

CHAPTER ONE

INTRODUCTION

Livestock represents the largest subsector of the Sudanese domestic economy and is a growing contributor to exports. Sudan is characterized by vast areas of range and arable land which is either rain-fed or irrigated. Although it has a large population of livestock estimated to be 103 million heads of cattle, sheep, goat, etc., but until now by international standards is not classified among the main meat producers or exporters. **(Mohamed and Elmardi, 2014)**

Meat is a food stuff that can be spoiled extremely quickly. Certain species of bacteria multiply on fresh meat thanks to its chemical composition, favourable water activity and pH. Their numbers soon reach levels that cause sensory deviations and lead finally to spoilage of the meat. **(Kamenik, 2013)**

Contamination of animal carcasses and raw meat by microorganisms, including spoilage and pathogenic types, is practically unavoidable. In general, animals may be infected, contaminated or be asymptomatic carriers of microbes, which, together with the environment serve as sources of contamination of carcasses during the slaughtering process of meat products during processing, storage and handling, or of water and other foods through contaminated manure. Contamination is introduced during growth and production at farms, ranches and feed yards, as well as during shipping, distribution, marketing, processing, retailing, preparation and consumption. Contamination sources include soil, decaying materials and animal waste, which contaminate water, air, animals, plants, processing facilities, equipment's and humans. All of these contribute to direct or cross-contamination, leading to a complete contamination cycle which is natural phenomenon that

cannot be prevented. Thus it is impossible to produce raw meat or other animal food products which are free of contamination. **(Koutsoumanies and Sofos, 2005)**

Although various foods can serve as sources of foodborne illness, meat and meat products are important sources of human infection with a variety of foodborne pathogens, i.e. *Salmonella* spp., *Staphylococcus* spp., *E.coli*, Coliform, moulds and yeasts, etc., all these may be harboured in the gastrointestinal tract of food-producing animals. Zoonotic pathogens in foods, including meats, have to be controlled through a complete, continuous farm-to-fork system and should take into account not only the risks but also technical possibilities, consumer's attitudes and behavior and cost-benefit analysis. However, some aspects of control systems are most efficiently controlled by the main interventions applied in the primary production combined with optimization of the slaughter hygiene. For some others, such as more environmentally ubiquitous like *Clostridium* spp. and *Staphylococcus aureus*, the main control measure are focused on later stages of the meat chain, Toldra (2009). Microorganisms introduced from environmental exposure, lack of sanitation in slaughtering premises, equipment and outfit, and operators' hands contaminate the meat product **(Sachindra et al. 2005; Kozačinski et al. 2006)**

Food consumers also comprise a link in the chain of food-borne bacterial illnesses with inappropriate storage and cooking of meat and meat products **(Sachindra et al. 2005; Kozačinski et al. 2006; Tachbele et al. 2006)**. Although many bacteria encountered in foods are harmless, some may be potentially pathogenic. Food-borne infections and intoxications can occur due to the presence of certain bacteria e.g. *E. coli*, *S. aureus* and *Salmonella* spp. **(Elmalı and Yaman 2005; Tachbele et al. 2006)**. Therefore, detection and identification of pathogenic bacteria in food as well as assessing the general microbial load of the food is of great significance. **(Kozačinski et al. 2006)**

Use of indicator bacteria is a common practice to evaluate the hygienic condition of foods and the possible presence of pathogens. Total number of bacteria, coliform bacteria and *E. coli* obtained via total viable counts may, in some instances, reflect the sanitary quality of food. Among all microorganisms, *E. coli* is most frequently the contaminating organism, and is generally a reliable indicator of fecal pollution in water, food, milk and other dairy products (**Soomro et al. 2002**). In addition to pathogenic bacteria, total count of aerobic mesophilic bacteria, *Pseudomonas* spp., yeasts and moulds are also used as general indicators of processing hygiene, storage conditions and spoilage in meat and poultry industries (**ICMSF 2005; Kozačinski et al. 2006**). It was important to study the microbiological status of fresh meat samples collected from local markets.

Main objective:

1- To study the microbiological characteristics of fresh meat cuts collected from local butcher shops and supermarkets from different locations in Khartoum State.

Specific objectives:

1- To determine the total number of the different types of microorganisms present in the collected fresh meat samples.

2- To determine the pathogenic microorganisms in the collected fresh meat samples.

CHAPTER TWO

LITERATURE REVIEW

2.1 Definition of meat:

In the broadest sense, meat is the edible postmortem component originating from live domesticated animal such as cattle, sheep, etc. (**Kauffman, 2001**). Meat is defined as the flesh of animals used as food. In practice this definition is restricted to a few dozen of the 3000 mammalian species; but it is often widened to include, as well as musculature, organs such as liver and kidney, brains and other edible tissues. (**Lawrei and Ledward, 2006**)

Lawrie (2006) defined meat as the flesh of animals used as food and claimed that this definition is often widened to include, as well as the musculature, organs such as liver and kidney, brains and other edible tissues. Consumption of meat is generally synonymous with human development.

It is a group of muscles, connective tissues and fats, in addition to some glands and internal organs (liver, heart, spleen, tongue, kidney, brain) that are taken from the carcasses of animals, and fit as beneficial food for human consumption, and it must be free from pests, diseases and consistent with the traditions of the group of consumers. (**Uoda, 1984**)

Meat means the whole or part of a carcass of any buffalo, camel, cattle, deer, goat, hare, pig, poultry, rabbit or sheep that is slaughtered. Meat flesh is defined as skeletal muscle to distinguish it from other parts of a carcass of meat such as offal, bone and bone marrow. Meat flesh includes any attached fat, connective tissue rind, nerves, blood vessels and blood, and skin. (**AFSC, 2001**)

2.2 Red meat:

They are found in Mammals such as (cattle - sheep - goats - buffalo - horses) which is taken from their muscles, they contains myoglobin which is the dye responsible for the color of meat, the concentration of the red color increases as the concentration of the dye increases, and this dye concentration depends on the type of animal, age and location of muscle on the body and also the type of practical activity. **(Fakhry, 1985)**

Red meat is taken to include the skeletal muscular tissue and associated materials (fat and other tissues) from the main commercial meat species i.e. cattle, sheep, pigs and deer. **(Rivas *et al*, 2014).**

2.2.1 Most important types of red meat:

(A) Mutton (sheep): One of the most important types of red meat. It is easy to digest when it is free from fats, with delicious taste and flavor and their color between the light pink to light red. **(Uoda, 1984)**

(B) Goat meat: Goat meat is the closest to mutton with the exception of a few amount of fat in goats and the color of the meat is red purple. **(Uoda, 1984)**

(C) Beef: Beef have high nutritional value for their meat, which contains a good amount of protein and vitamins. Color of beef is red cherry, increasingly color indicate the progress of the animal age. **(Uoda, 1984)**

(D) Camel meat:

Camel meat is tough because of thickness of the muscles fiber, characterized by having red color, quality of the meat determined by fat color in hump, and contains high percentage of water. **(Uoda, 1984)**

2.3 Description, composition and nutritional value of meat:

Meat animals contain, as a majority of their carcass weight, many muscles distributed in an unusually designed pattern to move the skeleton, for posture control, and for specialized functions such as respiration and swallowing. This musculature is categorized into two major types: striated and non-striated. The less voluminous non-striated muscles or smooth muscles have similar functions as striated muscles but possess different histological structures. Smooth muscles are primarily found in the lining of the gastrointestinal tract and the circulatory system as well as in specialized organs such as the gizzard of birds. Striated muscles are categorized as either cardiac or skeletal, cardiac muscles are confined to the heart and have the continuous responsibility of distributing and collecting blood throughout the body. **(Kauffman, 2001)**

The composition of meat differs according to the type, classification, structure and anatomy of animals. **(Uoda, 1984)**

Components involved in the composition of meat are water, protein, fat, sugars, minerals, vitamins and enzymes. According to Kauffman (2001) composition is defined as the aggregate of ingredients, their arrangement, and the integrated interrelationship that forms a unified, harmonious whole. The composition of meat can be approximated to 75% of water, 19.9% of protein, 3.5% of soluble, non-protein, substances and 2.5% of fat, but an understanding of the nature and behavior of meat, and of its variability, cannot be based on such a simplification. On contrary, it must be recognized that meat is the post-mortem aspect of a complicated biological tissue, and that the latter reflects the special features which the function of contraction requires, both in the general sense and in the relation to the type of action which each muscle has been elaborated to perform

in the body. The essential unit of muscular tissue is fiber which consists of formed protein elements, the myofibrils, between which is a solution, the sarcoplasm, and a fine network of tubules, the sarcoplasmic reticulum, the fiber being bounded by a very thin membrane (the sarcolemma) to which connective tissue is attached on the outside. The spatial distribution, between these structural elements, of the 19% of protein in the muscle. **(Lawrie and Ledward, 2006)**

2.3.1 Water:

Water is the major weight component, exactly the average of water is between (65-80%), it is also a part of the cell composition, it works as medium for transporting nutrients, outputs of metabolism, hormones and other chemicals such as minerals, organic substances that are soluble in water, and it's also involved in the preparation of meat colloids and emulsions during the processing. **(Uoda, 1984)**

2.3.2 Proteins:

Types of proteins (Actin + myosin, Tropomyosin, Troponin, Myoglobin, Enzymes, Collagen, Elastin) proteins are the most important components of muscles and meat, which is necessary to build the tissues of the body, as well as its effectiveness in interactions of bio-digestion and its role in protecting the fat from rancidity. Most of the protein in the meat is found within the muscle tissue and connective tissue and it appears in the form of fibers or semi-spherical or elongated particles according to the type of the protein. **(Fakhry, 1985)**

Meat is a major source of protein which helps to improve satiety and fills you up for longer. This makes protein-rich foods excellent for helping to control our weight so that we don't become overweight or obese. **(Red Meat and Health, 2016)**

2.3.3 Fat:

Muscle and meat contain different fat varieties, which contain natural fats (Glycerides and fatty acids) and its distribution differs in the muscle according to their location in the body. Fats are important in the cell composition, functions and the process of digestion. **(Fakhry, 1985)**

The fat content of red meat has been considerably reduced over the last few decades and the amount of fat in red meat is actually much lower than most people think. These reductions have been achieved by breeding techniques on the farm and new butchery techniques, which trim off most of the fat. Fully trimmed lean raw beef typically contains only 4.3% fat, fully trimmed lean raw pork only 4% fat and fully trimmed lean raw lamb only 8% fat. This compares well with a food such as cheddar cheese which contains an average of 34% fat. About half of the fat found in red meat is in the unsaturated form that is believed to be healthier. Surveys show that meat is a major contributor of mono-unsaturated fat in the diet. Choosing lean cuts of meat and trimming off any visible fat helps to reduce the saturated fat content further. **(Red Meat and Health, 2016)**

2.3.4 OMEGA-3 Fats:

Red meat contains very small amounts of omega-3 fats, which help to keep the heart healthy. With the exception of oil-rich fish, few foods contain good amounts of omega-3s. This makes the small amounts in red meat an important source, especially for people who eat little or no oily fish. **(Red Meat and Health, 2016)**

2.3.5 Sugars:

Muscles and meat in the animal's body are poor in sugars sources, but it has little amounts of starch in the muscles of animal (glycogen), and the average percentage of sugars between (0.5 - 1.5%), the proportion of glycogen in fresh liver between 2-8% of the weight, also in the heart muscle. Sugars play an important role in digestion, production of energy and the structure of muscle tissue. **(Fakhry, 1985)**

2.3.6 Minerals:

Muscle contains many inorganic compounds composed in cations and anions and physiological compounds such as calcium, magnesium, potassium, sodium, iron, phosphorus, sulfur, chlorine and other. The animal's body have a third of these elements, almost found in nature, the oxygen, ash, hydrogen and nitrogen constituents a ratio of up to 96% of the total animal weight. **(Fakhry, 1985)**

2.3.6.1 Iron:

Iron is a vital mineral for red blood cell formation. A deficiency of iron in the diet is the most common dietary cause of anemia. Certain groups of the population are at particular risk because of poor iron intakes. Currently a quarter of females aged 19-64 in the UK have iron intakes below the minimum amount to stay healthy. The type of iron found in red meat (haem iron) is more easily absorbed and used by the body than the iron in plant foods such as pulses, nuts, seeds and leafy green vegetables (non-haem iron). **(Red Meat and Health, 2016)**

2.3.6.2 Zinc:

Zinc is important for the healthy functioning of the immune system, growth, wound healing and fertility. Red meat is a source of readily absorbable zinc. We

get about 30% of our dietary intake of zinc from red meat and meat products. **(Red Meat and Health, 2016)**

2.3.6.3 Other minerals:

Red meat also provides other minerals such as potassium and for pork, selenium. Selenium is an important antioxidant, which has been linked to reducing the risk of heart disease and certain cancers. **(Red Meat and Health, 2016)**

2.3.7 Vitamins:

Vitamins found in meat: primarily vitamins B dissolved in water, and A, D, E, K soluble in fat, and sometimes vitamin C. **(Fakhry, 1985).**

Red meat is a source of a number of B vitamins: B3, niacin, B6 and B12 - a vitamin which is not found naturally in foods of plant origin and is important for healthy red blood cells, growth and the production of energy. It has also recently been found to make an important contribution to vitamin D intakes. Vitamin D works with calcium and phosphorous to build strong bones and teeth. **(Red Meat and Health, 2016)**

2.3.8 Enzymes

There are many groups of enzymes in meat necessary for nutrition, such as :(Lipase, Catalase, Diastase and Oxidase) the most important is Cathepsin and other enzymes. **(Uoda, 1984)**

2.4 Contamination of meat:

Food can be contaminated with pathogenic microorganisms. To evaluate the microbiological content of meat sixty samples of minced meat collected from various butcher shops and supermarkets in Istanbul were studied.

Following microbiological parameters were determined: total coliform, *Escherichia coli* and total viable counts and pathogenic microorganisms in addition to moulds and yeasts. Results indicated that aerobic plate count and the number of fecal coliforms such as *E.coli* and *S.aureus* were particularly high in almost all of the samples analyzed. It was found that the microbiological quality of all minced meat samples was inadequate and they exhibited high potential for food poisoning if consumed (**Erdem et al., 2014**)

Contamination of sterile animal muscle used as food is a direct consequence of slaughtering and dressing of animal carcasses. Wide ranges of microorganisms from different sources are introduced onto moist muscle surfaces that are rich in nutrients. It is argued that only a small portion (10%) of these microorganisms is capable of survival and proliferation during storage, distribution, and retail sales of meats. Additionally, an even a smaller portion will eventually predominate and cause spoilage. Survival and proliferation of microorganisms deposited on meat surfaces depends on their ability to withstand processing and storage conditions and to utilize available nutrients in the muscle through assimilation or proteolysis of complex molecules into readily utilizable substrates. The internal tissue of fresh meat does not contain any microorganisms except when the animal is infected by pathogens. Primary processing of livestock for red meat involves (**ICMSF, 2005**):

1. Stunning; (e.g. electrical / captive bolt / carbon dioxide gas);
2. Sticking (kill) usually by cutting the carotid arteries;
3. Bleeding;
4. Skinning (or scalding and dehairing for pigs/goats);
5. Evisceration (inspection of offal and corresponding carcass and head, edible offal's separated from other offal in a separate area of the abattoir);
6. Trimming and washing;
7. Air cooling of carcasses;

8. Grading/cutting and packaging.

2.4.1 Contamination from internal sources:

(A) Certain infectious diseases: that may infect animals such as tuberculosis and anthrax and septicemia, and others.

(B) Naturally occurring microbes: This is inside the animal body that may turn to be pathogenic because of the deterioration of the body immunity in some cases, such as heat stroke, hunger, stress and travel. **(Almorshidy, 1994)**

2.4.2 Contamination from external sources:

(A) The surface of the carcass: animal skin represents the main source of contamination of meat, especially in the winter, because his skin is very dirty, it contains huge numbers of soil bacteria (*Staphylococcus*) and many other types such as *E.coli*, *S.paratyphi*, and Coliform. The presence of these microbes on the surface of the carcass - which is usually transmitted during manual skinning - contributes in the contamination.

B) Equipment and machines: Knives used in the process of skinning play an important role in contamination.

C) Hands and workers: The folds of skin in the hands may contain Coliform, typhoid and dysentery.

(D) Air: Air can add bacteria or fungi to the surface of the meat and many types of pathogenic aerobic bacteria.

E) Flies and rodents: Flies and rodents have important role in the contamination of meat with different types of dangerous microbes; the flies have a mechanical role in transferring microbes from their locations to the meat and its products.

(F) Distribution and storage.

(G) Errors during the cavity: It is known that gastrointestinal tract of the animal, especially the stomach and intestines are full of pathogenic microbes, therefore any error during cavitations can add many microbes to meat, including *Escherichia coli* and *Staphylococcus*.

(H) The outer surface of the carcass touching the floor of the slaughter house or the skin: The floor of the slaughter house is often contaminated with all kinds of microbes so we must avoid subjecting the carcass to the floor of the slaughter house.

Each of these factors leads to the contamination by many types of bacteria, most important pathogenic Microorganisms are *Pseudomonas*, *Micrococcus*, molds and yeasts, *Staphylococcus*, *Flavobacterium*, *Achromobacter* and *Aerobacter*.
(Almorshidy, 1994)

2.4.3 Factors affecting the growth and proliferation of bacteria in the meat:

A) Temperature: at the higher temperature the rate of growth and reproduction of bacteria is faster.

(B) The degree of bleeding: all types of bacteria grow and multiply rapidly in poor bleeding meat.

(C) Primary number of bacteria: the shelf-life of meat will be longer when the primary number of bacteria is low and vice versa.

(D) The presence of oxygen: it is noted that the growth and reproduction of aerobic bacteria in the outside of the meat will increase whenever atmospheric air is

available, while the anaerobic bacteria are active and reproduce inside the meat because of the absence of oxygen.

(E) Moisture: it is the most important component for microbial life. Water is the solvent for most of the nutrients in the meat; this fact helps in preservation of meats by salting and drying. **(Almorshidy, 1994)**

2.4.4 Spoilage of meat:

Raw meat and poultry are highly perishable commodities subject to various types of spoilage depending on handling and storage conditions; because of this high potential for spoilage, the historical record reveals that early civilizations used techniques such as salting, smoking, and drying to preserve meat **(Sperber and Doyle, 2010)**. Today, more than ever, because of the globalization of the food supply, and increasing demands from exacting consumers, the control of meat and poultry spoilage is essential. Today consumers enjoy a variety of meat and poultry products. Each form or variety of meat and poultry has a specific range of shelf life primarily governed by its spoilage microflora.

Microbiological spoilage of meat and poultry is primarily due to the activity of psychrotrophic microbes that produce off-flavors, off-odors, and undesirable appearance at refrigeration temperatures. General factors that can mitigate against bacterial spoilage include **(Bailey, 1986)**:

- 1- Use of good sanitation practices during slaughter and processing to limit initial contamination;
- 2- Destroying or removing spoilage microorganisms;
- 3- Reducing the rate of growth of spoilage microorganisms by maintaining low temperature during processing, transportation, and storage; and

- 4- Knowing time–temperature response limitations for maintaining quality and turning product over within these limitations.

The spoilage of meat occurs if the meat is untreated, in a matter of hours or days and results in the meat becoming unappetizing, poisonous or infectious. Spoilage is caused by the practically unavoidable infection and subsequent decomposition of meat by bacteria and fungi, which are borne by the animal itself, by the people handling the meat, and by their implements. Meat can be kept edible for a much longer time though not indefinitely if proper hygiene is observed during production and processing, and if appropriate food safety, food preservation and food storage procedures are applied. (**Lawrie and Ledward, 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. (**Lawrie and Ledward, 2006**)

One source of infectious organisms is bacteraemia, the presence of bacteria in the blood of slaughtered animals. The large intestine of animals contains some 3.3×10^{13} viable bacteria, which may infect 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. (**Lawrie and Ledward, 2006**)

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 used for washing and cleaning, the dressing implements and the slaughterhouse personnel. (**Lawrie and Ledward, 2006**)

2.4.4.1 Symptoms of spoilage that depends on oxygen availability: (*Lawrie and Ledward, 2006*)

Aerobic bacteria

- Surface slime
- Discoloration
- Gas production
- Change in odor
- Fat decomposition

Yeasts

- Surface slime
- Discoloration
- Change in odor and taste
- Fat decomposition

Moulds

- Sticky and "whiskery" surface
- Discoloration
- Change in odor
- Fat decomposition

Anaerobic bacteria

- Putrefaction and foul odors
- Gas production
- Souring

2.4.5 Pathogenic bacteria in meat:

2.4.5.1 *Salmonella* spp.

Salmonella species are straight rods measuring 0.7 to 1.5 μm by 2 to 5 μm , motile by peritrichous flagella and have an optimum growth temperature of 37°C. The nomenclature of *Salmonella* has changed through the years and presently is comprised of only two species (*enterica* and *bongori*). *S. enterica* consists of six subspecies and each one contains multiple serotypes. Some *Salmonella* serotypes, *dublin* and *typhimurium* affect cattle and some, *choleraesuis* and *typhimurium* affect pigs and others, *pullorum* and *gallinarum* affect poultry. *Salmonella enterica* subsp. *enterica* serotype *typhi* and *paratyphi* A or B are human specific and can cause typhoid fever. The genus *Salmonella* is composed of more than 2300 serotypes. The main antigens used to distinguish between its serotypes are the somatic (O), flagellar (H), and capsular (K). *Salmonella* spp. has a wide occurrence in the natural environment. Intense husbandry practices in the meat, poultry, fish and shellfish industry, along with the recycling of offal into animal feed have favored the presence of this pathogen in the global food chain. This pathogen is a part of the microflora of many animals like chicken, cattle and reptiles. The predominance of *Salmonella* spp. in the poultry and egg industry has overshadowed its importance in meat, such as pork, beef and mutton. (Downes and Ito, 2001)

2.4.5.2 *Staphylococcus*:

Staphylococci are facultative anaerobic, Gram positive cocci. They ferment glucose, and are Catalase-positive, Oxidase negative and arginine-positive. These organisms are part of the normal flora of mammalian skin and mucous membranes. Some species are opportunistic pathogens, and can occur in high numbers in skin

lesions or the oro-nasal discharges from infected individuals. Enterotoxins produced during the growth of *S. aureus* and some other species in prepared foods are a major cause of food poisoning. The staphylococci characteristically divide in more than one plane, to appear under the microscope as tetrads and irregular clusters as well as single or paired cells. Being mesophiles, they do not contribute to the spoilage flora of chilled meat. **(GILL and GREER, 1993)**

Its cells measure 0.5 to 1.5 μm in diameter. This organism's optimum growth temperature is 30 to 37 C and is one of the hardiest nonspore forming bacteria that can survive extended periods of time on dry and inanimate objects. This organism is relatively heat resistant which allows it to survive in just about every environment in which humans coexist. The genus *Staphylococcus* is divided into 23 species and subspecies (*arlettae*, *aureus*, *auricularis*, *capitis*, *chromogenes*, *cohnii*, *epidermidis*, *gallinarum*, *haemolyticus*, *hominis*, *hyicus*, *intermedius*, *kloosii*, *lentus*, *lugdunensis*, *saprophyticus*, *schleiferi*, *sciuri*, *simulans*, *warneri*, and *xylosus*). The most ubiquitous of these species is *Staphylococcus epidermidis* which is found on the skin of humans and animals and rarely causes disease. The growth of *Staphylococcus aureus* in foods is a potential public safety hazard since many of its strains produce enterotoxins that cause food poisoning when ingested (Staphylococcal food poisoning). **(Downes and Ito, 2001)**

Staphylococcus intermedius and *hyicus* have also been shown to produce enterotoxins in food. *Staphylococcus saprophyticus* is known only to cause urinary tract infections. The coagulase test is used to differentiate *Staphylococcus aureus* from the other species since it is the only one to produce the coagulase enzyme. Humans are the main reservoirs of staphylococci involved in human disease, especially *Staphylococcus aureus*, the other species are considered to be normal inhabitants of the external parts of the body. *Staphylococcus aureus* usually colonize the external nostrils and are found in about 30% of healthy individuals.

This species also can be found on the skin, oropharynx and feces of humans and animals. *Staphylococcus aureus* can be isolated from fomites produced by food processing such as meat grinders, knives, saw blades and cutting boards or tables.

(Downes and Ito, 2001)

Staphylococcal food poisoning (SFP) is the fourth most common cause of food poisoning when ranked according to outbreaks and the most common when ranked according to the number of affected individuals. SFP is the classic example of short incubation time food poisoning cause by a toxin produced in food. Staphylococci produce many metabolites, but the enterotoxins pose the greatest risk to consumer health.

(Downes and Ito, 2001)

Staphylococcus aureus produces five distinct enterotoxins (type A through E) which are single polypeptide proteins with a molecular size from 22 to 28 kDa. The growth of 50 *Staphylococcus aureus* in foods may lead to the production of sufficient Enterotoxins which may cause illness when these contaminated foods are consumed. These toxins cause disease even in the absence of the organism. In the majority of cases SFP is associated with food being contaminated by the food handler who might have a minor *Staphylococcus aureus* infection such as a boil or cut. The contaminated food must be permitted to sit at an adequate temperature that will allow the Staphylococci to multiply and produce the toxin. Reheating the food before eating may kill the organism but does not eliminate the heat stable toxin. Some of the foods commonly linked with SFP are meat (beef, pork and poultry), meat products (sausages, hotdogs, ham), salads ham, chicken, potato), cream filled baked products and dairy products. **(Downes and Ito, 2001)**

2.4.5.3 *Escherichia coli*

Escherichia coli are straight rod measuring 1.1 to 1.5 µm by 2.0 to 6.0 µm which occur singly or in pairs and has an optimum growth temperature of 37 C.

Capsules or microcapsules occur in many strains and some strains are motile by peritrichous flagella. *Escherichia coli* are part of the normal flora of the intestinal tract of humans and various animals. It can be classified as an overt or an opportunistic pathogen and usually constitutes about 1% of the total biomass of feces. Most *Escherichia coli* do not cause gastrointestinal illnesses, but some can cause life threatening diarrhea and chronic sequelae or disability. *E. coli* is serologically classified on the basis of three major surface antigens: O (somatic), H (flagella) and K (capsule). The serogroup of the strain is identified by the O antigen and its combination with the H antigen identifies the serotype. There are more than 170 different serogroups of *E. coli* identified. Diarrhea causing *E. coli* isolates are classified into specific groups based on virulence properties, pathogenicity mechanisms, clinical syndromes, and specific O:H serotypes. These groups include enterotoxogenic *E. coli* (ETEC), enteroaggregative *E. coli* (EAEC), enteropathogenic *E. coli* (EPEC), enterohemorrhagic *E. coli* (EHEC), enteroinvasive *E. coli* (EIEC), and diffuse-adhering *E. coli* (DAEC). **(Downes and Ito, 2001)**

No recently described enteric pathogen has received as much scientific and medical examination as *Escherichia coli* O157:H7. There are many serotypes belonging to the EHEC group, but serotype O157:H7 is the predominant food pathogen. It was first recognized as a pathogen in 1982 when it was identified as the causative agent of two outbreaks involving the consumption of undercooked ground beef leading to hemorrhagic colitis. It is estimated that more than 700 individuals were affected and 4 deaths occurred between 1992 and 1993 due to this pathogen. According to CDC estimates there are more than 20,000 cases and 250 deaths annually in the U.S. due to *Escherichia coli* O157:H7 infections. Cattle are considered to be the main reservoirs of this pathogen and many outbreaks are

associated with the consumption of undercooked ground beef and unpasteurized milk. **(Downes, F. P. and Ito, K., 2001)**

2.5 Moulds and yeasts:

2.5.1 Moulds:

Moulds are microscopic plants. These plants consist of long filaments, interwoven in a complex web-like structure that gives them their characteristic (hairy or fuzzy) appearance. Moulds are composed of cells which together form the filaments, or hyphae. Hyphae collectively form the mycelium which is the main body of the mould. Moulds may be seen in many forms including the black growth on chiller walls, the grey / white fur-like deposit on bread and the green hairy growth on damp bags of meat, just to name a few. **(Husband, 1992)**

Reproduction is via spores (not to be confused with the spores associated with heat-resistant bacteria). Mould spores are like seeds that are easily windborne and can be carried over quite large distances on air currents. Moulds need some moisture to grow, however they can survive in seemingly quite dry environments. They grow best at room temperature but they will continue to grow at quite low temperature and some have even been known to grow on meat at 12°C. Moulds are very susceptible to high temperature however, and they are easily killed by hot water. Fluctuating ambient temperature and high relative humidity can cause products, such as meat meal, to become damp or sweaty, and such conditions provide an ideal environment for the growth of moulds. The problem is accelerated if the meat meal has too high a moisture content in the first place. **(Husband, 1992)**

2.5.1.1 Effect of mould growth

Mould growth is unsightly and can cause off odours. Also, mould may have a negative influence on the palatability of meat meal as stock feed.

Mould may also cause more serious problems. *Aspergillus*, a type of mould, can produce small amounts of aflatoxin, a poisonous compound known to be a powerful carcinogen. Even a very small quantity of this material is considered highly dangerous and, whilst it has been a problem mostly with peanuts, it is known to grow on meat and bone meal. **(Husband, 1992)**

2.5.1.2 Mould contamination:

Because moulds are easily transported around by wind currents and moisture, and because warm moist conditions are ideal for their growth, from the rendering process itself, together with the condensate that is commonly generated in several parts of the plant, provide ideal conditions for mould growth. Low-level mould contamination is impossible to avoid in the rendering plant because it is so easy for the spores to be transferred by wind currents, dust and other indirect means. **(Husband, 1992)**

Steps can be taken however to keep the number at a manageable level. Also, if the production process is properly managed such that meat meal with sufficiently low moisture content is produced, mould growth in meat meal will not be a problem. **(Husband, 1992)**

2.5.2 Yeasts:

Yeasts are unlike moulds in that they do not grow as plant-like structure. Moulds are unique in this regard in the world of micro-organisms. Yeasts do,

however, exist as cells as do all micro-organisms. Yeasts multiply by a process called budding. A small lump, or bud, appears on the side of the cell. This bud grows until it approaches the parent cell in size, at which time it separates and becomes a yeast cell in its own right. **(Husband, 1992)**

2.5.2.1 Effect of yeast growth:

Yeasts need the complete triangle of moisture, warmth and nutrition in order to grow, but they need more moisture than moulds. They are, however similar in terms of their lack of heat tolerance. They grow best at normal ambient temperatures and they are easily killed by hot water. In general terms, yeasts are more useful than otherwise. For instance, product such as bread and beer would not exist without yeasts. Yeasts do not cause serious problems in meat meal. If conditions in the plant or in the product are such that yeasts are able to grow, then it is likely that moulds are going to be of far greater concern than yeasts themselves. **(Husband, 1992)**

CHAPTER THREE

MATERIALS & METHODS

3.1 Materials:

3.1.1. Used media:

- Plate Count Agar
- Nutrient Agar
- Potato – Dextrose Agar
- MacConkey Broth
- Brilliant Green 2% Bile Broth
- EC Broth
- Eosin Methylene Blue Agar
- Selenite Cystine Broth
- Bismuth Sulphite Agar
- Triple Sugar Iron Agar / Mannitol Salt Agar (Substituted)
- Baird-Parker Agar
- Ceftrimide Fucidin Cephaloridine Agar (CFC)

3.1.2. Used diluent:

- 0.1% Peptone Solution
- **3.1.3. Meat samples:**

Twenty seven samples of fresh red meat were collected from different locations in Khartoum State. These samples were from close, semi-closed and open butcher shops.

3.2. Methods:

3.2.1. Sterilization:

3.2.1.1. Sterilization of glassware:

Petri dishes, test tubes, flasks, pipettes...etc., were sterilized in hot air oven at 160 – 180° C for 2 to 3 hours before they were put in the oven they were washed dried and packed in stainless steel cans or sometimes in aluminum foil

3.2.1.2. Sterilization of media:

Culture media were first adjusted to the required pH and then sterilized.

Sterilization was achieved by autoclaving at 121.5° C for 15 minutes.

3.2.2. Total viable count of bacteria:

It was carried out by using the pour plate count method as described by W. F. Harrigan (1998). Suitable medium for this purpose is Plate Count Agar

3.2.3. Preparation of serial dilutions:

Aseptically 10 grams of the sample were homogenized in 90 ml of sterile diluent (0.1% Peptone water). It was mixed well to give dilution (10^{-1}) by using sterile pipette 1 ml was transferred aseptically from dilution (10^{-1}) to a test tube containing 1 ml of sterile diluent (10^{-2}). In the same way the preparation of serial dilution was continued until the dilution (10^{-6}). One ml of each dilution was transferred into sterile petri dish, and then 15 ml of sterile melted Plate Count Agar medium were added to each plate. The inoculum was mixed with medium and allowed to solidify.

The plates were incubated at 37° C for 48 hours. A colony counter was used to count the viable bacterial colonies after incubation and the results were expressed as colony-forming units (CFU) per gram

3.2.4. Tests

3.2.4.1 Determination of Coliform bacteria:

It was carried out by using the Most Probable Number (MPN) technique.

3.2.4.2 Presumptive Coliform test:

1 ml of each of the three first dilutions (10^{-1} , 10^{-2} , 10^{-3}) was inoculated in triplicates of MacConkey Broth test tubes containing Durham tubes. The tubes were incubated at 37° C for 48 hours. The production of acid together with sufficient gas to fill the concave of the Durham tube is recorded as positive presumptive test.

3.2.4.3 Confirmed test for Total Coliforms:

From every tube showing positive result a tube of Brilliant Green 2% Bile Broth was inoculated by using a sterile loop. The tubes were inoculated at 37°C for 48 hours, and then the tubes showing positive and negative result were recorded. The Most Probable Number (MPN) of total coliform was found out by using the Most Probable Number (MPN) tables.

3.2.4.4 Confirmed E. coli test:

Medium used was EC Broth. From every tube showing positive result in the presumptive test inoculate a tube of EC Broth containing Durham tube were inoculated at 44.5° C for 25 hours. Tubes showing any amount of gas were considered positive, and then the Most Probable Number (MPN) was recorded. For

further confirmation of *E. coli* tubes of EC Broth showing positive results at 44.5° for 24 hours were streaked on Eosin Methylene Blue Agar (EMB) plates. The plates were incubated at 37° C for 48 hours. Colonies of *E.coli* are usually small with metallic green sheen on EMB Agar.

3.2.4.5 *Staphylococcus aureus* enumeration:

Medium used was Baird-Parker Agar; 0.1 ml from every dilution was transferred onto the surface of each well dried Baird-Parker Agar medium plates. The inoculum was spreaded all over the plate using sterile bent glass rod. The plates were incubated at 37° C for 24 hours, after that period of incubation the plates were examined. *Staphylococcus aureus* appear black shiny convex and surrounded by a zone clearing 2-5 mm in width of colony after 24 hours of incubation

3.2.5 Yeasts and Moulds:

From suitable dilutions of sample 0.1 ml was aseptically transferred onto solidified Potato-Dextrose Agar containing 0.1 gram chloramphenicol per one litre of medium to inhibit bacterial growth. The sample was spread all over the plates using sterile bent glass rod. Plates were incubated at 28° C for 72 hours. Colonies were counted using a colony counter and the result were presented as CFU/gram

3.2.6. Detection of *Salmonella*:

Ten gram of the sample were added to a conical flask containing 100 ml of sterile Nutrient Broth and incubated at 37° C for 24 hours. A loopful of 24 hours incubated Nutrient Broth was transferred to aseptically into sterilized Selenite Cystine Broth and incubated at 37° C for 24 hours. A loopful of 24 hours inoculum of Selenite Cysteine Broth was streak on Bismuth Sulphite Agar surface and

incubated at 37° C for 24 – 72 hours. Black metallic sheen discrete colonies indicated the presence of *Salmonella*.

CHAPTER FOUR

RESULT S AND DISCUSSION

4.1 Total viable count:

As shown in table 1, the total viable count of bacteria of meat samples collected from different sources in Khartoum State recorded 5.83, 4.55 and 4.70 (log cfu) for closed butcher shops in Omdurman, Khartoum and Khartoum North, respectively. The total viable count increased significantly in semi-closed and open butcher shops ($p < 0.05$) to 6.91, 4.96 and 5.83 and 9.60, 6.93 and 7.92, respectively due to lack of sanitary condition, exposure and poor hygienic and handling practices. Total viable counts of bacteria in samples of Khartoum and Khartoum North closed butcher shops decreased significantly ($p < 0.05$). Variation within the same type of butcher shops is due to the location of the butcher shops itself and the distance from street and other sources of contamination. Closed butcher shops in Khartoum locality proved to be the best source due to proper display and good handling practices. Erdem *et al.* (2014) reported that total viable count of bacteria of fresh meat ranged between 4.43 and 8.30 (log cfu) which is similar to the result obtained in this study.

4.2 Moulds and Yeasts:

Table 2 shows that moulds and yeasts were not detected in samples from closed butcher shops in Khartoum State which indicates good sanitary conditions. Moulds and yeasts in semi-closed butchers were only detected in Omdurman (2.53 log cfu). Open butcher shops samples recorded 2.90, 2.72 and 2.84 (log cfu) in Omdurman, Khartoum and Khartoum North, respectively. The results were significantly different ($p < 0.05$) and indicates that Khartoum was the best compared

to Omdurman and Khartoum North. Aslam *et al.* (2000) reported that moulds and yeasts count of raw minced beef ranged between 3.85 and 4.50 (log cfu). These results were in disagreement with the results obtained in this study due to large number of samples subjected to study in the former. Moreover, minced meat offers ample, desirable surfaces and thorough inoculation during grinding according to Banwart (1987).

4.3 *Staphylococcus aureus*:

Table 3 shows *Staphylococcus aureus* count of meat from closed, semi-closed and open butcher shops in Omdurman Khartoum and Khartoum North. Omdurman and Khartoum North samples recorded 2.59 and 2.52 (log cfu) respectively, that of Khartoum was significantly different ($p < 0.05$) and showed no contamination with *Staphylococcus aureus*, which indicates good handling and hygienic practices. However, these counts increased significantly ($p < 0.05$) in the samples from semi-closed and open butcher shops which indicates poor hygienic and handling practices. Results of semi-closed butcher shops in Khartoum North were significantly different ($p < 0.05$) compared to that of Omdurman. Open butcher shops samples showed higher contamination due to the exposure to garbage and contaminants surrounding and scattering near the shop. The results of this study were in disagreement with the findings of Erdem *et al.* (2014) who found that *Staphylococcus aureus* count of fresh meat ranging between 2.81 and 6.57 (log cfu). This variation may be due to huge number of samples subjected to study.

4.4 Total Coliform bacteria:

Table 4 shows the total coliform count in fresh meat samples collected from closed, semi-closed and open butcher shops in Omdurman, Khartoum and Khartoum North. Samples from closed butcher shops in Omdurman recorded 5.33

(MPN/g), that of Khartoum and Khartoum North showed no contamination with coliform bacteria. Samples from semi-closed butchers recorded 28.0, 12.0 and 19.0 (MPN/g) in Omdurman, Khartoum and Khartoum North respectively. The total coliform count decreased significantly ($p < 0.05$) in samples from Khartoum and Khartoum North. Samples from open butcher shops recorded 81.33, 21.00 and 36.67 in Omdurman, Khartoum and Khartoum North. There was a significant difference ($p < 0.05$) between samples from Omdurman open butcher and that of Khartoum and Khartoum North. Samples from closed butchers in Khartoum and Khartoum North proved to be the best sources. These results disagreed with the findings of Aslam (2000) who reported that most probable number of samples ranged from 39 to 2400.

4.5 *Escherichia coli*:

As shown in table 5, *E.coli* was not detected in samples from closed butcher shops which indicate good sanitary conditions. Samples from semi-closed butcher shops of Omdurman and Khartoum did not produce *E. coli*, unlike that of Khartoum North which recorded 4.00 (MPN/g). Samples of fresh meat from open butcher shops recorded 21.33, 7.33 and 13.00 in Omdurman Khartoum and Khartoum North. Counts decreased significantly ($p < 0.05$) in Khartoum and Khartoum North. Results of this study were in disagreement with the findings of Aslam (2000) who found that most probable number of meat samples ranged between 10 – 1100.

4.6 *Salmonella*:

Results of *Salmonella* detection in meat samples are presented in Table 6. Samples from closed butcher shops showed negative results which indicate proper handling and hygienic practices. Samples from semi-closed butchers showed

negative results as well, except for one sample from Omdurman. Open butcher shops showed only one negative result in Khartoum, this high contamination level may be due to exposure to the contaminated environment surrounding the shop, the work-place equipments and workers might be considered. Results of this study were in agreement with the findings of Hassanein *et al.* (2011) who found that *Salmonella* was found in 5 out of 27 samples of minced beef.

Table (1): Total viable count of bacteria ($\log_{10}$ cfu/g) of meat collected from different locations in Khartoum State

Source of meat	Closed system	Semi-closed system	Open system
Omdurman	5.83 ^a ±0.03	6.91 ^a ±0.01	8.60 ^a ±0.06
Khartoum	4.55 ^c ±0.06	4.96 ^c ±0.03	6.93 ^c ±0.04
Khartoum North	4.70 ^b ±0.03	5.83 ^b ±0.03	7.92 ^b ±0.02
Lsd_{0.05}	0.0894*	0.0632*	0.0894*
SE±	0.02582	0.0126	0.0258

Values are mean±SD.

Mean(s) bearing different superscript(s) in a column and rows are significantly different ($P \leq 0.05$) according to DMRT.

Table (2): Total count of yeasts and moulds ($\log_{10}$ cfu/g) of meat collected from different locations in Khartoum State

Source of meat	Closed system	Semi-closed system	Open system
Omdurman	0.00 ^a ±0.00	2.53 ^a ±0.04	2.90 ^a ±0.07
Khartoum	0.00 ^a ±0.00	0.00 ^b ±0.00	2.72 ^b ±0.03
Khartoum North	0.00 ^a ±0.00	0.00 ^b ±0.00	2.84 ^{ab} ±0.05
Lsd_{0.05}	0.526 ^{NS}	0.0632 [*]	0.1672 [*]
SE±	0.081	0.0126	0.0483

Values are mean±SD.

Mean(s) bearing different superscript(s) in a column are significantly different ($P \leq 0.05$) according to DMRT.

Table (3): *Staphylococcus aureus* count ($\log_{10}$ cfu/g) of meat collected from different locations in Khartoum State

Source of meat	Closed system	Semi-closed system	Open system
Omdurman	2.59 ^a ±0.01	2.90 ^a ±0.06	3.97 ^a ±0.05
Khartoum	0.00 ^b ±0.00	2.78 ^{ab} ±0.04	3.75 ^c ±0.03
Khartoum North	2.52 ^a ±0.03	2.69 ^b ±0.02	3.89 ^b ±0.04
Lsd_{0.05}	0.1548*	0.1413*	0.0632*
SE±	0.0447	0.0408	0.0183

Values are mean±SD.

Mean(s) bearing different superscript(s) in a column are significantly different ($P \leq 0.05$) according to DMRT.

Table (4): Total coliforms (MPN/g) of meat collected from different locations in Khartoum State

Source of meat	Closed system	Semi-closed system	Open system
Omdurman	5.33 ^a ±0.28	28.00 ^a ±1.00	81.33 ^a ±1.85
Khartoum	0.00 ^b ±0.00	12.00 ^c ±0.76	21.00 ^c ±1.03
Khartoum North	0.00 ^b ±0.00	19.00 ^b ±0.85	36.67 ^b ±0.53
Lsd_{0.05}	2.401 [*]	2.825 ^{**}	13.92 ^{**}
SE±	0.0694	0.8165	4.023

Values are mean±SD.

Mean(s) bearing different superscript(s) in a column are significantly different ($P \leq 0.05$) according to DMRT.

Table (5): *E. coli* (MPN/g) of meat collected from different locations in Khartoum State

Source of meat	Closed system	Semi-closed system	Open system
Omdurman	0.00 ^a ±0.00	0.00 ^b ±0.00	21.33 ^a ±1.54
Khartoum	0.00 ^a ±0.00	0.00 ^b ±0.00	7.33 ^c ±0.24
Khartoum North	0.00 ^a ±0.00	4.00 ^a ±0.73	13.00 ^b ±1.19
Lsd_{0.05}	0.526 ^{NS}	1.998 [*]	2.746 ^{**}
SE±	0.081	0.5774	0.7935

Values are mean±SD.

Mean(s) bearing different superscript(s) in a column are significantly different ($P \leq 0.05$) according to DMRT.

Table (6): Detection of *Salmonella* of meat collected from different locations in Khartoum State

Sample	Closed system	Semi-closed system	Open system
Omdurman	-	-	+
	-	+	+
	-	-	+
Khartoum	-	-	+
	-	-	-
	-	-	+
Khartoum North	-	-	+
	-	-	+
	-	-	+

CHAPTER FIVE

CONCLUSIONS & RECOMMENDATIONS

5.1 Conclusions:

From the results obtained in this study it can be concluded that:

- 1- The type of butcher shops from which the samples were collected have a major influence in the levels of contamination with different microorganisms in which pathogenic microorganisms are among them.
- 2- It was clearly obvious that this contamination was also influenced by the improper handling practices and the absence of sanitary conditions.
- 3- Highest contamination levels were obtained in the samples from open butcher shops while lowest levels were in the samples from closed butcher shops.
- 4- Khartoum butchers proved to be the best source of fresh meat

5.2 Recommendations:

- 1- Corrective measures for reducing risks associated in butcher shops should be identified and addressed.
- 2- The relevant authorities should develop minimum guidelines on basic hygienic practices in Khartoum State and ensure enforcement.
- 3- Short and long action plan should be in place regarding the restructure of butcher shops to enable proper hygienic practices with efficient monitoring.
- 4- Further studies are needed.

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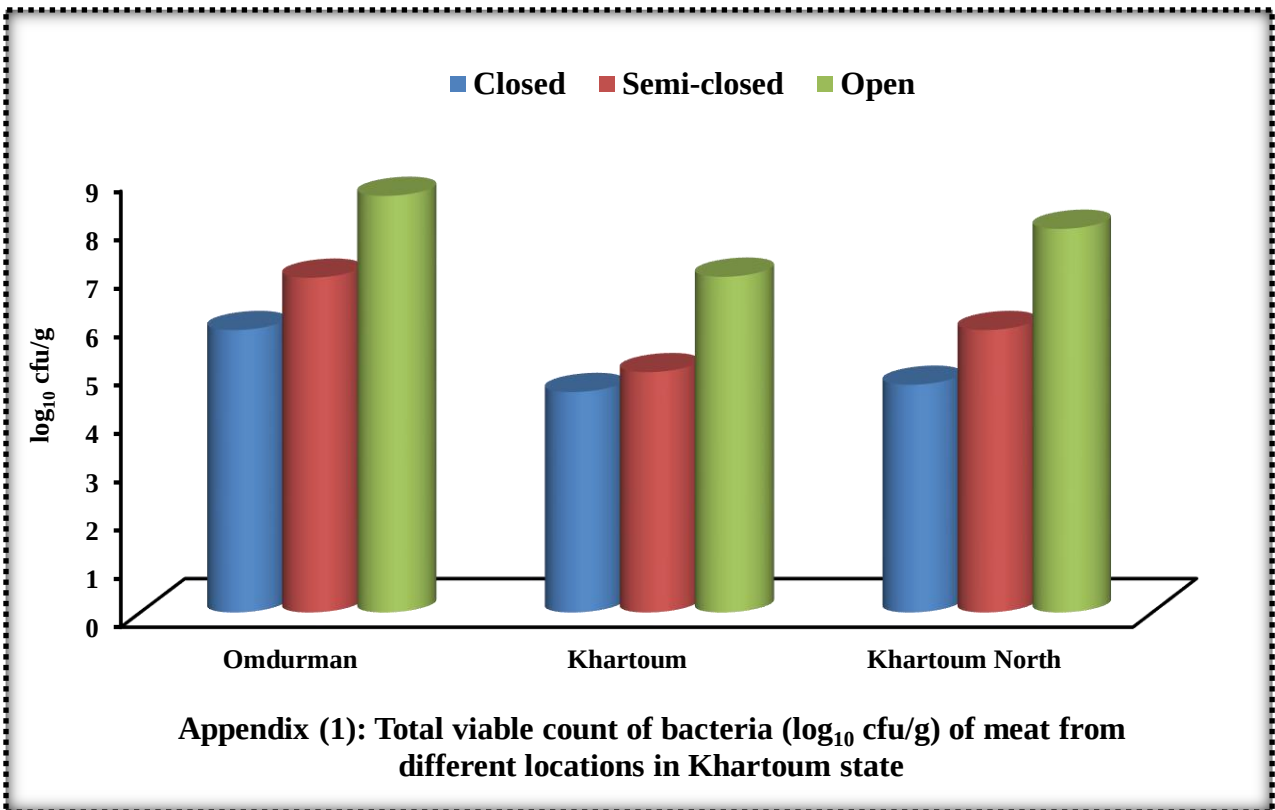
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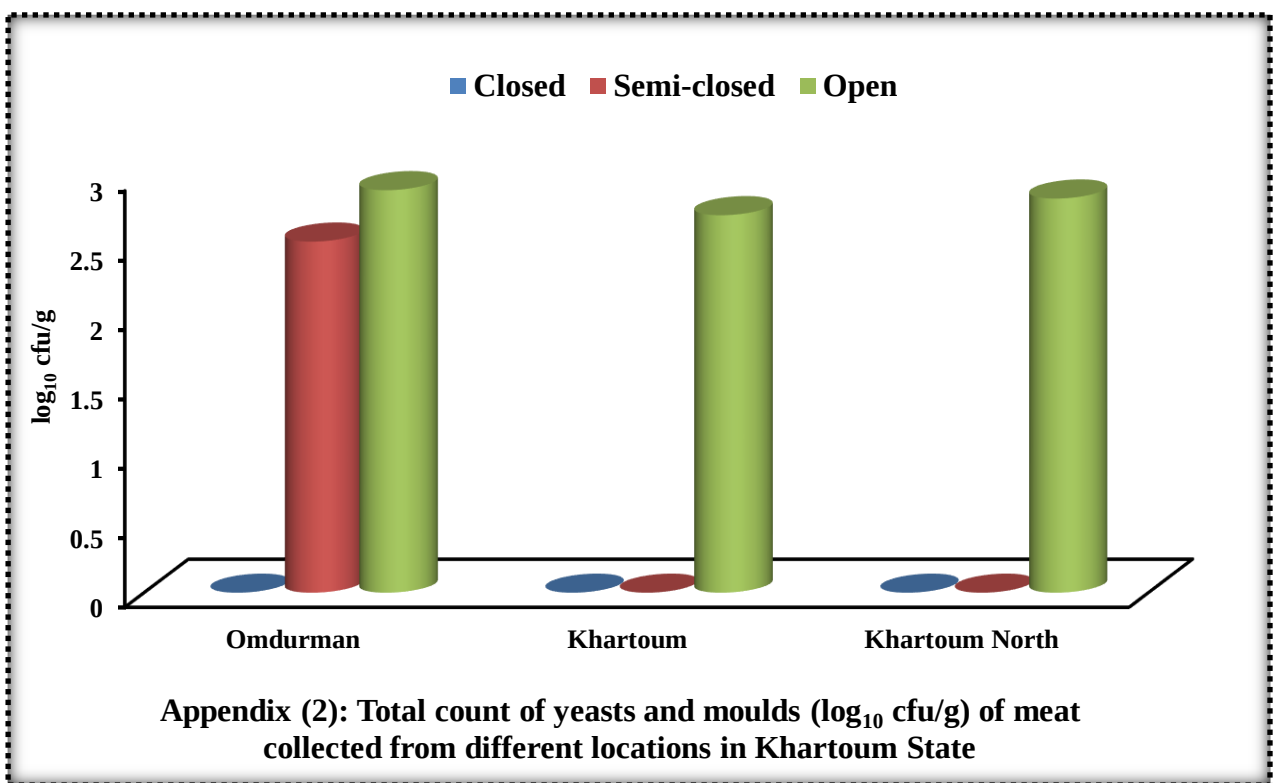
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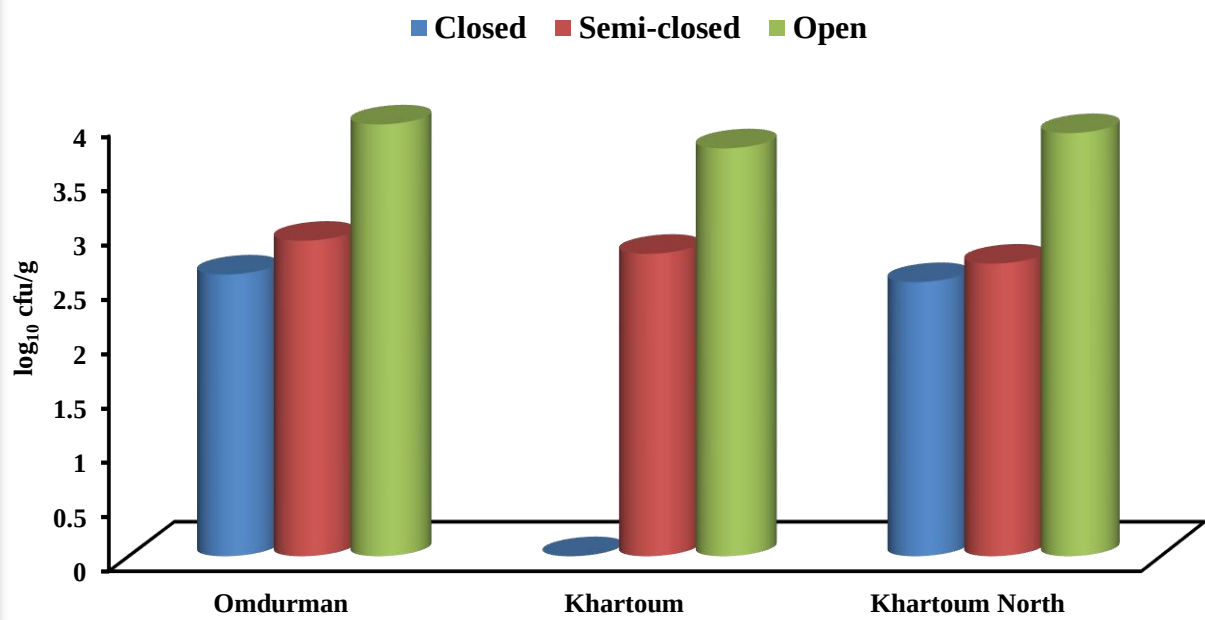
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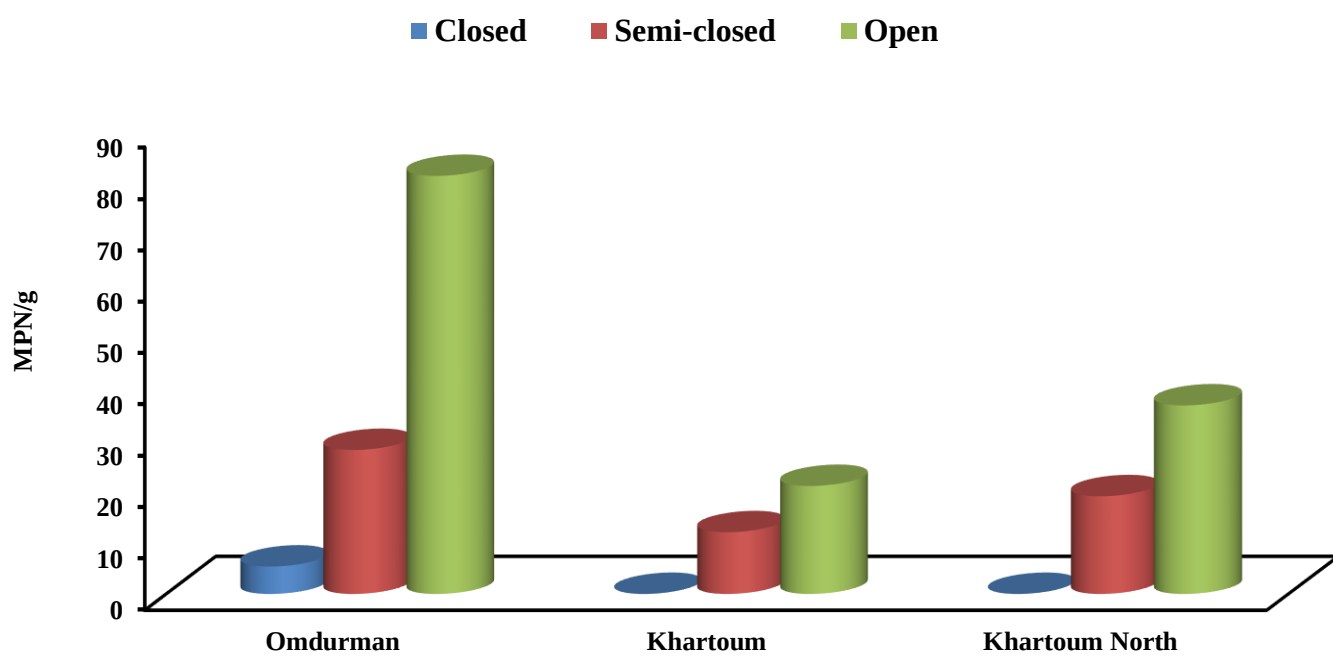
APPENDICES



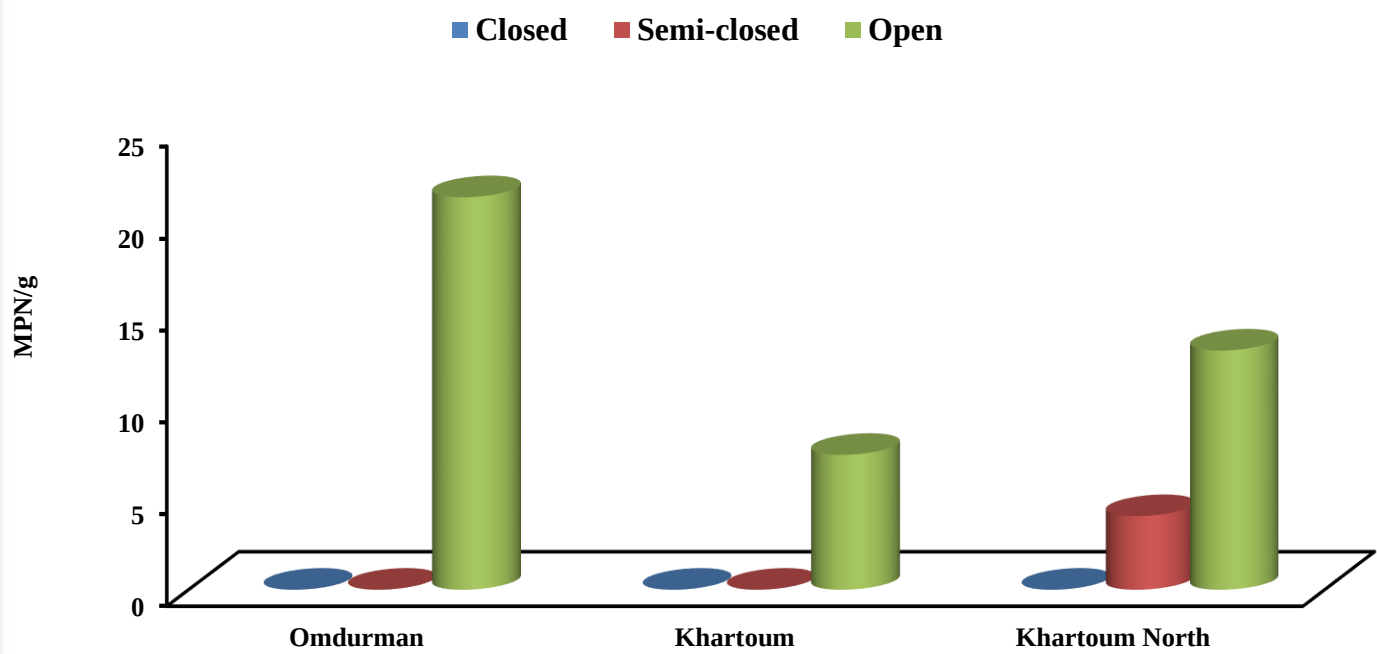




Appnedix (3): *Staphylococcus aureus* count (log₁₀ cfu/g) of meat collected from different locations in Khartoum State



Appendix (4): Total coliforms (MPN/g) of meat collected from different locations in Khartoum State



Appendix (5): *E. coli* (MPN/g) of meat collected from different locations in Khartoum State



Appendix (6): Closed butcher shop.



Appendix (7): Semi-closed butcher shop.



Appendix (8): Open butcher shop.