



**Contamination of Ready to Eat Vended Food of  
Meat Origin with Aerobic Bacteria  
in Khartoum State**

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## DEDICATION

*To Soul of my mother  
To sincerely my Father,  
To my sister and brothers  
To my friends  
For their tremendous support encouragement and patience.*

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## ABSTRACT

This study was carried out to isolate and identify Gram- positive and Gram-negative bacteria which contaminate food of meat origin.

Ninety samples of cooked, ready-to-eat Sandwiches (Beef-Burger, Shawarma, Kebab and Agashi) collected from different street food venders, samples were cultured on MacConkey's, nutrient and blood agar. Isolates, were purified and identified to species level according to their morphological and biochemical properties. A total of 76 isolates were obtained, 50 of them were Gram-negative bacteria which identified: *Klebsiella pneumoniae* (22%), followed by *Enterobacter spp* (15%), *Serratia marcescens* (11%), *Hafnia alvei* (7%), *Campylobacter spp* (2%), *Citrobacter freundii* (2%) and *Pseudomonas aeruginosa* and *Proteus vulgaris* (1%). 26 isolates were Gram-positive bacteria which identified: *Staphylococcus spp* (11%), *Bacillus cereus* (6%), *Listeria monocytogenes* (3%), *Bacillus mycoides* (2%), *Brochothrix spp* (2%), *Kurthia gibsonii* (2%), *Enterococcus spp* (2%) and *Micrococcus spp* (1%).

The study revealed that foods of meat origin are often contaminated with bacteria. The presence of *Klebsiella pneumoniae*, *Enterobacter cloacae*, *Campylobacter spp*, *Staphylococcus spp*, *Bacillus cereus* and *Listeria monocytogenes* was of medical significance. The presence of these Organisms in meat foods should receive particular attention, because their presence indicate public Health hazard and gives a warning signal for the possible occurrence of food borne diseases.

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## INTRODUCTION

The street food industry plays an important role in meeting the food requirements of urban dwellers in many cities and towns of developing countries. The industry feeds millions of people daily with a wide variety of foods that are relatively cheap and easily accessible. However, food-borne diseases of microbial origin are a major health problem associated with street foods (WHO., 2002). The traditional processing methods that are used in preparation, inappropriate holding temperatures and poor personal hygiene of food handlers are some of the main causes of contamination of street-vended food. Consumers who depend on such food are more interested in its convenience and usually pay little attention to its safety, quality, and hygiene (Mensah *et al.*, 2002; Muinde and Kuria, 2005 and Barro *et al.*, 2006). Street foods are frequently associated with diarrhoeal diseases which occur due to improper use of additives, the presence of pathogenic bacteria, environmental contaminants and disregard of good manufacturing practices (GMPs) and good hygiene practices (GHPs). Vendors are often poorly educated, unlicensed, untrained in food hygiene, and they work under crude unsanitary conditions with little or no knowledge about the causes of food-borne disease (Barro *et al.*, 2007). Most of foods are not well protected from flies, which may carry food borne pathogens. Safe food storage temperatures are rarely applied to street foods. Potential health risks are associated with contamination of food by *E.coli*, *Salmonella typhi*, *Pseudomonas sp.*, *Staphylococcus aureus* or *Proteus sp* during preparation, post cooking and other

handling stages (Hanoshiro, et al., 2004 and Ghosh, *et al.*, 2007). Even though, people are aware that food-borne diseases could occur due to consumption of street foods, the majority disregards these health hazards (Bryan, 1998). Human food-poisoning is commonly associated with bacteria originating from animal sources; in most cases, infection is contracted indirectly by eating contaminated meat and meat products (Report, 1970). Such contamination may occur within the slaughterhouse (Walton, 1970) or in processing and handling before sale (Foster, 1972; Casman, McCoy and Brandly, 1963; Timoney, Kelly, Hannan and Reeves, 1970; Gilbert, 1969; Gilbert & Watson, 1971). The high incidence of bacterial food-poisoning in man indicates that it is necessary to prevent contamination of meat and meat products in the food industry (Morisetti, 1971). Sliced cooked meats are important vehicles of bacterial food poisoning. Contamination of these foods may be associated with inadequate processing, or they may be contaminated after cooking from a source such as raw meat, the hands of personnel, or dirty equipment and work surfaces. One important vehicle appears to be the blades of food-slicing machines, which can spread bacteria from one slice of meat to the next (Gilbert, 1969; Bassett and Kurtz and Moore, 1978). Wiping cloths are also important reservoirs of bacteria for contamination of hands, equipment and surfaces (Davis, Blake and Woodall, 1968 and Tebbutt, 1984). In large establishments separate areas and personnel can be allocated to the preparation of raw and cooked meats. In small shops, unless an effective code of practice is used, frequent transfer by staff between raw and cooked food areas increases the risk of

cross-contamination. Poor personal hygiene, improper cleaning of storage and preparation areas and unclean utensils cause contamination of raw and cooked foods. Mishandling of raw and cooked foods allow bacteria to grow. The temperature range in which most bacteria grow is between 5 and 60 degrees C. Raw and cooked foods should not be kept in this danger zone any longer than absolutely necessary.

### **Objectives**

The objective of this work was to isolate and identify Gram- positive and Gram-negative bacteria from ready to eat vended food of meat origin in Khartoum State.

# **CHAPTER ONE**

## **LITERATURE REVIEW**

### **1.1 Sources of Meat Contamination**

Meat is considered to be spoiled when it is unsuitable for human consumption. Spoilage can be caused by a wide variety of factors, such as improper handling, exposure to air and high temperature, or conditions that trigger chemical reactions or microbial contamination, although the most common cause is the presence of microorganisms together with metabolite production. Spoiled meats and meat products are inedible mainly due to off-odor and flavor, but consumer rejection is also due to discoloration, blown packages, souring, surface slime, and other alterations of meat quality. However, meat may also contain pathogens without showing signs of deterioration (Zamudio, 2006). The organisms spoiling meat may infect the animal either while still alive ("endogenous disease") or may contaminate the meat after its slaughter ("exogenous disease"). There are numerous diseases that humans may contract from endogenously infected meat, such as anthrax, bovine tuberculosis, brucellosis, salmonellosis, listeriosis, trichinosis or taeniasis. Contaminated meat, however, should be eliminated through systematic meat inspection in production, and consequently, consumers will more often encounter meat exogenously spoiled by bacteria or fungi after the death of the animal. One source of infectious organisms is bacteremia (the presence of bacteria in the blood of slaughtered animals). The large intestine of animals contains some  $3.3 \times 10^{13}$  viable bacteria, which may contaminated the flesh after death if the carcass is improperly dressed. Contamination can also occur at the slaughterhouse through the use of

improperly cleaned slaughter or dressing implements, such as powered knives, on which bacteria persist. A captive bolt pistol's bolt alone may carry about 400,000 bacteria per square centimeter. After slaughter, care must be taken not to infect the meat through contact with any of the various sources of infection in the abattoir, notably the hides and soil adhering to them; Water is used for washing and cleaning, the dressing implements and the slaughterhouse personnel. Bacterial genera commonly contaminate meat while it is being processed, cut, packaged, transported, sold and handled include: *Salmonella spp.*, *Shigella spp.*, *E. coli*, *proteus*, *Staph. albus* and *Staph. aureus*, *Cl. welchii*, *B. cereus* and faecal streptococci. These bacteria are all commonly carried by humans. Infectious bacteria from the soil include *Cl. botulinum*. As these microorganisms colonize a piece of meat, they begin to break it down, leaving behind toxins that can cause enteritis or food poisoning, potentially lethal in the rare cases of botulism. The microorganisms do not survive a thorough cooking of the meat, but several of their toxins and microbial spores do. The microbes may also infect the person eating the meat, although against this the microflora of the human gut is normally an effective barrier (Lawrie and Ledward, 2006). Fast and accurate detection of spoilage, even before evident signs appear, is necessary to prevent losses during production, distribution, and storage of meat products. Microbial analysis by traditional methods evaluates freshness, spoilage, and safety of meat and meat products; these are precise but time-consuming methods. A similar situation occurs with the usually lengthy sensory analysis methods. Various authors report the advantages of analyzing the chemical compounds related to spoilage, mainly of microbial origin (Borch, Kant-Muermans and Blixt, 1996). Methods such as the electronic nose, biosensors, and fluorescence spectroscopy provide accurate

and fast tools for spoilage detection. Finally, molecular techniques present a new opportunity to determine the type and load of spoilage microorganisms (Bjorkroth and Korkeala, 1996).

## **1.2 The Gram-negative Bacteria associated with Meat Spoilage**

### **1.2.1 *Enterobacteriaceae***

The Enterobacteriaceae are Gram-negative bacteria, most of which are motile with polar flagella, they grow easily on the usual media in 24 h at 37°C aerobically and anaerobically, their nutritional requirements are generally reduced, most of them multiply in synthetic medium with a simple carbon source like glucose (Brenner, 1980). On the biochemical properties species of this family are generally oxidase negative, reduced nitrate to nitrite with the exception of some species of *Erwinia* and *Yersinia*. The name of Enterobacteriaceae was given because these bacteria are usually normal or pathological host, following the microbial species of digestive tract of humans and animals (Williams, 1965). Many members of this family are a normal part of the microbial community found in the intestines of humans and other animals (Hormaeche and Edwards, 1960), while others are found in water or soil, or are parasites on a variety of different animals and plants. *E. coli*, is one of the most important model organisms, and its genetics and

biochemistry have been closely studied. The accurate identification of Enterobacteriaceae and other glucosefermenting and nonfermenting gram negative bacilli has been the subject of many hundreds of publications over the years (O'Hara and Miller., 1965). The use of commercial kits, either manual or automated, to identify these organisms is common practice. Some species are difficult to identify with phenotypic and biochemical

identification schemes commonly used outside reference laboratories. 16S ribosomal DNA (rDNA)-based identification of bacteria potentially offers a useful alternative when phenotypic and biochemical characterization methods fail (Michel *et al.*, 2000).

### **1.2.2 *Campylobacter***

*Campylobacter* is Gram-negative, microaerophilic and/or anaerobic, mainly spiral-shaped bacteria. Most of them are recognized or suspected as human gastrointestinal pathogens (Skirrow, 1994). *Campylobacter jejuni* and *Campylobacter coli* are frequently associated with human campylobacteriosis (Skirrow, 1990). In many western countries, the incidence of campylobacteriosis is higher than diseases caused by *Salmonella* (Rohner *et al.*, 1997). Poultry and poultry products are known as important sources of human campylobacteriosis and play an important roles in disease transmission (Deming *et al.*, 1987 and Evans., 1992). However, in developing countries due to inappropriate detection method, a number of cases might have been undetected (Trachoo, 2003). The main transmission route of infection is the ingestion of food of animal origin (Butzler and Oosterom.1991). Symptoms of gastroenteritis caused by *C. jejuni* include diarrhea, sometimes bloody in the later stages, abdominal cramps, fever and vomiting (Bokkenheuser and Mosenthal. 1981). The onset of the symptoms occurs 2 to 7 days after exposure and the illness may last 7 to 10 days. Erythromycin and tetracycline have been used successfully to halt the progression of the infection (Blaser *et al.*, 1979).

### **1.2.3 *Hafnia alve***

*Hafnia alvei* is a Gram-negative facultatively anaerobic bacillus that belongs to the family *Enterobacteriaceae*, has been associated with the gaseous

spoilage of chub-packed ground beef (Gamage *et al.*, 1997), and other meats packaged under low oxygen atmospheres (Bersani *et al.*, 1980). Inoculation studies have also shown that *H. alvei* can grow in refrigerated, vacuum-packed beef (Kennedy *et al.*, 1980; Anderson *et al.*, 1989). This growth has been associated with hydrogen sulphide production which can contribute to gas accumulation under a packaging film (Hanna *et al.*, 1979). Albelda-Puig *et al.* (1986) found *H. alvei* in 47% of aerobically packaged retail ground beef samples. The presence of *H. alvei* on beef may be due to its prevalence on slaughtered cattle, (Maciak, 1977) found *H. alvei* on 21% of muscle, lymphatic glands and internal organs. While the presence of *H. alvei* on retail beef has been firmly established, its role in retail ground beef spoilage has not been widely studied as this microbe competes poorly with aerobic spoilage bacteria present under the aerobic conditions of retail packaging. *H. alvei* has also been isolated from various mammals (Sharma *et al.*, 1991). It is suspected to cause a variety of intestinal disorders, including gastroenteritis (Reina, 1993). In humans, *H. alvei* is a recognized cause of a number of illnesses, including pneumonia (Klapholz *et al.*, 1994). Meningitis (Mojtabae, 1978), abscesses (Agustin and Cunha. 1995), and septicemia (Moble, 1971).

#### **1.2.4 *Pseudomonas***

The predominant bacteria that are often associated with spoiled meat are members of the genus *pseudomonas*. There are strict aerobic microorganisms. Although these are the main organisms responsible for putrid odors, the volatiles produced appear only when the metabolized substrate changes to amino acids (Greer, 1989) producing bad odor, esters, and acid (McMeekin, 1981). They are polarly flagellated, Gram –negative,

rod shaped, aerobic bacteria (Anzai, Kim, Park and Wakabayashi 2000). *Pseudomonas aeruginosa*, probably the most well known of all *pseudomonas* strains, is an opportunistic pathogen that has the capability of adapting to many niches. It can infect animals and humans alike and can cross the species barrier and for this reason it is considered a potentially zoonotic pathogen. *Pseudomonas spp* are known to cause spoilage in food at low temperatures. These psychrophilic *Pseudomonas* strains include: *P. fragi* which causes spoilage of dairy products , *P. taetrolens* which causes mustiness in eggs and *P. mudicolens*, and *P. lundensis*, which causes spoilage of milk, cheese, meat, and fish. The term spoilage is used in food hygiene for a condition by which the food takes on an unpleasant texture, color change smell taste. This does not mean that the food has necessarily become a serious health risk in the same way as if meat were infected with *E.coli* or salmonella, for instance, but at the same time it is definitely not advisable to eat spoiled food. It means that the food has been infected with bacteria which cause the physical nature of the food to change. For instance, meat may become slimy to the feel or the outer lawyer of certain foods may become pulpy, it may also smell slightly sour and change color. This happens to food which has been stored in refrigerated conditions for long periods of time in places which have not been properly cleaned with anti bacterial disinfectants. This condition is often seen in places such as deli counters where meat is sliced in front of the customer. Spoiled food such as cured meats meat can cause stomach discomfort, diarrhea, vomiting or mild allergic symptoms in some people and may even become more serious in people whose immune system is compromised.

### **1.2.5 *Enterobacter***

*Enterobacter spp.* are Gram-negative motile rods and are usually

peritrichously flagellated. *Enterobacter* spp. are found in a wide variety of food as they can grow in a wide temperature range and are very adaptable in terms of the nutrients that they require to survive. *Enterobacter* spp. in meat products cause off-flavour, produce gas and form slime as a metabolic by-product.

#### **1.2.6 *Serratia***

*Serratia* spp are Gram-negative bacteria, classified in the large family of *Enterobacteriaceae*. *Serratia* spp can be distinguished from other genera by its production of three special enzymes DNAase, lipase and gelatinase (Giri *et al.*, 2004). *Serratia* spp occur in water and soil, on plant, in insects and in man and animal (Singleton and Sainsbury.2001).

#### **1.2.7 *Klebsiella pneumoniae***

*Klebsiella pneumoniae* is a Gram-negative, non-motile, encapsulated, lactose fermenting, facultative anaerobic, rod shaped bacterium found in the normal flora of the mouth, skin, and intestines (Ryan and Ray. 2004).

#### **1.2.8 *Citrobacter freundii***

*Citrobacter freundii*, is aerobic Gram-negative bacillus. *Citrobacter freundii* appears as long rod-shaped bacterium typically 1-5 µm in length. Most *C. freundii* cells are surrounded by many flagella, but a few are non-motile. Its habitat includes the environment (soil, water, sewage), food, and the intestinal tracts of animals and humans (Wang,Chang, Chen and Luh, 2000). As an opportunistic pathogen, *C. freundii* is responsible for a number of significant opportunistic infections. It is known to be the cause of a variety of nosocomial infections of the respiratory tract, urinary tract, blood and several other normally sterile sites in patients. *C. freundii* represents approximately 29% of all opportunistic infections (Whalen, Mully, 2007).

### **1.2.9 *Proteus vulgaris*:-**

*Proteus vulgaris* is a rod-shaped Gram-negative chemoheterotroph bacterium. The size of individual cells varies from 0.4~0.6µm by 1.2~2.5µm. *P. vulgaris* possesses peritrichous flagella, making it actively motile. It inhabits the soil, polluted water, raw meat, gastrointestinal tracts of animals, and dust. In humans, *Proteus* species most frequently cause urinary tract infections, but can also produce severe abscesses; *P. mirabilis* produces 90 percent of cases, and is encountered in the community, but *P. vulgaris* is associated with nosocomial infection (O'Hara *et al*,2000).

## **1.3 The Gram-positive Bacteria associated with Meat Spoilage**

### **1.3.1 *Bacillus cereus*:-**

*Bacillus cereus* is a sporeforming, Gram-positive, aerobic, rod bacterium. It has been long known as ubiquitous organism found in air, soil and water (Claus and Berkeley, 1986). *Bacillus cereus* is one of the potential spoilage bacteria associated with red meat (Nel *et al.*, 2004). In a study done by Mosupye and Von Holg (2000), *Bacillus cereus* was predominant in both raw and prepared food stuffs. Microorganisms control in meat products is the major concern in the preparation of high quality foods (Jo *et al.*, 2004). The hygienic state of animals prior, during and after slaughter can be critical to the finished product quality (Satin, 2002). During slaughtering process the meat is exposed to many sources of *Bacillus cereus* contamination (Lawrie, 1998). The incidence of *Bacillus cereus* is higher in cooked and processed (ground beef) meat than in raw meat samples (Nortje *et al.*, 1999; Mosupye & Von Holg, 2000). *Bacillus cereus* is the aetiologic agent of two distinct types of food poisoning characterized either by diarrhea and abdominal pain or by nausea and vomiting after ingestion of contaminated foods (Thayer

and Boyd, 1994; Nelms *et al.*, 1997)

### **1.3.2 *Listeria monocytogenes*:-**

*Listeria monocytogenes* is a Gram-positive, non-spore forming, and motile, facultatively anaerobic, rod shaped bacterium. It is catalase positive, oxidase negative (Farber and Peterkin (1991), *Listeria monocytogenes* is an important food-borne pathogen having common occurrence in a variety of foods (Farber and Peterkin,1991; Brett *et al*, 1998; CDC, 1999). The major outbreaks of listeriosis have been associated with consumption of foods of animals origin (Doyle, 1986) *Listeria monocytogenes* has been isolated from a wide variety of foodstuffs, including raw and cooked meat products. Conflicting results have been published about the growth of this pathogen on raw meat products. Nevertheless, growth appears to be highly dependent on pH, temperature, type of tissue and the competitive microflora (Farber and Peterkin 1991). In cooked meat products *L. monocytogenes* should be absent. The heating of these products for 2 min at 70°C, or to temperatures of 71°C, is likely to inactivate any *L. monocytogenes* present (Gaze *et al.* 1989, Mackey *et al.* 1990; Zaika *et al.* 1990). Although reports on survival of *L. monocytogenes* during processing have been published regularly, evidence for this is often obtained by heating meat products with un realistic high levels (107–108 cfu g<sup>-1</sup>) of added Listeriae (Boyle *et al.* 1990). The presence of heat-stressed cells is probably only of interest when cells have been exposed to sublethal temperatures for short periods of time, because this increases significantly the heat resistance (Linton *et al.* 1992). However, these results were also obtained in experiments using high initial levels of *Listeria*. Because raw meats generally contain low numbers of *Listeria* (less than 100 cfu g<sup>-1</sup>) (Sheridan *et al.* 1994), the decimal reductions obtained with the heating processes normally applied are

sufficient to ensure safe products (Mackey *et al.* 1990).

### **1.3.3 *Staphylococcus aureus***

*Staphylococcus aureus* is a bacterium with strains that are capable of producing a highly heat-stable toxin that causes illness in humans. Intoxication is caused by ingesting enterotoxins produced in food by *S. aureus*, usually because the food has been left at ambient temperature (Walls 1997). Foods that require considerable handling during preparation and that are kept without refrigeration are usually implicated in staphylococcal food poisoning. The toxin produced by *S. aureus* is very heat-stable and is not destroyed at normal cooking temperatures. Many kinds of cooked foods are known to be displayed in restaurant windows at ambient temperatures for several hours. Cooked meats, ham, poultry, egg products, tuna, potato and macaroni salads are good environments for this bacterium to produce the toxin. Many foods are often prepared under unsanitary conditions and stored for long periods at ambient temperature before being sold (for example street foods or ready-to-eat foods). Thus, the time lapse between food preparation and consumption is an important factor to consider in terms of hazard. For example, street vendors cook in the morning and then store it at ambient temperatures for the rest of the day (Mensah *et al.*, 2002; Caballerotorres *et al.*, 1998).

### **1.3.4 *Staphylococcus epidermidis***

*Staphylococcus epidermidis* is a Gram-positive, coagulase-negative coccus that is a part of human normal flora. Consequently, it is a true opportunistic pathogen, as it requires a major breach in the host's innate defenses. It is one of the leading pathogens of nosocomial infections (Nilsson *et al.* 1998). It causes endocarditis of native and prosthetic valves, peritonitis, bacteremia, osteomyelitis and urinary tract infection. (Theodore *et al.*, 1976).

### **1.3.5 *Brochothrix***

*Brochothrix* is a microorganism for which meat is considered an ecological niche. It is a Gram-positive non-spore-forming facultative anaerobe, reported to be one of the most important spoilage microorganisms in meat and meat products. Glucose is the only substantial component of meat that supports its growth. Under anaerobic conditions, its spoilage potential is very low, producing lactic acid and small amounts of volatiles, the result is a slight off-odor (Gill, 1995). In an aerobic complex medium such as meat, it produces highly odoriferous compounds such as acetoin, acetic, isobutyric, and isovaleric acids, and their aldehydes and alcohols (Dainty, Edwards and Hibbard, 1989). Its ability to grow under both aerobic and anaerobic conditions makes it a significant meat colonizer. The optimal temperature for growth is 20-25° C. The optimal pH for *B. thermosphacta* to grow is pH 7.0, but growth is seen within the ranges of pH 5-9. The species have often been isolated from irradiated meat and poultry. Although, they are an important spoilage organism found in prepacked meats and in meat stored in chill temperature.

### **1.3.6 *Enterococcus***

*Enterococcus* is a Gram-positive coccus that often occurs in pairs (diplococci) or short chains and is difficult to distinguish from *Streptococcus* on physical characteristics alone. Two species are common commensal organisms in the intestines of humans: *E. faecalis* (90-95%) and *E. faecium* (5-10%). There are rare clusters of infections with other species including *E. casseliflavus*, *E. raffinosus* (Gilmore, *et al.*, 2002). Species of the genus *Enterococcus* comprise a large proportion of the autochthonous microflora associated with the gastrointestinal tracts of animals and are frequently responsible for significant morbidity and mortality in predisposed humans

(Franz, Holzapfel, and Stiles. 1999). Enterococci are common components of the microfloral community of mammals, birds, insects, and reptiles and are commonly found in soil, on plants, and in water. These organisms are particularly challenging to eliminate because of their ability to adapt to environmental stresses. Thus, it is not surprising that antimicrobial-resistant variants of enterococci have been recovered from meats, dairy products, and ready-to-eat foods and have even been found within probiotic formulations (Giraffa, 2002).

## **CHAPTER TWO**

### **MATERIALS AND METHODS**

#### **2.1 methods of sterilization**

##### **2.1.1 Dry Heat**

###### **2.1.1.1. Hot air oven**

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

###### **2.1.1.2 Red Heat flame**

Red Heat flame was used for sterilization of wire loops, straight wires and forceps. It was done by holding the object as near as possible to the flame until it became red hot (Cruickshank *et al.*, 1972).

###### **2.1.1.3 Flaming**

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

##### **2.1.2 Moist Heat**

###### **2.1.2.1 Autoclaving**

Media, solutions, plastic wares such as rubber stoppers were sterilize by autoclaving it at 121 °C for 15min, under pressure of 15 pounds/sq. inch (Barrow and feltham.1993).

### **2.1.2.2 Momentary autoclaving**

In this technique the autoclave was turned off as soon as it reached 121°C. The valve of the autoclave was opened when the temperature reached 100°C and the autoclave is unloaded below the 80° C (Barrow and feltham.1993).

### **2.1.3 Disinfection of media-preparation room**

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

## **2.2 Diluents**

### **2.2.1 Normal saline**

About 8.5g of sodium chloride was added to 1 liter distilled water, mixed dissolved and sterilized by autoclaving at 121°C for 15 min (Cruick shank *et al.*, 1975).

## **2.3 Collection of blood**

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

## **2.4 Culture media**

All media were prepared according to the Oxoid Manual for culture media

Ingredients and Barrow and felthman (1993) as follows:

### **2.4.1. Solid media**

#### **2.4.1.1 Nutrient agar (Oxoid Code-CM3)**

Dehydrated nutrient agar (Oxoid Ltd., England) was prepared according to the manufacturer's instruction. This medium consists of yeast extract, sodium chloride, peptone and agar. Twenty-eight grams of the powder were dissolved in 1 liter of distilled water by boiling. The pH was adjusted to 7.4 and then the medium was sterilized by autoclaving at 121°C for 15 minutes, cooling to 50 -55°C and then distributed into sterile Petri-dishes, 20 ml in each.

#### **2.4.1.2. MacConkey's agar (Oxoid Code-CM3)**

Fifty-two grams, which consisted of 20g peptone, 10g lactose, 5g bile salt, 5g NaCl, 12g agar and 0.075g neutral red in 1 litre of distilled water, were boiled to dissolve completely, and sterilized by autoclaving at 121C for 15min after adjusting the pH to 7.4 The medium was poured onto sterile Petri-dishes and the plates dried before inoculation.

#### **2.4.1.3. Blood agar (Oxoid Code-CM3)**

Fourty grams, which consisted of 10g peptone, 5g NaCl, 15g agar and 10g of Lab-lemco "the blood agar powder" were suspended in 1 litre of distilled water and boiled to dissolve completely. The medium was sterilized by autoclaving at 121C for 15min after adjusting the pH to 7.3. The base was then cooled to 45-50C and 7% of defibrinated blood was added, mixed with gentle rotation and poured into sterile Petri dishes as 20ml in each dish.

#### **2.4.1.4. Baird Parker's medium (Oxide M<sub>2</sub>75)**

In a liter of distilled water, 63g of dehydrated (Oxide M<sub>2</sub>75) Baird Parker

medium were suspended, mixed and steamed to dissolve. The pH was adjusted to 6.8 before autoclaving at 121°C for 15 minutes. After cooling to about 45°C, 5 ml of egg yolk emulsion and 2 ml of potassium tellurite were added aseptically and mixed well before poured onto plates.

#### **2.4.1.5 Mannitol salt agar (Oxoid CM85)**

One hundred and eleven grams of (Oxoid CM85) were suspended in 1 liter of distilled water, mixed, steamed to dissolve and then the pH was adjusted to 7.5 it was then autoclaved at 121°C for 15 minutes, cooled and poured onto Petri dishes.

#### **2.4.1.6 Urea agar (Oxoid)**

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

#### **2.4.1.7 Simmon's citrate agar (Oxoid):**

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

#### **2.4.1.8 Ammonium salt sugars (ASS)**

This medium consisted of ammonium phosphate (1.0g), potassium chloride (0.2g), magnesium sulphate (0.2g), yeast extract (0.2g), agar (20g) and bromoeresol purple (0.04ml). It was prepared according to Barrow and Feltham (1993) by adding the solid to 1000 ml distilled water, dissolved completely by boiling and sterilized at 115 C for 20 minutes. The medium

was allowed to cool to about 55 C and the appropriate sugar was added as a sterile solution to give final concentration 1%. The medium was mixed and distributed aseptically into sterile tubes and allowed to set in the slope position.

#### **2.4.1.9 Nutrient gelatin (Oxoid Code-CM135a)**

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

### **2.4.2 Semi- solid Media**

#### **2.4.2.1 Hugh and leifson's (O.F) medium (Oxoid)**

The medium was prepared by dissolving 10.3 grams of solids in 1 liter of distilled water by heating, and the pH was adjusted to 7.1. filtered bromothymol blue (0.2% aqueous solutions) was added and then sterilized at 115 C for 20 minutes. Sterile solution of glucose was added aseptically to give final concentration 1%, mixed and distributed aseptically into sterile tubes.

#### **2.4.2.2 Motility medium: (Oxoid)**

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

### **2.4.3 Liquid media**

All liquid media were prepared according to Barrow and Feltham (1993).

#### **2.4.3.1 Nutrient broth**

This medium was prepared by dissolving 13g of the medium in 1 liter of

distilled water. The pH was adjusted to 7.4, distributed into screw-capped bottles 5 ml each and sterilized at 121 C for 25 minutes.

#### **2.4.3.2 Peptone water (Oxoid Code-CM9R4)**

Fifteen grams which consisted of 10g peptone and 5g NaCl were added to 1litre of distilled water, mixed well, distributed into tubes after adjusting the pH to 7.4 and sterilized by autoclaving at 121C for 15min.

#### **2.4.3.3 Peptone water sugars**

Nine hundred ml of peptone water was prepared and pH was adjusted to 7.1-7.3 before 10 ml of Andrade s indicator was added. Ten grams of the appropriate sugar was added to the mixture, distributed into tubes 5ml in each one. The peptone water was sterilized by autoclaving at 110° C for 10 minutes. The sugars used were Glucose, mannose, mannitol, maltose, sucrose lactose, fructose, raffinose, and trehalose.

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

Five grams of peptone and 5g of potassium phosphate were dissolved in 1 liter distilled water by steaming. The pH was adjusted to7.5 and 5g of glucose was added and mixed. The medium was distributed into test tubes 5ml each and sterilized by autoclaving at 110 C for 10 minutes.

#### **2.4.3.5 Nitrate broth**

One gram of nitrate was dissolved in 1 liter of nutrient broth, distributed into tubes and sterilized by autoclaving at 115 C for15 minutes.

#### **2.4.3.6 Cooked meat medium (Oxoid Code-CM81R20)**

Fifty five grams of granules which consisted of 10g peptone, 10g of "lab-lemco" powder,30g neutral heart tissue and 5g NaCl were added to 1 litre of distilled water,soaked for 15min and distributed into narrow containers.The meat pieces were kept in suspension so that a uniform layer was obtained,then the medium was sterilized by autoclaving at 121C for 15min.

## **2.5 Reagents**

### **2.5.1 Hydrogen peroxide**

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

### **2.5.2 Oxidase test reagent**

Oxidase test reagent was manufactured by British Drug House; a 1% tetramethyl-p-phenyldiamine aqueous solution was added to 1% ascorbic acid as fresh solution, before a 50x50mm of filter paper was impregnated with it, and then dried at 50 C.

### **2.5.3 Nitrate test reagent**

Nitrate test reagent consisted of two reagents (A&B), solution A consisted of 33% sulphanilic acid which was dissolved by heating in 5-N-acetic acid, while solution B consisted of 0.6% dimethyl-alpha-naphthalene amine, dissolved by gentle heating in 5-acetic acid (Barrow and Feltham, 1993).

### **2.5.4 Kovac's reagent**

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

### **2.5.5 Voges Proskauer test reagent**

Voges Proskauer test reagent consisted of two solutions:

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

### **2.5.6 Methyl Red**

Methyl Red is product of Hopkin and William, was prepared as 5% solution

and was used in methyl red test.

### **2.5.7 Andrade's indicator**

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

## **2.6 Sampling**

### **2.6.1 Description of samples**

#### **2.6.1.1 Sausage**

Sausage is food made from chopped or comminuted lean meat and fat, mixed with salt, spices and other ingredients, then filled into a container. The latter is often a casing, made from the cleaned intestines of cattle or sheep. In cleaning, much of the layered wall of the intestine is removed, leaving mainly just collagen. Artificial casings are now often used. These are made from reconstituted collagen derived from other parts of the animal, particularly the skin of cattle, and have the obvious benefit of uniformity of the product.

#### **2.6.1.2 Beef-Burger**

Beef-Burger consist of minced meat ,salt ,onion ,garlic .runner bean and spices.The mixture is pressed slightly on metal object to disc of 8-10 cm in diameter and 0.6-1.0cm in thickness.

#### **2.6.1.3 Shawarma**

Shawarma is food made by placing strips of beef, lamb or marinated chicken on a stick; an onion or tomato is placed at the top of the stack for flavoring. The meat is roasted slowly on all sides as the spit rotates in front of, or over, a flame for hours. Traditionally a wood fire was used; currently, a gas flame is common. While specialty restaurants might offer two or more meat selections, some establishments have just one skewer. After cooking, the

meat is shaved off the stack with a large knife, an electric knife or a small circular saw, dropping to a circular tray below to be retrieved. Shawarma is eaten as a fast food, made up into a sandwich wrap with pita bread or rolled up in an Armenian Lavash flatbread together with vegetables and dressing. Vegetables found in shawarma include cucumber, onion, tomato, lettuce, eggplant, parsley, pickled turnips, pickled gherkins and cabbage.

#### **2.6.1.4 Kebab**

Kebab is a wide variety of meat dishes originating in Persia , and now is found worldwide. In English, kebab with no qualification generally refers more specifically to shish kebab served on the skewer or döner kebab served wrapped in bread with a salad and a dressing. In Persia, however, kebab includes grilled, roasted, and stewed dishes of large or small cuts of meat, or even ground meat; it may be served on plates, in sandwiches, or in bowls. The traditional meat for kebab is lamb, but depending on local tastes, it may now be beef, goat and chicken.

#### **2.6.1.5 Agashi**

Agashi is delicious food made of meat on the African way in terms of cooking, spices, where it is put meat on sticks and add the spices Featured (mixture of spices) and then placed sticks lined up at the fire and the volatility until browned and then the spices are added again and cut it a little onion and a little lemon and it taken hot..

#### **2.6.2 Collection of samples**

Ninety samples of cooked, ready-to-eat Sandwichs (Beef-Burger, Shawarma, Kebab and Agashi) were collected from different street food vendors in each three areas which included: Khartoum, Omdurman, and Bahree. Samples were collected and transferred to the laboratory for bacteriological investigation.

### **2.6.3 Preparation of the samples**

. A small portion (25g) of each sample was mixed with 25ml of sterile normal saline crushed with sterile mortar and pestle and mixed gently to give a homogenous suspension. Small portion was streaked with a sterile loop on different types of solid media (Blood agar, Nutrient agar and MacConkey's agar) and incubated at 37C for 24-48h.

### **2.7 Examination of cultures**

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 48 hours and discarded if no growth was detected.

### **2.8 Purification of cultures**

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

### **2.9 Identification of isolated bacteria**

Identification was carried out according to the procedure described by Barrow and Feltham (1993).

#### **2.9.1 Primary identification**

##### **2.9.1.1 Preparation of smears**

Smears were prepared by emulsifying small inoculums of the bacterial colony in a drop of sterile normal saline on clean slide and spreaded it. The smears were allowed to dry and then fixed by gentle heating.

##### **2.9.1.2 Gram's technique**

This was done as described by Barrow and Feltham (1993).

Microscopic examination of isolates:

All isolation microorganisms were subjected to microscopic examination

under oil immersion lens and the shape, arrangement and Gram's reaction were recorded.

#### **2.9.1.3 Catalase test**

This test is used to identify bacteria which produce the enzyme catalase (Cheesbrough, 2000). A portion of the tested colony was placed on a drop of 3% hydrogen peroxide on a clean slide using a wooden stick. Production of air bubbles indicated a positive result.

#### **2.9.1.4 Oxidase test**

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

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

Two tubes of Hugh and Leifson's medium were inoculated with tested organism, one of them was covered with a layer of sterile paraffin oil. Tubes were incubated at 37° C and examined daily for seven days. Fermentative organisms produced a yellow color on both tubes while oxidative organisms produced a yellow color only in tubes without oil.

#### **2.9.1.6 Motility test**

Motility was tested by stabbing isolated bacteria with straight loop wire in semi solid medium (Craig tube method). The medium was then incubated for up to 3 days at 37° C together with an inoculated media as control. The growth of a non motile organism was confined to the stab, while that motile one spreaded out side the craige tube.

#### **2.9.1.7 Sugar fermentation test**

The sugar media were inoculated with 24 hours culture of tested organism. They were incubated at 37° C and examined daily for up to seven days. Acid production was indicated by the development of a pink color in the medium

and gas production was indicated by trapped air in the Durham's tube.

## **2.9.2 Secondary Identification**

### **2.9.2.1 Indole test**

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

### **2.9.2.2 Voges-Proskauer (VP) test**

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

### **2.9.2.3 Urease test**

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

### **2.9.2.4 Citrate utilization test**

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

### **2.9.2.5 Casein hydrolysis test**

Casein agar plate was inoculated with test organism and incubated at 37°C for 24-48 hours. Clear zone around the colonies indicated casein hydrolysis.

#### **2.9.2.6 Nitrate reduction test:**

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

#### **2.9.2.7 Sugar fermentation test**

The sugar medium was inoculated with bacteria grown in peptone water incubated and then examined daily for up to 7 days. Acid production was indicated by the development of pink color in the medium.

#### **2.9.2.8 Arginine hydrolysis test**

Inoculated 5 ml Arginine broth and after incubated for 24 hours added 0.25 ml Nessler's reagent. Arginine hydrolysis was indicated by the development of brown color.

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

Inoculated nutrient gelatin with a straight wire and incubated at 37°C for up to 14 days; every 2-3 days, place in a refrigerator for 2 h and then examined for liquefaction.

#### **2.9.2.10 Hydrogen sulphide production**

Inoculated the organism in nutrient broth or peptone water, and insert a lead acetate paper between the plug or cap and the tube. Examined daily for 7 days for blackening of the paper.

#### **2.9.2.11 Pigment production**

Nutrient agar plate was inoculated with a drop of light suspension of the organism, incubated for 24 h at room temperature and observed for up to 5 days for recording the color.

#### **2.9.2.12 Coagulase test**

0.5ml of 1/10 dilution of plasma was mixed in saline with an equal volume of an 18-24 hour broth culture of organism and Incubated at 37°C for 4 h. Examined after 1 and 4 h for coagulation. If negative, the tubes were left at room temperature overnight and then re-examined.

#### **2.9.2.13 Phosphatase test**

Phenolphthalein phosphate agar was inoculated to obtain discrete colonies, and incubated for 18 h. Placed 0.1 ml ammonia solution in the lid of the Petri dish phosphate-positive colonies became pink.

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

Organism was inoculated in nutrient agar NaCL 10% incubated at 37°C for growth.

#### **2.9.2.15 Growth at 50°C test**

Organism was inoculated in Nutrient agar media and incubated at 50°C for growth.

#### **2.9.2.16 Growth at 42°C test**

Organism was inoculated in N.A media and incubated at 37°C for growth.

#### **2.9.2.17 Lecithovitellin reaction**

Lecithovitellin agar was incubated with the test bacteria and incubated at 37°C for 5 days to examine the growth changes (opalescence under and around the growth). The appearance of pearly layer around the colonies constituted a positive reaction.

## CHAPTER THREE

### RESULTS

Ninety Samples were collected from cooked, ready-to-eat Sandwiches of Meat origin (31 Aqashi, 22 Kebab, 20 Shawarma and 17 Burger).

#### **The general isolates:**

From Ninety samples tested for bacterial isolates 76(84%) were positive for bacterial growth as shown in Table (1).

#### **Identification of isolates:**

According to microscopic appearance, the cultural and biochemical properties (table 4 and 5), the isolates were identified as:

1. Gram-negative bacteria. This group was represented by:

*Klebsiella spp, Enterobacter cloacae, Serratia marcescens, Hafnia alvei, Campylobacter spp, Proteus spp, Citrobacter freundii and Pseudomonas spp.*(Table 2 and Figure 2).

2. Gram- positive bacteria. This group was represented by:

*Bacillus cereus, Listeria monocytogenes, Staphylococcus spp, Brochothrix spp, Kurthia gibsonii, Micrococcus spp and Enterococcus spp.*(Table 3 and Figure 3).

Table 1: Sample positive for bacteria growth

No of samples	90
Positive for isolation	76
Percentage	84%

Table 2: The prevalence of Gram-negative bacteria in meat products

Gram-negative bacteria	No: of positive samples examined	No: of isolates	Percentage
<i>Klebsiella spp</i>	76	17	22%
<i>Enterobacter cloacae</i>	76	12	15%
<i>Serratia marcescens</i>	76	9	11%
<i>Hafnia alvei</i>	76	6	7%
<i>Campylobacter spp</i>	76	2	2%
<i>Citrobacter freundii</i>	76	2	2%
<i>Pseudomonas spp</i>	76	1	1%
<i>Proteus spp</i>	76	1	1%

Figure 2: The prevalence of Gram-negative bacteria in meat products

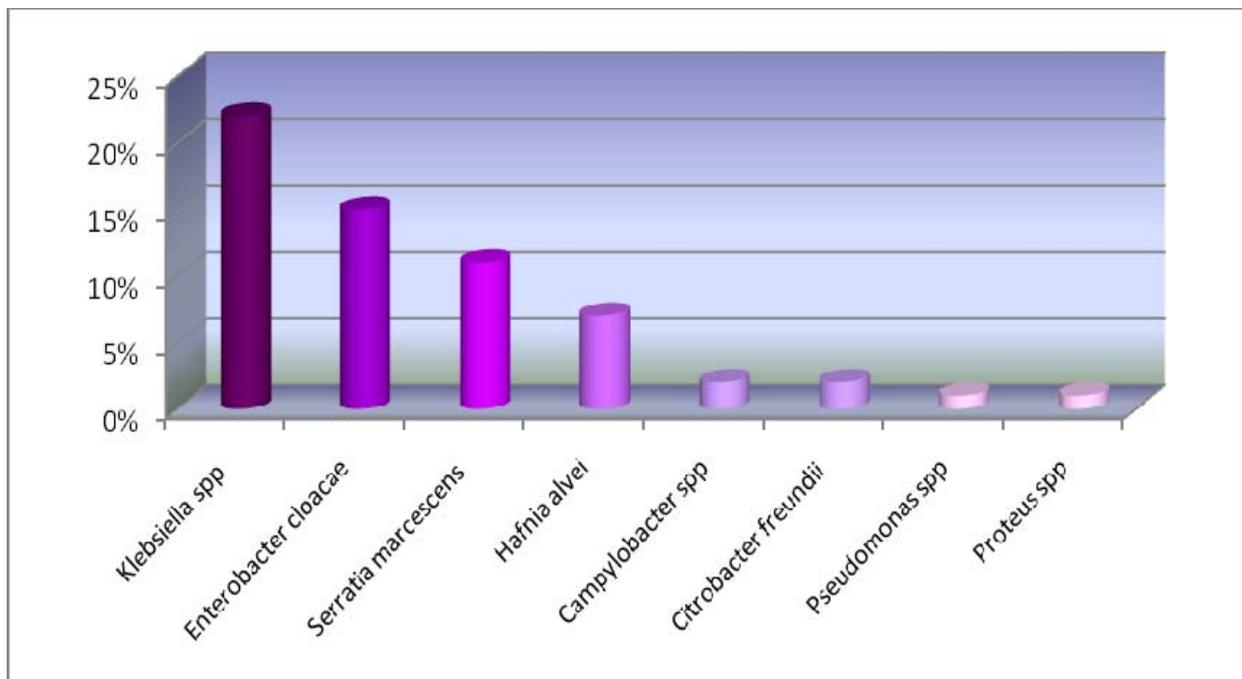


Table 3: The prevalence of Gram- positive bacteria in meat products

Gram- positive bacteria	No: of positive samples examined	No: of isolates	Percentage
<i>Staphylococcus spp</i>	76	9	11%
<i>Bacillus cereus</i>	76	5	6%
<i>Listeria monocytogenes</i>	76	3	3%
<i>Bacillus mycoides</i>	76	2	2%
<i>Brochothrix spp</i>	76	2	2%
<i>Kurthia gibsonii</i>	76	2	2%
<i>Enterococcus spp</i>	76	2	2%
<i>Micrococcus spp</i>	76	1	1%

Figure 3: The prevalence of Gram- positive bacteria in meat products

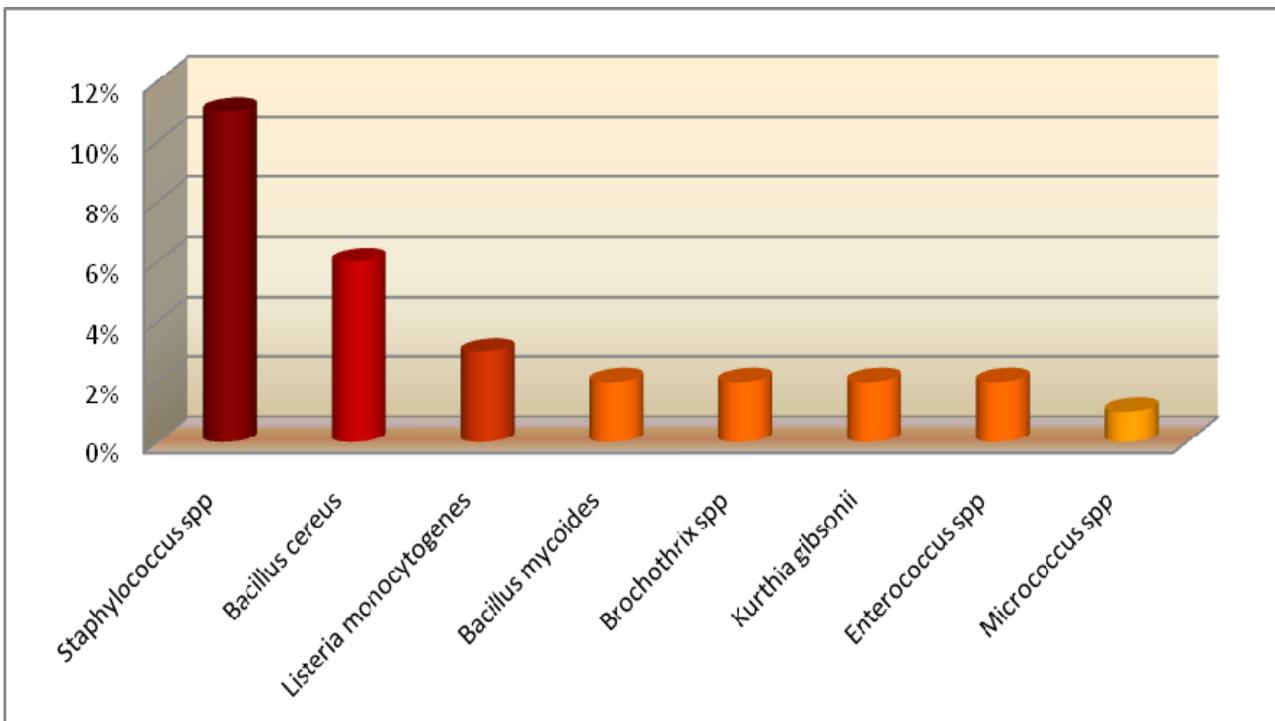


Table 4: Biochemical properties of Gram-negative bacteria

character	<i>Campylobacter</i>	<i>Pseudomonas spp</i>	<i>Enterobacter cloacae</i>	<i>Serratia marcescens</i>	<i>Haffnia alvei</i>	<i>Klebsiellae spp</i>	<i>Proteus spp</i>	<i>Citrobacter freundii</i>
shape	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod
Motility	+	+	+	+	+	-	+	+
Lactose	NT	+	+	-	-	-	-	+
Indole	-	+	-	-	-	-	-	+
Citrate	NT	+	+	+	+	-	-	+
Urease	-	+	-	-	-	+	+	+
H <sub>2</sub> S	+	-	-	-	+	-	+	+
MR	-	-	-	+	+	+	+	+
VP	NT	NT	+	-	+	-	-	-
Sucrose	-	+	+	-	-	+	-	+
Glucose	-	+	+	-	+	-	+	+
Mannitol	-	+	+	+	+	+	-	+

Table 5: Biochemical properties of Gram- positive bacteria

character	<i>Staphylococcus aureus</i>	<i>Bacillus cereus</i>	<i>Bacillus mycoides</i>	<i>Listeria monocytogenes</i>	<i>Brochothrix</i>	<i>Kurthia gibsonii</i>	<i>Micrococcus spp</i>	<i>Enterococcus spp</i>
shape	cocci	rod	rod	rod	rod	rod	cocci	cocci
Motility	-	+	-	+	-	+	-	-
Coagulase	+						-	
Novobiocin	S							
Oxidase	-	+	+	-	-		+	
Catalase	+	+	+	+	+	+		-
Mannose	+	+	-					
Sucrose	+					-		
Manitol	+	+				-		
Lactose	+					-		
Maltose	+	+			+	-		
Trehalose	-					-		
Xylose	-	-	-		+	-		
Rafinose		-	-			-		
Urease		+	+	+				
Haemolysis		+	+					+
Nitrate		+	+		-	-		

## CHAPTER FOUR

### DISCUSSION

Samples of meat sandwiches from three locations in Khartoum state (Khartoum, Omdurman, and Bahree) yielded markable growth of bacteria. The presence of these organisms on meat parts could be attributed to the fact that meat contains an abundance of all nutrients required for the growth of bacteria in adequate quantity.

The total of finding recorded in this study showed the microbial diversity (differences in form or species) in these locations. The contamination with these organisms in this study may be due to condition of the restaurants, street food tables, equipment and machines which had been used during food processing, preparing, and the hygienic practice employed by street sellers and handlers, This determined the variation of bacterial contamination. Although most of organisms found in this study are normal flora of different parts of man and animal body, some of them have been associated with many disease problems. They might cause disease in their presence in the animal body or by contamination of food. The microflora of meats available to consumers is the total sum of microorganisms acquired during processing of animal muscle food. Animal health, dressing skills, personnel hygiene, abattoir cleanliness, and adequate storage and holding temperature during distribution and retail influence the constitution and number of microorganisms present (Hudson, Mead, and Hinton. 1996).

Our results are similar to the result of Collins (1995) who isolated Gram-positive and Gram-negative bacteria from meat products.

The result of Cox and Mercuri (1978) who isolated *Citrobacter freundii*, *Enterobacter cloacae*, *Hafnia alvei* and *Klebsiella pneumoniae* from

meat products agree with the result of this study.

Garcia-Lopez, Preto, and Otero (1998) isolated Gram- positive and Gram-negative bacteria from cooked ready to eat sausages and they reported that The presence of these bacteria on fully cooked products is the result of post-heating contamination, usually during casing removal, slicing, and subsequent handling during packaging.

Meats products sold to the public in open markets or by streets vendors are grossly contaminated with coliform bacteria as well as other bacterial forms. The finding of this study revealed that meats products are contaminated with pathogenic Gram- positive and Gram-negative bacteria. This also implies that these meats are viable source of various diseases. Some of these diseases could spread and acquire epidemic status which poses serious health hazards. Since improper handling and improper hygiene might lead to the contamination of meats products and this might eventually affect the health of the consumers (Okonko et al., 2008b,c,d,2009a,b). It is, therefore, suggested that meats products processors and sellers should be educated on the adverse effect of contamination. However, the processors, handlers and sellers should observe strict hygienic measures so that they may not serve as source of chance inoculation of microorganisms and fecal contamination of fresh meats and meat products.

The presence of organisms examined in this study is of special concern and perhaps the greatest danger associated with meats used for food preparation, eating purposes and for other human consumption is contamination by human excreta (Okonko et al., 2008a, b, c, d, 2009a, b). It demonstrates a potential health risk as the organism is pathogenic and causes complications in children (Taulo et al., 2008). The need for microbial assessment of fresh meats and other meat products processed and cooked for human

consumption is therefore emphasized and recommended to reduce possible contamination (Okonko et al., 2009a, b). The presence of these organisms in meat foods should receive particular attention, because their presence indicates public health hazard and give warning signal for the possible occurrence of food borne intoxication (Kabir, 2009). Since control of fecal-orally transmitted pathogens is inadequate in many developing countries, in particular, in sub-Saharan Africa (Okeke et al., 2007) and acquired resistance to antimicrobial drugs is becoming more prevalent among diarrheagenic pathogens in this region (Okeke et al., 2007),

The value of microbiological sampling in the prevention of food-borne disease associated with catering premises is uncertain. It is generally accepted that random food sampling is not worthwhile. A programme which concentrates on specific aspects of hygiene and on cleaning procedures may help to establish and monitor a good code of working practice. It is not clear, however, which food types should be sampled, and whether or not total bacterial counts or specific bacteria should be looked for.

## **CONCLUSION AND RECOMMENDATIONS**

### **Conclusion**

From the results this investigation can be concluded that:-

- 1- food served in informal vending operations is not relatively safe for consumption. However, ample evidence indicates potential risks of contracting food-borne disease(s) by consumption of contaminated food and there have been reports of serious consequences as a result of contaminated street food.
- 2- the presence of specific microorganisms in foods and on surfaces, even when detected in low numbers, indicates the need for improving infrastructure, especially in terms of the provision of proper sanitation facilities.

### **Recommendation**

(1) Recommended that complete separation of raw from cooked meats. This must include the provision of different surfaces and equipment for handling, cutting, slicing, weighing, display and sale of such meats.

(2) Recommended that washing (sanitizing) of hands and equipment before handling a cooked product. Preferably, different staff should be employed on the cooked meats

section of the premises. Where possible, tongs, forks or other suitable instruments should be made available for handling a cooked product.

(3) Recommended that hold cooked meats under refrigeration, i.e. < 4.40 C. with free circulation of air. Since pathogenic bacteria grow between 4.40 and 48.80 C. Cooked meats should not be permitted to be held in this range for long periods.

(4) Recommended that facilities for curing must be provided, preferably under refrigeration, in a separate room or an area remote from the shop

proper. Immersion brines should be filtered or clarified before reuse.

(5) Recommended that all persons engaged in the handling of meat, both in the factory and in the retail trade, must be provided with suitable protective clothing (protection for the meat, not the worker).

(6) Recommended that any person suffering from an infection of the stomach or intestine (vomiting, diarrhoea), septic cuts or boils must not handle meat (raw or cooked) until the condition has disappeared .

(7) Recommended that environmental health officers should look for in their inspection, and a more critical examination of hygienic practices and cleaning schedules to determine their meat hygiene effectiveness and whether or not they are actually used is probably necessary.

(8) ) Recommended that local government should intervene to ensure that the standard of safety of street foods is the best attainable under the circumstances of the prevailing situation.

(9) Such training should preferably be made available to the street food vendors free of charge.

(10) Support from local authorities in the form of adequate infrastructure with services such as water supply, toilets, refuse disposal and waste water disposal facilities, is also needed

(11) Risk assessment, risk management and risk communication as a mean of organizing available information, identifying data gaps, quantifying risk for specific pathogens and foods, and presenting strategies for improvement are need for education to reduce the incidence of food borne illness and complements regulatory and other activities.

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