

## INTRODUCTION

Meat is considered as an important source of proteins, essential amino acids, B complex vitamins and minerals. Due to this rich composition, it offers a highly favourable environment for the growth of pathogenic bacteria Gill( 1998). Meat products are perishable and unless processed, packaged, distributed and stored appropriately can spoil in relatively short time Sofos, (2005).

Raw retail meats have been identified as potential vehicles for transmitting food-borne diseases, and hence the need for increasing implementation of hazard analysis of critical control point (HACCP) and food safety education efforts (Zhao *et al.*,2001). Sources of bacterial contamination of meat are hide, hooves, soil, intestinal contents, air, water supply, knives, cleavers, saws, hooks, floors and workers (Haines, 1933, Empey and Scott, 1939).

Many bacterial pathogens were reported as meat contaminants. CDC (2000) reported that, pathogenic microorganisms that were involved in outbreaks associated with consumption of meat and meat products during the period 1993-1997 were *Bacillus cereus*, *Campylobacter*, *Clostridium botulinum*, *Clostridium perfringens*, entero-pathogenic *E. coli*, *salmonella*, *shigella*, *Staphylococcus aureus* and *Yersinia enterocolitica*.

Food-borne illness is most often linked to bacteria, and less common to viruses, parasites, prions, and molds Rahuman,( 2007). The HACCP program is a preventive approach to consistent safe food production (Marriott and Gravani,2006) HACCP interventions may be applied at any of a number of positions on the production line, and more than one may be used Sosos, (2005).

In Sudan, hygienic measures to control microbial contamination of meat it unsatisfactorily applied. Storage at refrigerated temperatures is still one of the

most effective practices for improving the safety of fresh meat. However, some butcheries still use poor refrigeration; in addition, the retail raw meat in most of butcheries is presented exposed to environmental pollution, which might lead to increased bacterial contamination.

**Objectives:**

- To determine the bacterial count in beef in small butcher.
- To isolate *E. coli* and *Salmonella* in small butcher in Alhaj yousef area.

# Chapter One

## Literature Reviews

### 1.1 Source of contamination

There were several genera of bacteria specially associated with the hands and nasal cavities and mouth, the important of there are *Micrococcus* and *Staphylococcus*, (Bryan, 1978, Jay, 1986)

Frazier (1967) showed that any contaminating bacteria on the knife would soon be found on meat in various parts of the carcasses as it is carried by the blood. The contamination of carcasses come from different sources including environment and the equipment's with which meat comes in contact during slaughtering and processing but hides remain as an important source of contamination.

Jepsen (1967) noticed that bacteria were carried to the abattoir on skin, hooves and body cavities of animals.

Frazir and Westhof (1988) emphasized that the importance of contamination from external source during bleeding, skinning and cutting. These include the knives, air ,hands and clothes of workers .They also reported that during handling contamination came from carts, boxes, and other contaminants.

Hussein (1971) isolated bacterial contaminant from fresh meat of the gastro internal tract and hides of the slaughtered animals and the water, halls and deposits.

During harvesting processing, distribution, and preparation, food is contaminated with soil, air, and water-borne microorganisms. Hence high numbers of microorganisms are found in animal intestinal traces, and some of these microorganisms find their way to the carcass surfaces during evisceration. When

carcasses and cuts are subsequently handled through the food distribution channels, where they are reduced to retail cuts, they are subjected to an increasing number of microorganisms from the cut surfaces (*Marriott and Gravani,2006*) .At each stage of beef processing after slaughtering, different microbes get introduced and these tend to contaminate the meat *Ebel et al .(2004)*

Utensils, equipment's and cutting board surfaces were identified as a major source of contamination in meat processing plant *Rahman,( 2007)*.

The hands, hair, nose, and mouth harbor microorganism that can transferred to the food during processing, packaging, preparation, and service by touching, breathing coughing, or sneezing. *Marriot and Gravani,( 2006)*.

*Jay (2000)* suggested that the microorganism, individually and as a group, grow over a very wide range of temperatures, therefore, it is well to consider at this point the temperature growth ranges for organisms of importance in foods as an aid in selecting the proper temperature for the storage of different types of foods. The lowest temperature at which a microorganism has been reported to grow is  $-34^{\circ}\text{C}$ , somewhere in excess of  $100^{\circ}\text{C}$ , it is customary to place microorganisms into three groups based on their temperature requirement for growth in the following:

- Those organism that grow well at or below  $7^{\circ}\text{C}$  and have their optimum temperature between  $20^{\circ}\text{C}$  and  $30^{\circ}\text{C}$  are referred to as psychrophiles.
- Those that are grow well between  $20^{\circ}\text{C}$  and  $45^{\circ}\text{C}$  with optima between  $30^{\circ}\text{C}$  and  $40^{\circ}\text{C}$  are referred to as mesophiles.
- Where those that grow well at and above  $45^{\circ}\text{C}$  with optima between  $55^{\circ}\text{C}$  and  $65^{\circ}\text{C}$  are referred to as thermophiles.

*Roders and Fletcher (1966)* noted that sychrophilic and mesophilic type of bacteria are the most important.

Gracey (1985) reported that bacteria associated with meat depend on bacteriology of the soil on which the animals were kept prior to slaughter. The bacteria were transferred to the hides and then to the exposed meat.

Gracey (1980) reported that there are different sources of meat contamination for example, invasion of blood vessels by bacteria from the intestine of weakened or ill animals just prior to slaughter. The animal's digestive tract was claimed to carry dangerous load of bacteria actual contagion with dirty hands, clothing and equipment's are important factors in the presence of bacteria in frozen meat in chilling storage.

*George (1989) isolated from fresh meat samples, Staph epidermidis, Micrococcus spp. Escherichia coli, Proteus sp.* Gracey (1980) reported that there are different sources of meat contamination for example, invasion of blood vessels by bacteria from the intestine of weakened or ill animals just prior to slaughter. The animals digestive tract was claimed to carry dangerous load of bacteria actual conagion with dirty hands, clothing and equipments are important factors in the presence of bacteria in frozen meat in chilling storage, *Aeromonas spp. and Achromobacter spp.*

*Fatima (1982) isolated Sallmonella spp., Clostridium perfringes, Staphylococcus and E. coli* from processed meat.

Lawris (1979) reported that if a contaminated knife was used or organisms were in advertently introduced from the skin where the main blood vessels were severed could as source of contamination of tissues

Slantez. *et al.* (1963) suggested that the spoilage of fresh meat was associated with the growth of Proteus, Pseudomonas and Escherichia. In addition to Gram-positive bacteria such as Bacillus and Micrococcus SPP.

According to Dolman (1967) meat provide excellent medium for *Staphylococcal* Proliferation and if the temperature is warm, enough only few hours it needed for the production of effective amount of enterotoxin.

In Sudan Salih (1971) isolated from fresh meat samples spoilage bacteria of genera *Micrococcus*, *Streptococcus*, *Bacillus*, *Coli aero genes*. He also isolated hemolytic and coagulase positive *Staphylococci* from ovine and bovine liver and rumen samples obtained from Omdurman Central Abtior and isolated *Micrococci* and *Salmonella Doblin* from ovine and bovine offal's.

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

## **1.2 The importance e of meat contamination:**

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

The members of genera *Pseudomonas*, *Actinobacter* and *Moraxella* dominated the bacterial content of unprocessed meat exposed to air chill temperature (International Commission for Microbiological Specification for Food, 1980).

Gracey (1980) stated that the main types of bacteria involved in spoilage are from the Gram-positive genera *Micrococcus*, *staphylococc-us*, *Streptococcus*, *Lactobacillus* *Leuconostoc*, *Bacillus* *Clostridium* *Corynebacterium* and *Microbacterium*. Meat spoilage

Brahmbhalt and Anjaria (1993) examined samples of raw meat obtained from shops, they isolated *E coli*, *Staphylococcus epidermidis*, *Staphylococcus aurus*, *Micrococcus luteus*, *Citrobacter freundii* *Bacillus cereus*, *Sterptococcus faecalis*, *Enterobactere aerogenes*, *Proteus mirabilis* *Bacillus subtilis*, *Aeromonas*

*liquifaciens*, *Proteus vulgaris*, *Kelebsilla poneeumonia* and *Pseudomonas deruginosa*.

John and Anthony (1974) stated that Lactobacteriaceae may be the eventual cause of meat spoilage, under some condition in meat handling, where it enters the product through contamination from plant equipment or handlers of the product.

Lawarie (1991) found that the organisms derived from infected personal or healthy carriers include *Sallmonella SPP.*, *Shigella Spp.*, *Escherichia coli*, *Bacillus*, *Proteus*, *Staphylococcus albus* and *Staphylococcus aurues*, *Colstridium welchii*, *bacillus cerues*, *Bacillus faecal* and *Streptococcus spp.*

Among the bacteria present in the air and dust are bacillus and Micrococcus spp., which were able to tolerate dryness to varying degrees Jay ( 1984)

The microbial status of the product that reaches the consumer in either raw or processed meat will depend on the exposure to contamination and it is control during subsequent chilling, processing, handling, distribution and preparation (Sofos *et al*, 1999).

Soyiri *et al.* (2008) reported that the butchers of retail beef in Asaiman market –Ghana, which under unhygienic practices and poor handling of beef, contaminated with aerobic *mesophiles*, *Staphlococcus aureus* *Bacilus cereus*, *Clostridium perfringens* and *Escherichia coli*.

Khalid (2004) reported that the contamination of beef in Khartoum slaughterhouse by the aerobic bacteria from the Gram-postive genera were *Micrococcus sp.*, *Staphylococcus sp.* and *Bacillus cereus*, while the Gram-negative species was *E.coli*.

## **1.3 Pathogen associated with meat**

### **1.3.1 Gram positive cocci**

#### **1.3.1.1 Micrococcus spp**

Organisms that fit current description of micrococci are commonly encountered in routine laboratories either as environmental contamination or as commensals from normal skin and only occasionally from infections. The difficulty is recognized when these colony distinct required (Barrow and Feltham, 1993).

#### **1.3.1.2 Staphylococcus spp**

The most important one is *S. aureus*, it is a pathogenic microorganism that may cause infections as well as food-borne intoxications. It is a gram-positive cocci, facultative anaerobic microorganism, but it grows faster in aerobic conditions with remarkable resistance in environments *S. aureus* will grow in the temperature interval 7° C - 48° C, with optimum temperature around 37° C. Production of enterotoxin can occur between 10° C and 48° C. They are easily killed by heat. *Staphylococci* are naturally present on the skin and mucous membranes of animals, including healthy animals and man Editor (2009). It has been shown that 20-30% of the population are permanent carriers of *Staphylococci*, whereas 60% of the population are intermittent carriers Klytman and Wertheim (2005).

A total of 71 isolates of gram positive catalase positive cocci were isolated from 112 abscesses observed during inspection of slaughtered animals (sheep, cattle, pigs, and goats) .Amongst 35 coagulase positive isolates were classified as *Staphylococcus aureus*, while among negative isolates five of the coagulase negative isolates were *Staphylococcus hominis* and four were *Staphylococcus xylosum* Menes *et al.*, (1984)



### 1.3.1.2 Gram positive rods

#### 1.3.1.2.1 Bacillus cereus

*Bacillus cereus* is widely distributed in nature including soil, dust, cereal crops, vegetables, animal hair, fresh water and sediments (Kramer and Gilbert, 1989). Foods implicated with *B. cereus* intoxications are meats vegetables, dairy products, suces, pudding and foods containing rice Kramer and Gilbert, (198,) Griffiths and Schraft,( 2002).

*Bacillus cereus* group, such as *B. cereus*, *B.thuringiensis*, *B anthracis* and *B mycooides* strains have been isolated from foods and samples associated with food –poisoning outbreaks Hsieh *et al.* (1999).

In addition to *Bacillus cereus* other *Bacillus Spp*, mainly *B. mycooides*, *B. lentus*, *B. thuringiensis*, *B. polymyxa*, *B. carotarum*, *B.pasteurii* were found to be enterotoxin- producers and have been isolated from foods .They apparently produce vero-cell toxin Jay (2000)

Similar enterotoxigenic profile could be found among strains of. *cereus*, *B. mycooides* and *thuringiensis* .Thus all *B. cereus* group strains B may be potentially toxinogenic and the detection of *B. cereus* group cells on food samples is important Hirsh *et al* (1999).

*B. cereus* toxin causes food poisoning.The toxin is produced when the *Bacillus* sporulate, usually in rice or other cereals that have been cooked and then stored in warm temperatures. Occasionally, *B. cereus* opportunistic infections in immunocomperised persons Cheesbrough. ( 2000).

Food poising caused by *B. cereus* has two distinct: the emetic type, associated with fried rice, and the diarrheal type, associated with meat dish and sauces. *B. cereus* produces toxins that cause a disease which is more than less an

intoxication than a food-borne infection. The emetic form is manifested by nausea, vomiting, abdominal cramps and occasionally, diarrhea.

### **1.3.2 Enterobacteriaceae**

#### **1.3.2.1 *Salmonella* spp.**

*Salmonella* is a Gram-negative rod, facultative anaerobic, it grows at temperatures range between 5°C – 46°C , *Salmonella* is found in the environments and in the gastrointestinal tract of animals, it was reported in all farm animals, but most frequently in poultry. In red meat animals *Salmonella* are most frequent in pigs, followed by cattle, infected cattle may not show symptoms but it can excrete the organisms in their faeces, which cause continuation of water, environment and other animals and meat Editor, (2009). *S. enteritidis* and *S. typhimurim* are the most commonly implicated serovars, while foods of animal origin, particularly meat and eggs seem to be the most common source Todd (1997).

The prevalence of *Salmonellae* on beef, sheep, and pig carcasses varies widely. Sometime *salmonellae* are rarely found Biemuller *et al*, (1973).

In a large survey in the United State, *Salmonellae* were found on 1% of excised 25g samples of brisket from 3075 chilled carcasses of steers, heifers, bulls, and cows and on 5% of samples from 397 calves Hogue *et al.* ( 1993).

USDA (1994) detected *Salmonellae* on 1% of samples excised from chilled steer and heifer carcasses.

#### **1.3.2.2 *Klebsiella* spp.**

*Klebsiella pneumonia* is present in the respiratory tract and faeces of about 5% normal individuals (Brooks *et al.* 2001). *K. pneumortiae* is an opportunistic pathogen of respiratory tract, where as *k. aerogenes* is associated with infection of wounds and urinary tract Cheesbrough( 2000).

*Klebsiella spp* have been implicated in cases of acute gastroenteritis due to consumption of contaminated raw foods Lindsay( 1997). *K. pneumoniae* has also been reported as a cause of superior vena cava syndrome Kim *et al.* ( 1997) and retropharyngeal abscess Pontell *et al.* (1995) in humans.

### **1.3.2.3 *Citrobacter spp.***

They are opportunistic pathogens, *Citrobacter freundii* is the most prevalent species of this genus in foods, while other species are not common on fresh meats Jay, J. Loessner ( 2005).

### **1.3.2.4 *E. coli***

*E. coli* is a Gram-negative rod, facultative anaerobe, and generally motile organism. *E. coli strains* are, in general, non-pathogenic and exist harmlessly in the intestinal tract of humans and animals. Pathogenic *E. coli* strains cause a variety of diseases including gastroenteritis, dysentery, haemolytic uremic syndrome, urinary tract infection, septicemia, pneumonia, and meningitis. However, the major concern in recent years has been the increasing numbers of outbreaks of enterohemorrhagic *E. coli*, due to consumption of contaminated meat, fruits, and vegetables Bhandare *et al.*( 2008). *E. coli* is common in the intestinal microflora of warm-blooded animals. It is routinely shed into the environment through faeces and can contaminate water and soil. Meats are also a common source of *E. coli* contamination, which may be acquired during slaughter through faecal contact Bhandare *et al* (2008) *E. coli* outbreaks have been associated with meat (especially group beef) and dairy products. The pathogen is generally present in the intestine of animals, particularly in cattle, without causing disease. *E. coli* also have been isolated from the faeces of chicken, goats, sheep, pigs, dogs, cats, and sea gulls, Bhandare *et al* (2008). Food of animal origin, especially ground beef, has been implicated in many outbreaks in the U.S, Europe, and Canada; however, in late

2006, a major outbreak involving 26 states was associated with spinach and lettuce.

Mackey *et al.* (1980) reported that *E. coli* in the stationary growth phase survives well in frozen and non-frozen meat and grows in meats at improper storage temperatures.

#### **1.3.2.5 *Listeria* spp.**

*Listeria monocytogenes* is the causative agent of 0.02% of total annual food-borne illnesses in the US. However, listeriosis accounts for approximately 28% of the total deaths due to food poisoning Mead *et al.* (1999). It is widely distributed in nature, including soil, decaying vegetation, animal and human faeces, sewage, silage and water.

#### **1.3.2.6 *Pseudomonas* spp.**

They are soil and water contaminants, which are widely distributed among foods, especially fresh meats and meat products. They are by far the most important group of bacteria that bring about the spoilage of refrigerated fresh foods since many species are psychrophiles Jay( 2000).

*Pseudomonas aeruginosa*, sometimes colonizes human tissue and it is the major human pathogens of the group. *P. aeruginosa* is invasive and toxigenic and produce infections in patients with impaired body defenses. It is an important nosocomial pathogen Brooks *et al.*( 2001).

#### **1.3.2.7 *Moraxella* spp.**

*Moraxella* spp. are Gram-negative, non-motile, non-fermentative, aerobic coccobacilli. *M. bovis* occurs worldwide in bovine conjunctiva and upper respiratory mucosa, often without clinical manifestation. *M. bovis* causes infections

bovine keratoconjunctivitis (IBK), one of the most troubling diseases in cattle Hirsh and Zee ( 1999); there was no evidences for human infection.

#### **1.4 The HACCP and sanitation system**

Raw retail meats have been identified as potential vehicles for transmitting food-borne diseases, and hence the need for increasing implementation of hazard analysis of critical control point (HACCP) and consumer food safety education efforts Zhao *et al*( 2001).

According to Scarfoni (1957) about 90% of food potsoning cases caused by meat and meat products may be attributed to post-mortem operations and handling and about 10% of the cases are due to diseases already present in the living animals. The HACCP program is a preventive approach to consistent safe food production and this program is based on two important concepts of safe food production, prevention and documentation Marriott *and* Gravani ( 2006).

The HACCP can be achieved by the following principles.

Brown ( 2000) Conduct a hazard analysis. Identify the potential hazards associated with food production at all stages up to the point of consumption, assess the likelihood of occurrence of the hazards and identify the preventive measures necessary for their control.

The determination of the critical control points (CCP), and the Identification the procedures and operational steps that can be controlled to eliminate the hazards or minimize the likelihood of their occurrence.

Also Establishment of critical limit(s), set target levels and tolerances which must be met to ensure that the CCP is under control, using of monitor control of the CCPs and The corrective actions to be taken when monitoring indicates that a particular CCP is not under control.

Establishment of procedures for verification to confirm that the HACCP system is working effectively. Establishment documentation concerning all procedures and records appropriate to these principles and their application.

Sanitation is “the creation and maintenance of hygienic and healthful conditions”. It is the application of a science to provide wholesome food processed, prepared, merchandised, and sold in a clean environment by healthy workers; and to minimize the proliferation of food spoilage microorganisms. Marriott and Gravani ( 2006), and it can be achieved by Chlorine, Iodine, Peracetic acid and Quaternary ammonium compounds which are applied on all food contact surfaces and also for environmental control; walls, drains and tiles.

According to FAO/WHO (2003) official or officially recognized program for specified zoonotic agents should include measures to:-

- Control and eradicate their presence in animal populations or subsets of populations.
- Prevent the introduction of new zoonotic agents.
- Provide monitoring and surveillance systems that establish baseline data and guide a risk –based approach to control of such hazards in meat.
- Control movement of animals between primary production units and abattoirs where trade animal populations are under quarantine restrictions.

### **1.5 Meat hygiene and safety**

It is generally assumed that preventing visible contamination or removing visible contamination from carcasses will enhance the microbiological safety of meat. They have a potential impact on the food safety or bacteriological quality of poultry carcasses.

Heemskerk (2005) reviewed the recent literature on the slaughter process and came to the conclusion that improvements on the hygienic could only be obtained by intervention at several places in the slaughter process at the same time.

### **1.7 Conception of HA CCP system**

On 1996, the food safety and inspection service (FSIS) of the United States department of agriculture (USDA) published a final rule on pathogen reduction hazard analysis and critical control point (HACCP) system (PR/HACCP).

The (PR/HACCP) rule requires meat and poultry plants under federal inspection to take the responsibility for other things reducing the contamination of meat and poultry products with disease causing (pathogenic) bacteria and reducing the number of deaths and illness linked to meat and poultry products. The preamble to the final rule describes on overall system in which preventive and corrective measures. The HACCP system, which is a science, based and systematic identifies specific hazard and measures for their control to ensure the safety of food HACCP is a tool to asses hazards and establish control system that focus on prevention rather than relying mainly on end products testing. HACCP system is capable of accommodation change such as advance in equipment design processing.

HACCP can be applied throughout the food chain from primary production to final consumption. The HACCP system program is a preventive approach to safe food production. It was based on the two important concepts of safe food production, which are prevention and documentation. The major thrusts of HACCP are to determine how and where safety hazards may exist and their prevention. The documentation concept is essential to verify that potential hazards have been controlled. The primary objective of HACCP is to insure that effective sanitation

and hygiene and other optional consideration be conducted to produce safe product to provide proof that safe practices have been followed

### **1.8 History of HACCP**

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

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

### **1.8 Application of the HACCP**

The application of the HACCP system can aid inspection by food control regulatory authorities and promote international trade by increasing buyer s confidence. Any HACCP system should be capable of accommodating change, such as advances in equipment design, changes in processing procedures or



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

## **Chapter Two**

### **Materials and method**

#### **2.1 Area of study**

This study was carried out in Alhaj Yousef area in small four butcher.

#### **2.2 Sampling method**

Samples were taken from four butchers the swabs were taken from five sites of each butcher(meat, knife, butcher's hands and surface -table and cutting board)) the total were 80 swabs then the swabs were transferred in ice box to the microbiology laboratory of collage of Veterinary Medicine of Sudan University of Scince and Technology.

##### **2.2.1 Preparation of the samples**

Samples were inoculated onto liquid broth and incubated over night then dilutted with normal sline then, Small portion was streaked with a sterile loop on solid media (Deoxy Cholate Citarate and MacConkey's agar) and incubated at 37°C for 24.

For isolation and identification of Gram-negsitive organisms were inoculated in. MacConkey agar and Deoxy Cholate Citarate agar and incubated aerobically at 37°C for 24 h.

## **2.2.2 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 48hours and discarded if no growth was detected.

## **2.2.3 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.

## **2.2.4 Identification of isolated bacteria**

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

## **2.3 Bacteriology**

The samples were examined for isolation and identification of *E coli* and *Salmonella*.

### **2.3.1 Cultural media**

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

#### **2.3.1.1 Solid culture media**

##### **2.3.1.1.1 Blood agar**

Fourty grams of the base powder were added to one liter of distilled water. The mixture was then boiled until the powder dissolved completely. The solution was autoclaved at 121°C and 15 pound per square inch for for 15 minute, it was then called to 45-50°C. About 7% of sterile blood was added with gentle rotation

and then poured into Petri dishes (15-20 ml) and left to solidify. The poured Petri dishes were kept in refrigerator (about 4°C) until it is used.

#### **2.3.1.1.2 Nutrient agar**

The medium was prepared as described by (Oxoid lab) 25grams of the powder were added to one liter of distilled water and brought to boil to dissolve the powder completely .It is sterilized by autoclaving for 15 minutes at 121° c and 15 pounds per square inch. Then pounred aseptically as 18-20 ml in Petri-dishes.

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

Fifty-two grams of Mac Conkey's agar powder (Oxoid 1982) were added to one liter of distilled water and brought to boiling until dissolved completely. The PH was+ or- adjusted to 7.4 then sterilized by autoclaving at 121°C for 15 minutes. Then it was aseptically distributed in sterile Petri –dished as 15-20ml portion and left to solidify.

#### **2.3.1.1.4 Deoxy Cholate Citarate Agar**

It prepared by 70.52 grams in1000ml of distilled water then heat to boiling to dissolve the medium completely don't autoclave and avoid excessive heating.

### **2.3.2. Semi-solid media**

#### **2.3.2.1 Motility medium**

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

#### **2.3.2.2 Hugh and Liefson's (o/F)medium**

Hug and Liefso's (O/F) medium Cowan and Steel (1974) contained peptone (2g), NaCL (5g), KHPO4 (0.3G), agar (3g), distilled water (1000ml) and

bromocrysol purple, and 0.2% aqueous solution (15ml). The Solids were dissolved by heating in the water. The pH was adjusted to 7.1, the medium was filtrated. The indicator was added. Sterilization was done by autoclaving for 15 minutes and pressure of about 15lb per square inch. Sterile glucose (1%) was added to the mixture was and they distributed aseptically in ten ml volumes into sterile test tubes with cotton plugs of not more than 16mm diameter.

### **2.3.3 Liquid cultural media**

#### **2.3.3.1 Peptone water**

Peotone water was prepared according to *Cruikshank et al*, ( 1975) ten grams peptone and 5 grams NaCL were dissolved by heating in 1000 ml distilled water. The PH was adjusted to 7.2 and the medium was distributed in test tubes (5ml) and serialized by autoclaving at 115° C for 15minutes under pressure 15lb per square inch. The stock was preserved in the refrigerator.

#### **2.3.3.2 Nutrient broth**

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

#### **2.3.3.3. MR-VP medium**

MR-VP medium (Oxoid lab) contained peptone (5g), dextrose (5g) and phosphate buffer (5g). The PH was adjusted to 7. One liter distill water was mixed well with 15 gram of the medium .Then distributed in test tubes with cotton

plugs and sterilized by autoclaving at 121°C for 15 minutes under pressure 15lb per square inch.

#### **2.3.3.4 Nitrate broth**

Nitrate broth (Cowan and Steel, 1985) contained KNO<sub>3</sub> (1g) and 13 grams of nutrient in 1000 of distilled water. Then the medium was distributed in sterile test tubes with cotton plugs and then sterilized by autoclaving at 121°C for 15 minutes under pressure 15lb per square inch.

#### **2.3.3.5 Carbohydrates liquid medium**

Carbohydrates liquid medium was prepared *Cruickshank et al* ( 1975). The sugars used were glucose, maltose, sucrose, lactose and monitor all percent Andrade s indicator. The medium was distributed in 5ml amount in test tubes with cotton plugs and autoclaved under 10lb pressure per square inch for 5 minutes.

### **2.4 Sterilization**

#### **2.4.1 Hot air oven**

This method was used for sterilization of clean glass containers which were wrapped in paper or put in stainless steel cans, and the temperature was 160°C *Stainer et al* (1986).

#### **2.4.2 Sterilization by red heat**

The method was used for sterilization wire loops, straight wire and tissue forceps it was done by holding the object over the flame as near and vertical as possible until it becomes red-hot *Cruickshank et al* ( 1975).

### **2.4.3 Sterilization by autoclaving**

This method was used for sterilizing of culture media and for materials that could not with stand the dry heat .The temperature was 115°-121° C under 10-15 pounds pressure for 15-20 minutes Barrow and Feltham, ( 1993).

## **2.5 Biochemical Tests**

### **2.5.1 Oxidase test**

Tetra methyl-pheynlen-diamine dihydro chloride was prepared as 1% aqueous solution. Filter paper of 50x50 millimeter size were impregnated in reagent before and dried at 50°c. A sterile platinum loop was used to spread the isolated colony on oxidase paper. Color change (violet) indicated appositive reaction Barrow and Feltham ( 1993).

### **2.5. 2 Hydrogen peroxide**

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

### **2.5.3 Kovac's reagent**

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

### **2.5.4 Bromocrysol purple and phenol red indicator**

Bromocrysol purple and phenol red indicators were obtained from Britch Drug House. Methyl red was a product of *Hopkins and William*, s. it was prepared as 5% solution for use in methyl red test .it was of acid fuchsin (5g) distilled water 1000ml and N-NaOH)150-180) ml. The acid fuchin was dissolved in distilled

water and 150ml of alkaline solution were added. It was used in sugars test as one percent volume indicator was prepared according to *Cowan and steel* (1974).

### **2.5.5 Catalase test**

Using sterile glass rod apart of isolated colony was emulsified in one drop of hydrogen peroxide on a clean slide. Gass bubbles indicated positive reaction *Barrow and Feltham* (1993).

### **2.5.6 Motility test**

The isolates were studied for motility by Craigie technique Cruickshank *et al*, (1975) in which the bacteria was inoculated into a central tube containing semi solid agar placed in test tube using straight wire. After incubation at 37°C for 24 hours, the tubes were examined for migrating of bacteria outside the tube.

### **2.5.7 Hugh and Leifson's test**

Hugh and Leifson's test or oxidation fermentation test (O/F) was done as shown by Cruickshank *et al* (1975). Duplicate tubes of freshly prepared medium were inoculated by stabbing with straight wire. One of the inoculated media was immediately covered with layer of sterile liquid paraffin to a depth of one ml and examined daily for up to 14 days. A colour change from green to yellow in both tubes indicated a fermentative organism but change in the uncovered tube only indicated that the organism was oxidative.

### **2.5.8 Nitrate reduction test**

Nitrate reduction test was carried out as described by *Cowan and Steel* (1985). Nitrite broth was inoculated and incubated for up to five days. One ml nitrate solution 1 was added followed by one ml of solution 2. A red coloration within five minutes, powdered Zink was added and allowed to stand. Development



of red colour indicated that nitrate was present. Absence of red coloration in this case indicate absence of nitrate.

### **2.5.9 Carbohydrates fermentation tests**

Carbohydrates fermentation tests were carried out as described by Cruickshank *et al.* (1975). 5 carbohydrates media, glucose, lactose, manitol, sucrose and maltose, were inoculated with peptone water culture by a sterile loop a fermentation reaction was indicated by change of color of the medium to pink.

### **2.6 Gram Stain**

Using a sterile wire loop apart of isolates colonies four primary plots and pure were taken and spread on microscopes slides to make thin smears. They were fixed with heat and placed in staining rack. They were covered by crystal violet for two minutes and washed off by tap water, then decolorized with acetone for few seconds and washed off by tap water, then covered with carbol fuchsine for thirty seconds. Finally, the stained smears were washed and air-dried. Then they were examined under oil immersion lens (100°). The gram positive and negative organisms shape and arrangement of organisms were identified according to *Barrow and Feltham* (1993).

### **2.7 Bacteria Isolation and Identification**

For *E. coli* identification, 1 ml of the dilutions were inoculated on McConkay agar (Oxoid Ltd., Basingstoke, Hampshire, England) and incubated aerobically at 37°C for 24 hrs. Colonies that were suspected to be *E. coli* were isolated and confirmed using gram staining and other biochemical tests.

*Salmonella spp.* were also identified by inoculating 1ml of the dilutions on SS agar (Oxoid Ltd., Basingstoke, Hampshire, England) and incubated for 24 hrs.

at 37°C. Colonies that were considered to be *Salmonella* spp. were also further identified using gram staining technique and other biochemical tests.

### **3. Statistical analysis**

The data of bacteria count collected was analyzed by the computer program SPSS, used A nova test.

## Chapter Three

### RESULTS

Eighty Samples were collected from four butchers, from five sites (40 Meat, 10 butcher hand, 10 knives, and 20 surfaces which are cutting board table). During sample collection, observation of the operations of the study butcheries revealed that study butcheries had similar operating conditions and hygiene practices. All the butcheries displayed meat (beef) mixed with offals openly on tables and wooden logs had no screens which let flies into the butcheries, floors were not clean, knives and other cutting tools were handled carelessly, weighing scales were unclean and all the butcheries lacked hand washing facilities. The butcher men did not wear protective gear such as white coats, caps and gumboots and the same people handled meat and received money. The butcheries were located along the road side probably for display and marketing purposes but this exposed the meat to dust raised by automobiles. Several butcheries were located next to each other and the butcher men shared weighing scales and stones. All of that lead to result below in figure (1) which explains that their hands had the highest mean of TVC, surfaces come in second place then knives and finally the meat had least contamination by bacteria. On the same scale the figure (2) explain the highest mean of general bacterial TVC appeared on butcher B, C, D and A respectively.

Figure (1.1) points' bacterial counts means of  $\log_{10}$ :-

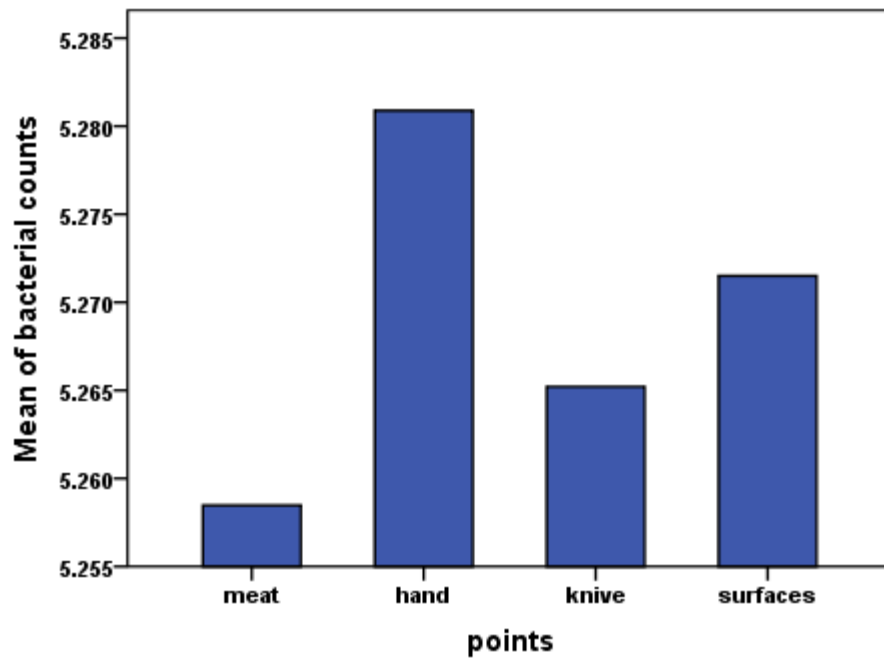
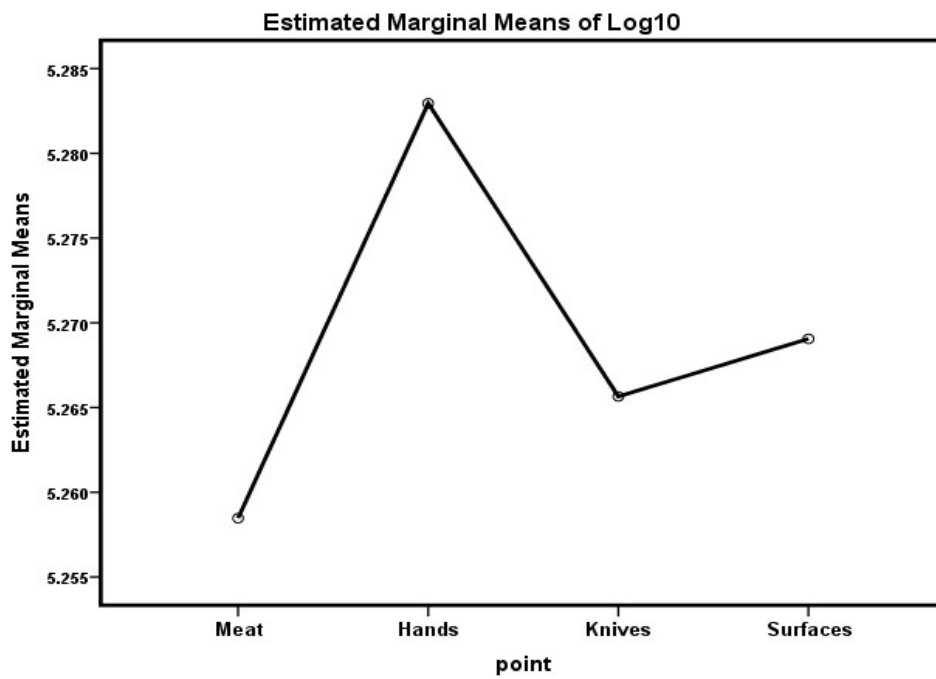
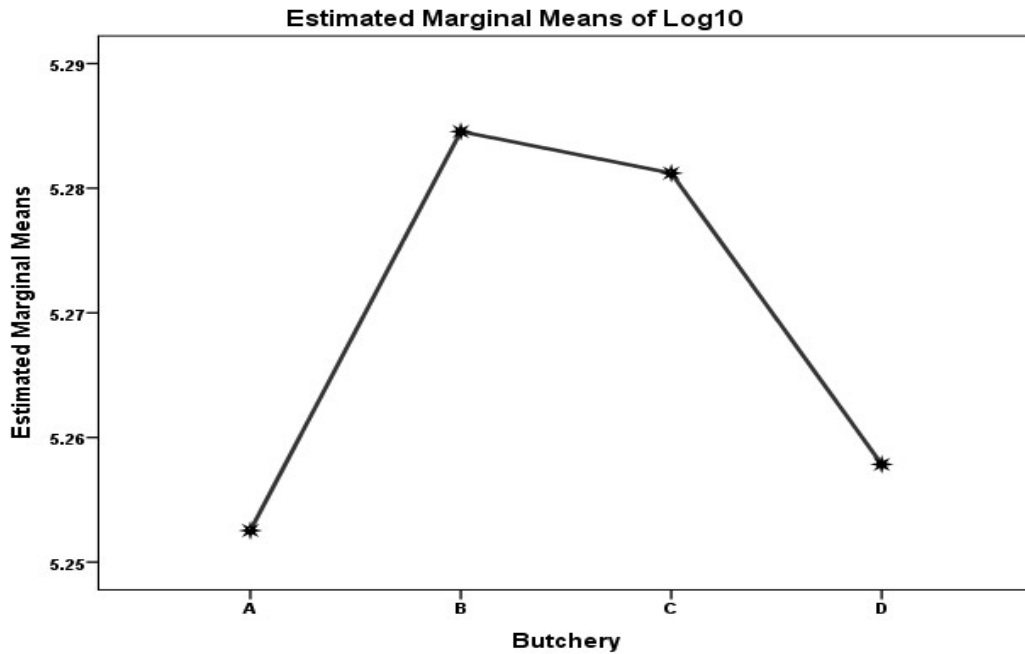


Figure (1.2) points' bacterial counts means of  $\log_{10}$ :-



**Figure (2) Butcherries bacterial counts means of log<sub>10</sub>:-**



Despite the difference in the mean of bacterial TVC in butchers and point but the result shown that There is no significant difference in bacterial count of butcherries with ( $p \leq 0.05$ ) in table one below, also table (2) shown there is no significant difference in bacterial count between points at ( $p \leq 0.05$ ).

**Table (1) Estimating variance of butcherries:-**

Bacterial count	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	0.013	3	0.004	1.636	.188
Within Groups	0.203	76	0.003		
<b>Total</b>	<b>0.216</b>	<b>79</b>			

**Table (2) Estimating variance of points:-**

<b>Bacterial count</b>	<b>Sum of Squares</b>	<b>Df</b>	<b>Mean Square</b>	<b>F</b>	<b>Sig.</b>
<b>Between Groups</b>	0.005	4	0.002	0.607	0.613
<b>Within Groups</b>	0.211	75	0.003		
<b>Total</b>	0.216	79			

**Table (3) Mean of total viable count of bacteria (log<sub>10</sub>) ± Sd at different points in different butcheries:-**

	<b>Meat</b>	<b>Hands</b>	<b>Knives</b>	<b>Surfaces</b>	<b>Total</b>
<b>Butchery(A)</b>	5.25±0.05	5.29±0.03	5.23±0.03	5.24±0.06	5.25±0.05
<b>Butchery(B)</b>	5.26±0.05	5.27±0.03	5.30±0.04	5.31±0.05	5.29±0.05
<b>Butchery(C)</b>	5.27±0.05	5.32±0.07	5.25±0.06	5.29±0.05	5.28±0.05
<b>Butchery(D)</b>	5.26±0.07	5.26±0.04	5.29±0.07	5.23±0.07	5.26±0.06
<b>Total</b>	5.26±0.05	5.28±0.04	5.27±0.05	5.27±0.06	5.27±0.05

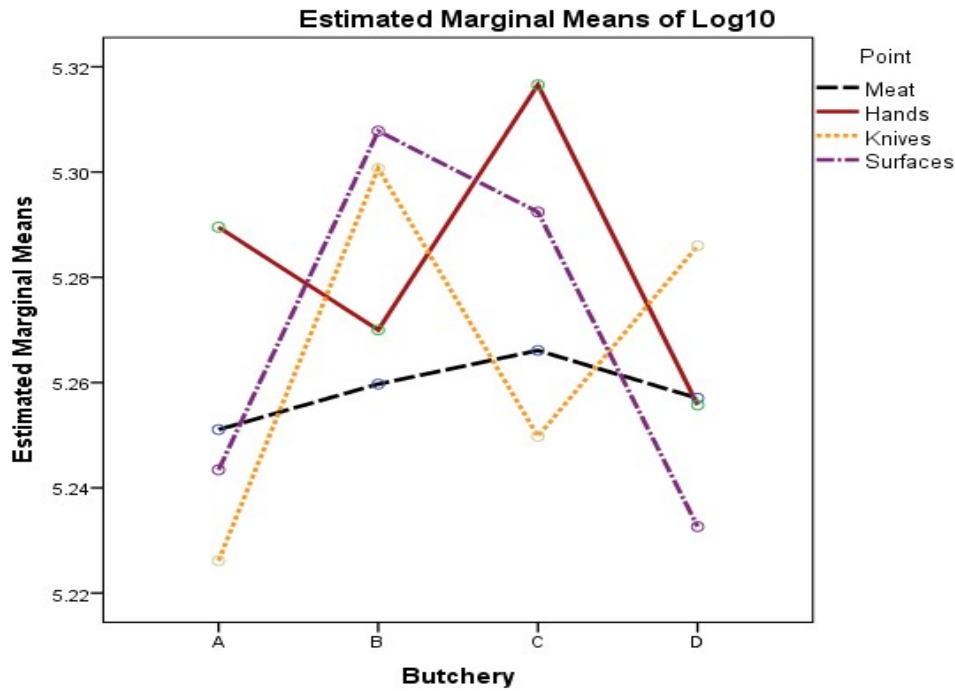
As shown in table (3) above the TVC of bacteria revealed the highest contamination level of the butcher A , B, C ,and D at the points where the samples was taken. In the meat point butcher C Log<sub>10</sub>CFU/ cm<sup>2</sup> TVC 5.27±0.05, B 5.26±0.07 ,A 5.25±0.05 and D 5.26±0.07 respectively ,at the hands point butcher C with a highest mean 5.32±0.07,A with a highest mean 5.29±0.03, B 5.27±0.03 and D with5.26±0.07 respectively .

At the surfaces point the highest contamination butcher B with 5.31±0.05 butcher C with 5.29±0.05, butcher, butcher A with 5.25±0.05 and butcher D with 5.23±0.07 respectively.

At the knives point the highest contamination in butcher B with 5.31±0.04, butcher D with 5.29±0.07, butcher C with 5.25±0.06 and butcher A with 5.23±0.03 respectively.

So from the result above on table (3) and figure (4) below conclude that, the butcher B had the highest level of contamination with bacteria due to rising of TVC of bacteria on surfaces (cutting board/ table) and knives, on the opposite butcher A had the lowest level of contamination for the same reason. Although contamination in the hands higher in C but it seems the effects of contamination in the surfaces and knives together are the strongest.

**Figure (4) points' bacterial counts means of  $\log_{10}$  among butcheries:-**



**Identification of isolates:**

According to microscopic appearance and biochemical properties were identified the bacterial culture as in table (4) and (5):-

**Table (4) Number and percentage of Salmonella among the points:**

Points	Positive (%)	Negative (%)	Total (%)
Meat	24(30)	16(20)	40(50)
Hands	5(6.2)	5(6.2)	10(12.5)
Knives	7(8.8)	3(3.8)	10(12.5)
Surfaces	7(8.8)	13(16.2)	20(25)
Total	43(53.8)	37(46.2)	80(100)

As shown in table (4) the highest contamination by Salmonella was in meat, which have 30%, surfaces and knives had an equal percentage 8.8% and the last point was the hands 6.2%.

**Table (5): Number and percentage of E coli among the points**

Points	Positive (%)	Negative (%)	Total (%)
Meat	20(25)	20(25)	40(50)
Hands	5(6.2)	5(6.2)	10(12.5)
Knives	4(5)	6(7.5)	10(12.5)
Surfaces	13(16.2)	7(8.8)	20(25)
Total	42(52.5)	38(47.5)	80(100)

The highest contamination point by E coli was also meat 25%, surfaces 16.2%, hands 6.2% and the last point knives which had 5% respectively.

**Table (6):Bacteria isolated and identified**

Isolate	E. coli	Salmonella
Test		
Gram stain	-ve	-ve
Mac conckey	+	-



DCA	-	+
Oxidist	-	-
Motility	+	+
Indole	+	-
Citrate	-	-
Urease	-	-
KIA	Yellow butt Yellow slop -ve to H <sub>2</sub> S +ve to gass	Yellow butt Red slop +ve H <sub>2</sub> S +ve to gass

## CHAPTER FOUR

### *Discussion*

Meat samples from four small butchers yielded marked growth of bacteria. The presence of these organisms on meat could be attributed to the fact that meat contains an abundance of all nutrients required for the growth of bacteria in adequate quantity.

At the total of eighty sampels hands had the highest mean of TVC, surfaces come in second place then knives and finally the meat had least contamination by

bacteria. the highest mean of general bacterial TVC appeared on butcher B, C, D and A respectively.

The highest contamination level of the butcher A, B, C, and D at the points where the sample taken. In the meat point butcher C 5.27 Log<sub>10</sub>CFU/ cm<sup>2</sup> TVC ±0.05, B 5.26 Log<sub>10</sub>CFU/ cm<sup>2</sup> TVC ±0.07, A 5.25 Log<sub>10</sub>CFU/ cm<sup>2</sup> TVC ±0.05 and D 5.26 Log<sub>10</sub>CFU/ cm<sup>2</sup> TVC ±0.07 respectively, at the hands point butcher C with a highest mean 5.32 Log<sub>10</sub>CFU/ cm<sup>2</sup> TVC ±0.07, A with a highest mean 5.29±0.03, B 5.27±0.03 and D with 5.26 Log<sub>10</sub>CFU/ cm<sup>2</sup> TVC ±0.07 respectively.

The highest contamination point by Salmonella was meat, which have 30% and the lowest point was the hands 6.2%.

The highest contamination point by E coli was also meat 25%, and the lowest point was knives 5%.

E. coli, which are normal flora of the human and animal intestine, have been identified as a leading cause of food borne illness all over the world. E. coli and E. coli 0157: H7 strain has previously been isolated from meat samples (Hussein, 2007). E. coli 0157: H7 strain was not detected in any of the fresh meat samples examined. However, diarrhea caused by enterotoxigenic E. coli (ETEC) is highly prevalent in young children in developing countries as well as in travelers. It spreads through contaminated water and food (Qadri et al., 2005).

Salmonella species such as *Salmonella typhi* is a bacterium that causes typhoid fever (enteric fever), an acute, life-threatening febrile illness (CDC, 2008). The disease is a cause for concern and a major public health problem in developing countries (Asia, Africa); especially in Nigeria due to poor sanitary conditions and lack of or inadequate potable water (Ibekwe et al., 2008). It is mainly transmitted through food or drink or water, contaminated with urine or faeces of infected people or a chronic carrier (CDC, 2008; Ibekwe et al., 2008).

The contamination with these organisms in this study may be due to condition of the handling, tables, equipment and machines, which had been used during processing, preparing, and the hygienic practice, employed by butchers. 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).

This study agree with Bhandare *et al.*(2007), due to improper/unhygienic handling and processing of the meat, meat is normally transported to the markets in unhygienic meat vans, taxis, motor cycles, motor kings, and sometimes on bicycles. It is also a common practice to see people carrying carcasses on their bare shoulders. According to Bhandare *et al.* (2007), the unhygienic practices of meat processing in developing countries results in these meat been contaminated with microorganisms. Meat sellers were also observed busily conversing, coughing, and

sneezing, which might result in contamination through introduction of saliva on the meat. Okonko *et al.* (2008) stated that, food can be infected with microorganisms as a result of “coughing” and “sneezing” from those who handle and process these foods. Koffi-Nevry *et al.* (2011) also stated that, “careless sneezing and coughing among butchers can this in accord with the results of Gill (1998) who reported bacterial contamination of meat during butchering and skinning.

Another study by Jeffery (2003) revealed that the workers hands and the equipment were the sources of meat contamination and these results are in accord with the present results.

Also another study by Agbeyegbe and Uraih (1982) reported high prevalence rate of *E. coli* in raw meat samples. Also Enabuleleand Uraih (2009) reported *E. coli* prevalence rate to be 85.65% in a study with the fresh meat samples from abattoir and traditional open market each, recording 100% *E. coli* prevalence.

This result agrees to Soyiri *et al.* (2008) reported that the butchers of retail beef in Asaiman market –Ghana, which under unhygienic practices and poor handling of beef, contaminated with aerobic mesophiles, *Staphylococcus aureus*, *Bacillus cereus*, *Clostridium perfringens* and *Escherichia coli*.

Mackey *et al.* (1980) reported that *E. coli* in the stationary growth phase survives well in frozen and non-frozen meat and grows in meats at improper storage temperatures.

Meat 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 meat is 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 meat and this might eventually affect the health of the consumers (Okonko et al., 2008b,c,d,2009a,b). It is, therefore, suggested that meat 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 these organisms in the meat 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**

- The general sanitary conditions at the meat shops in addition to poor hygienic practices by the butcher are probable contributors to the microbial contamination on the beef.
- Ensuring good hygienic standard at the various meat shops
- Potential pathogenic bacteria such as E. coli and Salmonella were isolated and they could constitute a public hazard.

## **Recommendations**

- Training and guidance programs should be started in order to develop awareness among butchers
- Education and application of control measures suitable to prevent meat contamination, from slaughtering to selling, is an important recommendation.
- To always present quality meat, health and standards authorities' should keep follows up through testing of samples and executing of regular studies.
- It highly recommended to eat meat after proper cooking.



## References

- Agbeyegbe JM, Uraih N (1982).** The prevalence of *E. coli* in meat samples in Benin City, Nigeria. *Microbios Lett.* 20: 21–28.
- Barrow, G. I. and Feltham, R. K. A (1993).** *Cowan and steel's Manual for the identification of Medical Bacteria.* London: Cambridge university press.
- Bhandare, S. G., Sherikarv, A. T., Paturkar, A. M., Bhunia, A. K. (2008)..** *Food-borne Microbial pathogens.* Springer science + business media, LLC.
- Biemuller, G. W.; Carpenter, J. A. and Reynolds, A. E. (1973).** Reduction of bacteria on port carcasses. *J. food. Sci.* 38: 261-263.
- Brahmbhatt, M. N. and Anjaria, J. M. (1993).** Isolation of bacteria from market goat meat foods. Incidence of *Escherichia Coli* in the processed meat and meat products. *Ind. Vet. J.* 70: 373-473.
- Brooks, G. F.; Butel, J. S. and Morse, S. A. (2001).** *Hawetz, Melnick & Adelberg's Medical Microbiology,* 22nd ed. McGraw-Hill companies, Inc.
- Bryan, F.L. (1978).** Factors that contribute to our breaks of food-borne disease *JOURNAL OF FOOD PROTECTION* .41; 816-827 *carcass at a small abattoir. Can. Vet. J.* 44: 1 *carcasses in a modern Indian abattoir and traditional*

- CDC (2000).** Surveillance for food-borne disease outbreaks – United States, 1993-1997. *Morbidity and Mortality Weekly Report*. 49(SS-1),1-51 .
- Cheesbrough, M. (2000).** District oratory practice in tropical countries, part 2. Cambridge university press. Comparison of microbial contamination on sheep/goat
- Cowan, S. J and steel K.J and (1985).** Manual for identification of medical bacteriology. 2<sup>nd</sup> London: Cambridge University press reprinted 1985.
- Cruickshank, r.; Duguid, G. P.; Marmion, B.P and Swan, R.H.A. (1975)** Medical Microbiology, Vo1.2 the practice of medical microbiology 12<sup>th</sup> edition. London: Churchill Livingstone.
- Dolman, C. E. (1967).** Epidemiology of meat borne diseases. In meat hygiene Geneva: world health organization.
- Ebel, E.; Schlosser. W.; Kause, J.; Orloski, K.; Roberts. T.; Narrod, C.; Malcolm, S.; Coleman, M. and Powell, M. (2004).** Draft risk assessment of the public health impact of Escherichia coli O157:H7 in ground beef. *J. food Prot.* 67: 1991-1999.
- Editor, F. T. (2009).** Food microbiology and food safety. Springer science + Business Media, LLC.
- Empey, W. A. and Scott, W. G. (1939).** Investigation of chilled beef. Part 1. Microbial contamination acquired in the meat works. CSIRO Aust. Bull. No. 126.
- Fajobi, E. A. (2008).** Assessment of bacteriological

- FAO/WHO (2003)** Report of the 9<sup>th</sup> session of the codex committee on meat and poultry hygiene. Wellington, New Zealand, 17-23 February 2003, food and Agriculture Organization of the United Nation .Pp.38/45.
- Fatima, E. M. (1982).** Bacteriology of processed meat in Sudan, M.Sc., thesis, university of Khartoum.
- Food Safety Inspection Service, United States department of agriculture (USDA), (1997).** Meat and poultry products hazard and control guides, washing.
- Frazier, W. C. (1967).** Food Microbiology, New York: Mc. Graw-Hill.
- Frazier, W. C. and Westhoff, D. C. (1988).** Food microbiology 3<sup>rd</sup>. ed. USA: Mc. Graw Hill Inc.
- Garcia–Lopez, M. L.; Prieto, M. and Otero, A. (1998).** The physiological attributes of Gram-negative bacteria associated with spoilage of meat and meat products. In: Davies, A. and Board, R. G. (eds). The microbiology of meat and poultry. Blackie academic and professional, London, pp. 1-34.
- George, J. Banwart (1989)** Basic food microbiology, An AVI book, library of congress cataloging in publication data, QR115. B34, 88-20837, 576:163.
- Gill, C. O. (1998).** Microbiological contamination of meat during slaughter and butchering of cattle, sheep and pigs. In: Davies, A. and Board, R. (eds). The microbiology of meat and poultry Blackie academic and professional, London, pp. 118-157.
- Gracey, J. F. (1980).** Thornton’s Meat Hygiene. 7<sup>th</sup> ed. London: Bailliere Tindal.

- Gracey, J. F. (1985).** Thornton's Meat Hygiene. 7<sup>th</sup> ed. London: Bailliere Tindal.
- Gracey, J. F.; Collins, D. S.; and Huey, R. I. (1999).** Meat hygiene, 10<sup>th</sup> ed. London: Harcourt Brace and company.
- Griffiths, M. W. and Schratt, H. (2002).** Bacillus cereus food poisoning. In: Cliver, D. O. and Riemann, H. P. (eds). Foodborne disease, 2nd ed. San Diego, CA Academic press, pp. 261-270. HACCP system for the control of microbiological contamination of pig.
- Haines, R. B. (1993).** Observations on the bacterial flora of some slaughter houses. J. Hygiene, Cambridge.
- Heemskerk, W.J.C. (2005)** Improving processing hygiene by mechanical removal of faeces before scalding. XVIIth Eur. Symp. on the Quality of Poultry meat and XIth European Symposium on the Quality of Eggs and Egg Products, Doorwerth, the Netherlands 23-26, p37
- Hirsh, D. C. and Zee, Y. C. (1999).** Veterinary microbiology Blackwell Science. Inc.
- Hogue, A. T.; Dreesen, D. W.; Green, S. S.; Ragland, R. D.; James, W. O.; Bergeron, E. A.; Cook, L. V.; Pratt, M. D. and Martin, D. R. (1993).** Bacteria on beef briskets and ground beef: correlation with slaughter volume and ante mortem condemnation. J. food Prot. 56: 110-113. of ground beef. Inter. J. food microbial. 6(4): 269.
- Hussein, A. H. (1971).** Aaerobic bacteria in fresh and refrigerated beef. M. V. Sc. Thesis: University of Khartoum.
- Hussein, M. S. E. (1987).** Studies on bacteriological quality of fresh meat. Khartoum: Ph.D. thesis, university of Khartoum.

- Ibekwe AC, Okonko IO, Onunkwo AU, Donbraye E, Babalola ET, Onoja BA.** **Baseline Salmonella agglutinin** titres in apparently healthy freshmen in Awka, South Eastern, Nigeria. Scientific Research and Essay, 2008; 3 :225-230
- International commission on Microbiological specifications for food (ICMSF).** (1980). Microbial ecology of foods CH. II. Meat and meat products, academic press, New York, London.
- Jay, J. M. (1986).** Modern food microbiology. 3<sup>rd</sup> ed. New York: Van Nostrand Reinhold Company Inc. New York. PP. 679-680
- Jay, J. M. (2000).** Modern food microbiology 6th ed. Gaithersburg, M. D. Aspen Publishers.
- Jay, J. M., M. L. Loessner, and D. A. Golden. (2005).** Protection of foods with low temperature, p. 395–409. In Modern Food
- Jeffery B, Donald AB, Gill CO (2003).** Implementation of validated.
- Jepsen, A. (1967).** Bacteriological examination of manufactured meat products. In meat hygiene. FAO agricultural Studies No. 34. Rome.
- John, F.; Timoney, J. H.; Gillespie, F.; Seott, J. and Barlough, E. (1988).** Hagan and Varner's Textbook of Microbiology and infectious diseases of domestic animals. 8<sup>th</sup> ed. P. 89, Cornell University Press, New York. USA.
- John, T. N. and Anathony. J. S. (1974).** Microbiology of food and food processing, New York. American Elsevier publishing co. Inc.
- Khalid, K. A. (2004).** Meat hygiene assessment in Khartoum state.

- Kim, J. Y.; Lim, C. M. Koh, Y.; Choe, K. H.; Kim, W. S. and Kim, W. D. (1997).** A case of superior vena cava syndrome caused by *K. pneumoniae*. *Eur. Respir. J.* 10(12): 2902-2903.
- Klytmans, J. A. J. W. and Wertheim, H. F. L. (2005).** Nasal carriage of *staphylococcus aureus* and prevention of nosocomial infection 33:3-7.
- Kramer, J. M. and Gillbert, R. J. (1989).** *Bacillus cereus* and other *Bacillus* species. In: Doyle, M. P. (ed). *Food – borne Bacterial pathogens*. New York, Marcel Dekker, pp. 21-70.
- Lawrie, R. A. (1991).** *Meat science*. 5<sup>th</sup> ed. Tokyo. Pergamon press.
- Lindsay, J. A. (1997).** Chronic sequelae of food-borne disease. *Emerg. Infect. Dis.* 3: 443-452.
- Mackey, B. M.; Derrick, C. M. and Thomas, J. A. (1980).** The recovery of sublethally injured *Escherichia coli* from frozen meat *J. Appl. bacteriol.* 48: 315-324.
- Marriott, N. G. and Gravani, R. B. (2006).** *Principles of food sanitation*, 5th ed. Springer science + business media, Inc.
- McNamara, A. M (1997).** Generic HACCP applications in broiler slaughter and processing. *National Advisory Committee on Microbiological Criteria for Foods. J Food Port.* 5, 462-604.
- Mead, P. S.; Slutsker, L.; Dietz, V.; McCaig. L. F.; Bresee, J. S.; Shapiro, C.; Griffin, P. M. and Tauxe, R. V. (1999),** food-related illness and death in the United States. *Emerg. Infect. Dis.* 5: 607-625. meat shops. *Food Control.* 18, pp. 854-868.

- Menes, I.; Garcia, M. L.; Moreno, B.; Gutierrez, I. and Polledo, J. J. (1984).**  
Staphylococci isolated from abscess in slaughtered animals.  
Characterization and epidemiological studies. Zentralbl. Bacteriol.  
Microbiol Hyg. 9B. 178(5-6): 551-561.
- Okonko, I. O., Ukut, I. O. E., Ikpoh, I. S., Nkang, A. O., Udeze, A. O.,  
Babalola, T. A., Mejeha, O. K. and Fajobi, E. A. (2008).**  
Assessment of bacteriological quality of fresh meats sold in Calabar  
Metropolis, Nigeria. Electronic Journal of Environmental.
- Qadri F, Khan AI, Faruque ASG, Begum YA, Chowdhury F, Nair GB, et al.**  
**Enterotoxigenic Escherichia coli and Vibrio cholerae diarrhea, Bangladesh,**  
**2004. Emerging Infectious Diseases, 2005 Jul [cited 2009 August 18].**  
Available from <http://www.cdc.gov/ncidod/EID/vol11no07/04-1266.htm>.
- Rahman, M. S. (2007).** Handbook of food preservation, 2nd ed. Taylor & Frances  
Group. LLC. P. 176. retail sale in Cote d'ivoire. American Journal of  
Food.
- Roders, A. and Fletcher, D. L., (1966).** Principles of industrial microbiology.  
Pergramon Press. London.
- Salih, M. S. E. (1971).** Studies on bacteriological quality of fresh meat. Thesis for  
Ph.D. degree, university of Khartoum.
- Scarafoni, G. S. (1957).** Technical Organization of Slaughter Houses World  
Health Organization Monograph Series 33, 125-136.
- Slantez, L. W, Chichester, C. O, Gauffin, A. R and Oral, Z. J. (1963).**  
Microbiological quality of food. London, Aca-demic press.

- Sofos, J. N. (1999).** Microbial growth and its control in meat, poultry and fish P. 356-403; quality attributes and their measurement in meat, poultry and fish product. A. M. Pearson and T. R. Dutson (Eds) Blakie, Academic and professional Glasgow, United Kingdom.
- Sofos, J. N. (2005).** Improving the safety of fresh meat, 1st ed. Woodhead publishing Ltd and CRC press LLC.
- Soyiri, I. N. Agbogli, H. K. and Dongdem, J. T. (2008).** A pilot microbial assessment of beef sold in the Ashaiman market, a suburb of Accra, Ghana. Afr. J. food Agric. Nutr. Dev. 8: 91-103.
- Stanier, R. X.; Ingraham, J. L.; Eeelis, M. L. and Paintec, P. P. (1986).** The microbial world. 5<sup>th</sup> ed. New Jersey: Prentice Hall. Technology. 6(9), pp. 835-842.
- Todd, E. (1997).** Epidemiology of food-borne disease a worldwide review. World. Health stat. Q. 50(1-2): 30-50.
- USDA (1994).** USDA nationwide beef microbiological baseline data collection program: steers and heifers (October 1992- September 1993). US Department of Agriculture, food safety and Inspection service. Washington. DC.
- Waskar, V. S., and Zende, R. J. (2007).** A comparison of microbial contamination on sheep/goatcarcasses in a modern Indian abattoir and traditional meat shops. Food Control. 18, pp.854-868.
- Zhao, C., Ge., B., De. Villena, j. Sudler, R, Yeh, E, Zhao. S, White, D.G, Wanger D and Meng, J (2001).** Prevalence of Campylobacter SPP, Escherichia coli and Salmonella serovars in retail chicken, turkey,



pork, and beef from the greater Washington, D.C. Area. *Appl. Environ. Microbiol.* 67:5431-5436.