carcass is normally washed from the upper part. Another possible explanation to the differences of the points of the highest TVC could be due to multiple contacts of carcasses with contaminated slaughtering utensils and hands of workers (Jeffery, 2003; Nouichi and Hamdi, 2009 Shuaib *et al*, 2015).

The high level of bacterial viable counts after post washing of bovine carcasses in this study is in agreement with Abdalla *et al* (2009b) who evaluated the degree of contamination in indigenous cattle carcasses in a slaughterhouse where they revealed high level of bacterial viable counts after post washing of bovine carcasses. Similar results were obtained by Ali (2007) who found that the highest contamination was at the point of washing on different sites of examination of bovine carcasses.

The variability in microbial counts especially after washing could be attributed to the absence of prerequisite programmers. Contrary to the above mentioned findings Abdallat *et al* (2009) and Magada *et al* (2014) revealed that sterilization by hot water ( $82^\circ \text{C}$ ) to knives in all slaughtering operations and fairly warm water to workers' hands resulted in reduction in TVCs of worker's hands and their knives with statistically significant difference ( $P < 0.05$ ).

Washing of the carcasses and the commitment of labors to completely wear protective cloth reduces the level of organisms (Magada *et al.*, 2014). The workers' hands and equipment in the processing facilities are also sources of meat contamination (Jeffery *et al.*, 2003). This agreed with the findings of my study.

The TVC of the workers' hands at post skinning, post evisceration and post washing were  $5.00 \pm 0.27$ ,  $5.52 \pm 0.23$  and  $5.00 \pm 0.20 \log_{10} \text{CFU/cm}^2$ , respectively with no significant differences ( $P > 0.05$ ) between them. This finding disagrees with Abdalla *et al* (2009 b) who found statistically significant difference.

Also this study recorded that the TVC in knives after skinning and evisceration were  $5.30 \pm 0.50$  and  $5.04 \pm 0.49 \log_{10} \text{CFU/cm}^2$ , with no statistically significant difference ( $P > 0.05$ ). This result agrees with Abdalla *et al* (2009 b).

This study revealed three types of bacterial contamination of the carcasses. The relative frequency of isolates was *Staphylococcus aureus*, 49.3%, *Escherichia coli*

32.0 % and *Salmonella* species 18.2%. This agrees with Khalid (2004) who isolated *Staph. aureus*, *Escherichia coli* .no *Salmonella*.

This study revealed a statistically significant difference at *P-value* ( $p \leq 0.05$ ) at the different operational points between the samples tested from slaughterhouse after skinning, after evisceration and after washing respectively. These findings are similar to those obtained by Gill (1998) who reported bacterial contamination of meat during the different operational steps, and with Abdalla *et al* (2009 b) who found statistically significant different of total viable count of aerobic bacterial after skinning, after evisceration and after washing, but disagrees with Shuaib *et al* (2015) who found no statistically significant difference between their studied samples.

## CHAPTER FIVE

### CONCLUSION

- 1- This study revealed high level of contamination of bovine carcasses at ElGadaraf slaughterhouse with organism which reduce the quality of meat.
- 2- Potential pathogenic bacteria such as *Staphylococcus aureus*, *Escherichia coli*, *Salmonella spp.* were isolated from bovine carcasses
- 3- Food poisoning bacteria such as *S. aureus* was isolated in most of stages of carcass processing.
- 4- The highest level of contamination with regard to the critical control point was found at the point of washing than skinning & eviseration.

## **CHAPTER SIX**

### **RECOMONDATION**

Meat is a highly perishable food and must reach the consumer suitable for human consumption. Due to the high levels of contamination of meat that was found in the present study, it was strongly recommended that:

1. Strict meat hygiene system\*s which control contamination in slaughterhouses including: sanitation of animal, personal hygiene and staff handling meat must be followed.
- 2-Washing of the animal prior to slaughtering to reduce the contamination of meat by *E.coli* from fecal matters on hide and skin is recommended.
- 3-Water sources and utensils must be checked frequently for bacterial load to control any contamination.
- 4-The floors, walls, slaughtering equipments and clothes of workers at slaughterhouse must be cleaned with effective disinfectant to reduce or kill microbial population.
- 5-Departments of public health and veterinary authorities should take adaptive measures to train personal for slaughtering operations in order to reduce contamination source and protection of people against food-borne infections using good sanitary measures.



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# Appendix

## Appendix (1)

Worker at the slaughterhouse are not committed to wear protective clothes





## Appendix (2)

The carcasses skinning take place adjacent to rumen content due to lack dumpster





### Appendix (3)

Poor hygiene in the slaughter house putting edible offal's in the skin or in the floor



## Appendix (4)

Carcasses washing is done manually by bottles



