

BACTERIAL LOAD IN FRESH AND CHILLED MUTTON INTENDED FOR EXPORT

BY

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يَتَأْتِيهَا الَّذِينَ ءَامَنُوا لَا

تُحْرِمُوا طَيِّبَاتِ مَا أَحَلَّ اللَّهُ لَكُمْ وَلَا تَعْتَدُوا ۗ إِنَّ اللَّهَ لَا يُحِبُّ الْمُعْتَدِينَ ﴿٨٧﴾ وَكُلُوا

مِمَّا رَزَقَكُمُ اللَّهُ حَلالًا طَيِّبًا ۗ وَاتَّقُوا اللَّهَ الَّذِي أَنْتُمْ بِهِ مُؤْمِنُونَ ﴿٨٨﴾

88 - 87 :

PREFACE

This work has been carried out at the department of preventive medicine and Veterinary public health, faculty of Veterinary medicine, University of Khartoum.

Under the supervision of prof. Abdel Aziz Eltayeb Ibrahim.

DEDICATION

To my mother

To my father

To my brothers, sisters and my family.

To my Supervisor

To my friend

To whom I love

A KNOWLDGEMENT

First, thank to Allah for giving me strength and patienc and descernment to do this work.

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ABSTRACT

The present study was carried out in a slaughterhouse in Khartoum state intended in export, to assess the meat hygiene and safety of meat prepared for export.

Two conditions were selected in mutton line, which include: before chilling (after skinning and washing) and after chilling, the samples were taken by swabs from: back, neck, forelimb and hind limb.

Fifty six samples were examined in the laboratory to identify the bacteria which were present in the various sites.

Both gram-positive and gram-negative bacteria were isolated.

The gram positive were *Micrococcus* spp. And gram-negative were *Citrobacter* spp.

The Highest number of isolated was found after skinning and washing (before chilling)

The most predominant organism isolated in this study was *Micrococcus* spp.

The observation in this study is that the most common contaminated bacteria is *Micrococcus* spp. which was come with water of washing and air, and *Citrobacter* spp which came from intestine during the preparing of carcass.

ملخص الإطروحة

هذه الدراسة أجريت في احد المسالخ الحكومية بولاية الخرطوم التي تعمل في مجال صادرات لحوم الضأن ، وذلك لقياس صحة وسلامة اللحوم التي تجهز للتصدير. تم إختيار مرحلتين من مراحل تجهيز الذبيح هما قبل التبريد (بعد السلخ والغسيل) وبعد التبريد . أخذت هذه العينات بواسطة مسحات من : الظهر، العنق ، الرجل الأمامية والرجل الخلفية .

حوالي 56 عينة تم أخذها واختبارها في المعمل وذلك لتعريف البكتريا التي توجد في هذه الأجزاء المختلفة من الذبيح . كل من البكتريا الموجبة للجرام والسالبة للجرام قد تم تشخيصها، ووجد أن هذه البكتريا هي *Micrococcus spp.* (موجبة للجرام) ، و *Citrobacter spp.* (سالبة للجرام). العدد الأكبر من البكتريا التي وجدت كان في مرحلة ما قبل التبريد, وقد إنخفض العدد بعد التبريد .

أغلب المكروبات التي عزلت في هذه الدراسة هي *Micrococcus spp.* من الملاحظ في هذه الدراسة أن معظم البكتريا الملوثة للحوم في المسلخ هي *Micrococcus spp.* والتي مصدرها الهواء والماء المستخدم في الغسيل ، و *Citrobacter spp.* والتي مصدرها من الإمعاء اثناء عمليات تجهيز الذبيح.

INTRODUCTION

Food can be unsafe for human consumption due to change in its biological, chemical or physical properties (Food safety inspection service, 1997).

According to Gracey (1981) the contamination by bacteria or their toxins was most important and frequent type of food poisoning.

Meat is defined as those animal tissues, which are suitable for use as food. The majority of meat consumed is derived from domesticated mammals and birds.

The contamination of meat has been reported to be due to presence of microorganisms or their toxins in amounts that render the meat unacceptable or potentially harmful to consumers.

Bownlie, (1966) reported that the keeping quality of meat and meat products depend on the number and types of the contaminating bacteria and their metabolism and rate of growth, it also depend on the physical or chemical environment.

In Sudan more problems of slaughter houses are due to poor waste disposal systems and environmental sanitation, lack of workers training and understanding of the importance of sanitation (Ibrahim ,1989).

Objectives of the study:

The present work was carried out to;

1. Assess meat hygiene in some slaughterhouses in Khartoum state.
2. Measure the bacterial load in fresh and chilled mutton produced in a slaughterhouse in Khartoum state.
3. Judge the safety of the meat produced.

CHAPTER ONE

LITERATURE REVIEW

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

1.1 Sources of contamination:

Haines,(1933); Empey and Scott, (1939) found that the sources of the bacterial contamination of meat are hides, hooves soil adhering to the hide, intestinal contents, air, water supply, knives, cleavers, saws, hooks, floors and workers.

Frazier (1967) showed that any contaminating bacteria on the knife would soon be found on meat in various parts of the carcass as it is carried by the blood. The contamination of carcasses come from different sources including environment and equipments 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, hoofs and body cavities of meat animals.

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

Hussien (1971) isolated bacterial contaminants of fresh meat from the gastro- intestinal tract and hides of the slaughtered animals and the water, halls and air deposits.

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.

According to Storke and Redmond (1966) and Druce and Thomas (1970) the bacteria which cause spoilage of chilled meat are common in soil, water and vegetation.

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

Forrest et al (1975) considered inedible offal's as potential source of meat contamination.

Lawrie (1979) reported that if a contaminated knife was used or organisms were inadvertently introduced from the skin where the main blood vessels were severed bleeding could lead to contamination of the tissues.

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

During processing contamination came from special equipments (grinders, sausage stuffers, Fillers Spices and casings).

1.2 The type of microorganisms, which cause contamination of meat:

The significance of bacteria in meat was recognized during the Era of Pasteur (1880). It was then evident that meat favors multiplication of many kinds of bacteria which may reach it from various sources besides the air (Miller, 1951).

Frazier (1967) found that meat was an ideal environment and culture medium for the growth of bacteria especially when it is minced.

Mohamed (1970) suggested that in meat industry, bacteria is classified according to their temperature requirement into three groups:

1. Psychrophilic: which grow comparatively and rapidly at temperatures below 5°C e.g. *Pseudomonas sps* and *streptococci sps*. The growth of this type is not slowed down by refrigeration.
2. Mesophilic: which grow at temperatures between 15 – 40 °C, it includes most food poisoning bacteria.
3. Thermophilic: which grow at higher temperatures 40°C and above.

Rodes and Fletcher (1966) proved that the Psychrophilic and mesophilic types of bacteria were the most important ones.

Banwart (1981) reported that the gaseous atmosphere surrounding the food might determine the types of organisms, which become dominant. Oxygen favors the growth of aerobes while lack of oxygen will allow facultative anaerobes to dominate. A source of bacteria in the carcass is the lymph node, which filter out bacteria from the lymph.

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 are needed for the production of the effective amounts of enterotoxin.

Salih (1971) isolated from fresh meat samples spoilage bacteria of the genera *Micrococcus*, *streptococcus*, *Bacillus*, *Colstridium* *Pseudomonas* and *Coli-aerogenes*. He also isolated hemolytic and coagulase positive *Staphylococci* from ovine and bovine liver and rumen samples obtained from Omdurman Central Abattior and isolated *Micrococci* and *Salmonella doblin* from ovine and bovine offals.

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

The most frequent coliform bacteria present in meat were *Escherichia coli*, *Klebsiella spp*, *Citrobacter spp*, *Enterobacter cloacae* and *Arizona spp*. (Fatima, 1985).

Hussein (1987) reported that the aerobic organisms isolated from fresh meat were *Bacillus spp*, *staphylococcus spp*, *Diphtheroides spp*, *Micrococcus spp*, *Streptococcus spp*, and *Lactobacillus spp*. While gram-negative isolates were dominated by *Escherichia coli*, *Citrobacter freundii*, *Proteus morgani*, *Alcaligene spp*, *aeromonas spp*, and *Pseudomonas spp*.

Hussein (1971) isolated from fresh meat samples *Staph epidermidis*, *Micrococcus spp*, *Escherichia coli*, *Proteus spp*, *Aeromonas spp*, *Pseudomonas spp* and *Achromobacter spp*.

1.3 The importance of meat contaminations:

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

The members of the genera *Pseudomonas*, *Actinobacter* and *Moraxella* dominated the bacterial content of unprocessed meat exposed to air at chill temperature (International Commission For Microbiological Specification for Food – I. N. C. M. S. F, 1980).

Gracey (1980) stated that the main types of bacteria involved in the spoilage are from the Gram- positive genera *Micrococcus*, *Staphylococcus*, *Streptococcus*, *Lactobacillus* *Leuconostoc*, *Bacillus* *Clostridium* *Corynebacterium* and *Microbactrium*. Meat spoilage may also be caused by bacteria from the genera *Pseudo-*

monas, Flavobacterium, Actinobacter, Achromobacter, Alcaligenes, Halobacterium, Moraxella, Escherichia and *Kelbsiella*.

Fatima (1982) isolated *Salmonella* spp, *Colostridium perfringens*, *Staphylococcus aureus* and *E. coli* from processed meat.

John et al (1988) reported that *Proteus species* are important in the spoilages of meat, because they grow and spread readily on moist surface at low temperatures and produce a number of proteases.

According to Holy and Holzopfel (1988) *Pseudomonads* are susceptible to freezing and thawing.

Brahmbhalt and Anjaria (1993) examined samples of raw meat obtained from shops; they isolated *E. coli*, *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Micrococcus luteus*, *Citrobacter freundii*, *Bacillus cereus*, *streptococcus faecalis* *Enterobactere aerogenes*, *Proteus mirabilis* *Bacillus subtilis*, *Aeromonas liquifaciens*, *Proteus vulgaris* *Kelbsiellea poneumonias* and *Pseudomonas deruginosa*.

The Foods safety and inspection Services in the USA (1997) reported the following pathogenic bacteria in meat and meat products; (*Bacillus cereus*, *Clostridium botulinum*, *Clostridium perfringes*, *E.coli*, *Salmonellae*, *Staphylococcus aureus* and *Yersinia enterocolitica*).

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 personnel or healthy carriers include *Salmonella spp*, *Shigella spp*, *Escherichia coli*, *Bacillus*, *Proteus*, *Staphylococcus albus* and *Staphylococcus aureus*, *Colstridium welchii*, *Bacillus cerues*, *Bacillus faecal* and *Streptococcus spp*.

According to Gracy (1999) describing a study in North Ireland that showed a wide range of organisms isolated from all area of the abattoir were mainly gram-positive *Bacillus*, *Coryneform*, *M. thermospactum*, *Enterobacteriaceae*, *Aeromonas*, *Vibrio*, *citrobacter*, *Hafnia*, *Serratia*, *Klebsiella like organism*, *Yersinia enterocolitica like bacteria* and *Salmonella dublin*.

Among the bacteria present in the air and dust are *Bacillus* and *Micrococcus* species, 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 its control during subsequent chilling, processing, handling, distribution and preparation (Sofos et al, 1999).

According to Ajit et al. (1990) *Salmonella* was isolated from lymph nodes of slaughter sheep. The isolates from muscles included *Escherichia coli*, *Proteus*, *Pseudomonas*, *klebsiella* and *Citrobacter*. Also the study reported that contamination after slaughter was probable in many cases.

Fatima (1990) found the aerobic plate count (APC) of meat before processing in Khartoum was 10^3 CFU/gram.

Entisar (1998) recognized different types of aerobic bacteria present in Omdurman slaughterhouse and Khartoum north retail markets, the bacteria isolated were *Micrococci and Enterobacteria*.

Salih (1971) reported heavy contamination of fresh meat in Khartoum state with spoilage bacteria of genera *Micrococci, Streptococci, Bacilli, Pseudomonas and Aerogenes*.

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

1.4 Procedure adopted in slaughterhouses to ensure hygienic safe meat production:

Dickson (1988) and Hennlich and Verny (1990) emphasized that hygienic measures promote the quality and safety of meat and improves its shelf life.

The keeping quality of meat is primarily determined by the nature and degree of initial contamination of the carcass surface (Haines, 1933; Ingram, 1972).

Meat inspection was practiced in France as early as 1162, in Britain in about 1319, in Germany in 1383, while in U.S.A meat inspection was carried out in 1884 (Ibrahim, 1990).

According to Thornton (1968) based on FAO / WHO expert committee reports (1954) and (1961) the efficient meat hygiene

practices begin in the farm. It should be maintained in the animal collection centers, markets, during transportation of animals for slaughter, in abattoirs, during transport of meat to butcheries and even at the consumer home.

To execute such programs necessary laws and guiding instructions should be laid out vividly and firmly. On the other hand basic knowledge about hygiene and sanitation should be disseminated among people especially those directly concerned with meat hygiene and quality control, i.e. farmers, butchers and consumers. This knowledge would contribute positively to the understanding of laid out policies and to establishment of proper standards. It is also necessary to study and assess the influence of social traditions and religion in the community and also the economical and environmental conditions in a particular area for achieving the goals of meat hygiene programs (Kaplan, (1957), Mann, (1960) and Echert et al, (1981).

The main objective of meat hygiene and inspection is to prevent meat spoilage and meat borne infections. The meat hygiene, inspection and control practices are based on the concept of the transmissibility of diseases through either consumption or handling of meat (Ibrahim, 1990).

Meat hygiene is essentially a public health function, the primary role of which is to safeguard against infectious disease, to prevent its transmission to human and provide safe wholesome-

meat and meat product for human consumption Goetzche, (1958) and Ibrahim and Salih, (1970).

The effective operation of meat hygiene services is multidisciplinary. They involve the veterinary medicine and engineering professions. The veterinarian is the one who is trained to deal with diseases transmitted through meat (WHO series, 1957).

Salih (1969) proposed that in order to improve the standards of meat hygiene laws of animal health should include meat hygiene regulations. He noted that there is lack of proper training of the various staff members working in the meat inspection services. He suggested that programmes should be formulated to improve their academic and technical abilities, and also suggested the establishment of a meat research center where data pertaining to meat hygiene (Number of slaughtered animals, condemnation and reasons for condemnation through out the country could be collected and analyzed). Regarding the slaughterhouses he suggested that they should be run on sound economical bases and they should be able to make some financial benefits.

1.5 Low Temperature Food preservation and characteristics of Psychrophilic microorganisms:

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

reactions of microorganisms are catalyzed and the rate of enzyme reactions is dependant on temperature.

The term Psychrophile was coined by Schmaltz-Nielsen (1902) for microorganisms that grow at 0°C. This term is now applied to organisms that grow over the range of subzero to 20°C, with an optimum range of 10 –15°C.

The Psychrotrophs are organisms able to grow at 5°C or below. It is now widely accepted among food microbiologist that Psychrotroph is an organism that can grow at temperature between 0 – 7°C and produce visible colonies (or turbidity). Within 7 –10 days. Because some Psychrotrophs are organisms that can grow at temperature at least as high as 43°C they are in fact, Mesophilles. By these definition Psychrophilles wold be expected to occur only on products from extremely cold climate. The organism that causes the spoilage of meat in the 0- 5°C range would be expected to be pschrotrophs.

There are three destined temperature ranges for low-temperature stored food: Chilling temperature: are those between the usual refrigerator (5 – 7°C) and Ambient temperature: usual about (10 – 15°C).

Freezer temperature are those at or below –18°C under normal circumstance growth of all microorganisms is prevented at freezing temperature, never the less some can grow within the freezer range but at extremely slow rate.

1.6 The effect of freezing on microorganisms:

In consider the effect of freezing on those microorganisms that are unable to grow at freezing temperature, it is well known that freezing one means of preserving microbial cultures with freezing, drying being perhaps the best method known. However freezing temperature have been shown /to effect the killing of certain microorganisms of importance in food. Ingram summarized the salient fact of what happens to certain microorganisms upon freezing.

There is sudden mortality immediately on freezing, varying with species, the proportion of cell surviving immediately after freezing die gradually when stored in frozen state.

This decline in number is relatively in rapid at temperature just below the freezing point especially about -2°C , but less so at lower temperature, and it is usually slow below -2°C .

Bacteria differ in their capacity to survive during freezing, with cocci being generally more resistant than gram-negative rods of the food poisoning bacteria, salmonella are less resistant than *staphylococcus aureus* or vegetative cell of *clostridia*, where as endospores and food poisoning toxins are apparently unaffected by low temperature.

CHAPTER TWO

MATERIALS AND METHODS

This work had been done in Khartoum state in one of government's slaughter houses which is utilized for export of mutton.

2.1 Collection of Samples:

The aim of investigation was to measure the bacterial load of export mutton as a parameter of meat hygiene. The samples were collected during the period of Jun – October 2006.

2.2 Swabs:

The samples were taken from four sites of carcasses which were i.e.: Forelimb, hind limb, neck and back samples were taken from the slaughterhouse twice, the first one was taken after skinning and washing, the second one after chilling.

2.3 Culture media:

2.3.1 Blood agar:

As prescribed by (Oxoid Lab) 40 grams of the base powder were added to one liter of distilled water. The solution was then boiled until the powder dissolving completely. The mixture is autoclaved at 121°C and 15 pound per square inch for 15 minute. It was then cooled to 45 – 50°C. 7% of sterile blood was added with gentle rotation and then poured in to petri dishes and left to solidify. The poured petri were kept in the refrigerator (about 4°C) until it is used within one day.

2.3.2 MacConkey agar:

This medium was prepared as prescribed by (Oxoid Lab) methods. 59 grams of powder were dissolved in one liter of distilled water. The solution was swirled for ten minutes until the powder was dissolved completely. It was then sterilized by autoclaving for 15 minute at 12°C and 15 pound per square inch. The Macconkey agar was cooled to 47°C, mixed well, poured in petri dishes and left to dry by the partial exposure to the air at 37°C.

2.3.3 Nutrient agar:

The medium was prepared as described by (Oxoid Lab) 25 grams of powder were added to one liter of distilled water and brought to boil to dissolved the powder completely. It is sterilized by autoclaving for 15 minute at 121°C and 15 pound per square inch.

2.3.4 Motility medium:

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

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; temperature must be 160°C for one hour (stainer et al, 1986).

2.4.2 Sterilization by red heat:

This method was used for sterilizing wire loops, straight wire and tissue forceps. It was done by holding the object over 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 115-121oC under 10- 15-pound pressure for 15-20 minute. (Barrow and Feltham, 1993).

2.5 Reagents:

2.5.1 Nitrate test reagent:

Accordint to (Bio Merieux) it consist of tow separate solution first of them the sulfanilic acid reagent was prepared by dissolving 0.4 gram of sulfanilic acid in 100 ml Acetic acid. The other solution a;pha-naphthylamine was prepared by dissolving 0.6 gram of N.N-dimethyl-1-naphylamine in 100 ml acetic acid.

2.5.2 Hydrogen peroxide:

30% Hydrogen peroxide produced by B. D. H (British Drug House) was diluted to 3% aqueous solution for catalase test.

2.5.3 Potassium hydroxide and Alphanaphthol:

According to Cowan and Steel (1974) the reagent prepared as 40% potassium hydroxide and 5% Alphanaphthol for use in Voges-proskauer test V. P.

2.6 Diluents:

2.6.1 Physiological saline:

It was prepared by dissolving 8.5 grams sodium chloride in one liter distilled water then dissolved and sterilized by autoclaving at 121°C for 15 minutes under 15 pounds per square inch pressure (Cruickshank et al, 1975).

2.7 Culturing, microscopy and identification of bacteria:

2.7.1 primary culturing and purification:

Blood Agar, MacConkey Agar and Nutrient Agar were used and Streaked By swabs from samples and then incubated at 37 °C for 24 hours. The primary culture was examined for differentiation of colonies according to the Hemolysis, size, color, surface and shape. Different types of colonies were sub cultured for purification and incubated at 37°C for 24 hours.

2.8 Staining:

Smears were prepared by emulsifying part typical and well-isolated colony in a drop of sterile and spread in a clean slide. The smears were then allowed to dry by air then fixed by gentle flaming, all smears were examined by Gram stain.

2.8.1 Gram stain:

Using sterile wire loop, part of isolates were taken and spread on microscopes slides to make thin smears. They were fixed with heat and placed on staining rack. They were covered by crystal violet for two minute and washed off by tap water, then covered with logul's iodine for one minute and washed of by tap water, then decolonization with acetone for few seconds and washed off by tap water, then covered with carbol fuchsin for thirty seconds. Finally the stained smears were washed and air dried. Then they were examined under oil immersion lens (100x). The Gram positive and negative organisms, shape and arrangement of organisms were identified according to Barrow and Feltham (1993).

2.9 Bacterial cell counting techniques:

2.9.1 Miles-Misra technique:

This method has the advantage of being economical with agar media. Lines can be drawn on the bottom of an agar plate with plate with a waterproof marker dividing it into 8 sectors.

An inoculum of 0.02 ml. Delivered as drops placed on the agar in each sectors. At least 4 drops per sample dilution should be used. The inocula are allowed to dry and the plate at 25 – 37°C

for 24-48 hours. A sample dilution yielding about 30 colonies per drop should be selected. (Anon (1982)).

2.10 Biochemical test:

2.10.1 Voges-Proskauer Test:

This reaction depends on production, from dextrose, of acetylmethylcarbinol (or acetoin). This is then oxidized, by the addition of alkali, to diacetyl, which gives pink colour.

Method: To a 2-day dextrose broth culture of the organism under test add 1ml of 10% potassium hydroxide leave at room-temperature for 1 hour.

Pink colour-positive.

No change-negative.

2.10.2 Catalase Test:

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

2.10.3 Urease test:

A slope of urea agar medium was inoculated with the test organism and incubated at 37°C for 5 days. A change of the color to red indicated a positive reaction.

2.10.4 Citrate utilization:

Inoculate Koser's citrate with straight wire or Simmon's citrate agar from a light suspension of the organism in quarter strength Ringer solution. Incubate at the optimum temperature for the test organism. Turbidity in the Koser's citrate tube or growth of a blue colour on the Simmon's agar indicates a positive result (citrate utilized).

2.10.5 Malonate utilization:

A tube of malonate-phenylalanine medium was inoculated lightly and incubated for 18-24 hours. The tube was examined for colour change and kept for phenylalanine deamination test. A positive malonate reaction was indicated by a deep blue colour, a negative reaction by the unchanged greenish colour of the medium.

2.10.6 KCN test:

A bottle of KCN broth of 1 ml amount was inoculated with one loop-full of an overnight broth culture. The bottle was screw-capped tightly and incubated for up to 48 hours. The bottle was examined after 24 hours and 48 hours for turbidity indicating growth, which constituted a positive reaction.

2.10.7 Oxidation-Fermentation (O-F) test:

Two tubes of Hugh and Leifson's medium were inoculated with the test culture, one being covered with a layer of sterile soft paraffin to a depth of about 1-2 cm. Both tubes were then incubated at 37°C and examined daily for up to 14 days. Fermentative

organism produced a yellow color in both tubes, while oxidative organism produced a yellow color in the unsealed tube only.

2.10.8 Motility test:

The isolates were studied for motility by Craigie's 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 migration of the bacteria out side the Craigie tube.

Table (1): Identification of Gram-positive isolates

Site of isolate	O.F	Motility	Nitrate	Glucose gase	GPM V.P	Anaerobic growth	Catalase
Hind limb/1/BA/1	-	-	-	-	-	-	+
Fore limb/1/BA/2	-	-	-	-	-	-	+
Back/1/BA/3	-	-	-	-	-	-	+
Neck/5/BA/1	-	-	-	-	-	-	+

BA: Blood Agar.

Table (2): Identification of Gram-negative isolates.

Site of iso- late	KCN	O.F	Malonate	Citrate	Ureas e	Motility	GPM V.P
Hind limb/1/Mc/1	+	-	+	+	-	+	-
Fore limb/1/Mc/2	+	-	+	+	-	+	-
Back/1/Mc/3	+	-	+	+	-	+	-
Neck/1/Mc/5	+	-	+	+	-	+	-

Mc: MacConky.

CHAPTER THREE

THE RESULTS

3.1 Identification of the isolates:

All samples were examined after counting of aerobic micro-organisms which were (24.046×10^6 isolates) which were Gram-positive and Gram-negative bacteria as shown as in table (3) and (4).

3.2 The frequency of isolates from different sites of the carcass in blood and MacConky agar:

Here we count the frequency of isolates from different sites in both blood and MacConky agar. The result showed that hind limb had higher frequency in blood agar as in table (5), and neck had higher frequency in MacConky agar, as in table (6).

3.3 The frequency of isolates from total samples:

The frequency of different genera was counted from the total count of the contaminating bacteria, and we found that *Micrococcus spp* was higher than *Citrobacter spp*. *Micrococcus spp* was 74.9% and *Citrobacter spp* was 25.1% as in table (7).

3.4 The frequency of isolation from the different sites of the carcass:

The results showed that the frequency of contamination of the hind limb was the highest as compared to the other sites of the car-

cass, mean that the back was the least contaminated as shown in table (8) and histogram No.(1).

Table (3): Types of aerobic bacteria isolated from carcasses before chilling:

Gram-positive bacteria	Gram-negative bacteria
<i>Micrococcus spp</i>	<i>Citrobacter spp</i>

Table (4): Types of aerobic bacteria isolated from carcasses after chilling:

Gram-positive bacteria	Gram-negative bacteria
<i>Micrococcus spp</i>	<i>Citrobacter spp</i>

Table (5): The frequency of the isolate from different sites of the carcass in blood agar:

Site of the carcass	Frequency of the isolation	Total count
Hind limb	52.18	8.962×10^6
Neck	27.86	4.784×10^6
Fore limb	11.27	1.936×10^6
Back	08.69	1.492×10^6

Table (6): The frequency of the isolates from different sites of the carcass in MacConky agar:

Site of the carcass	Frequency of the isolation	Total count
Neck	55.56	3.818×10^6
Hind limb	23.66	1.626×10^6
Forelimb	19.40	1.333×10^6
Back	01.38	0.095×10^6

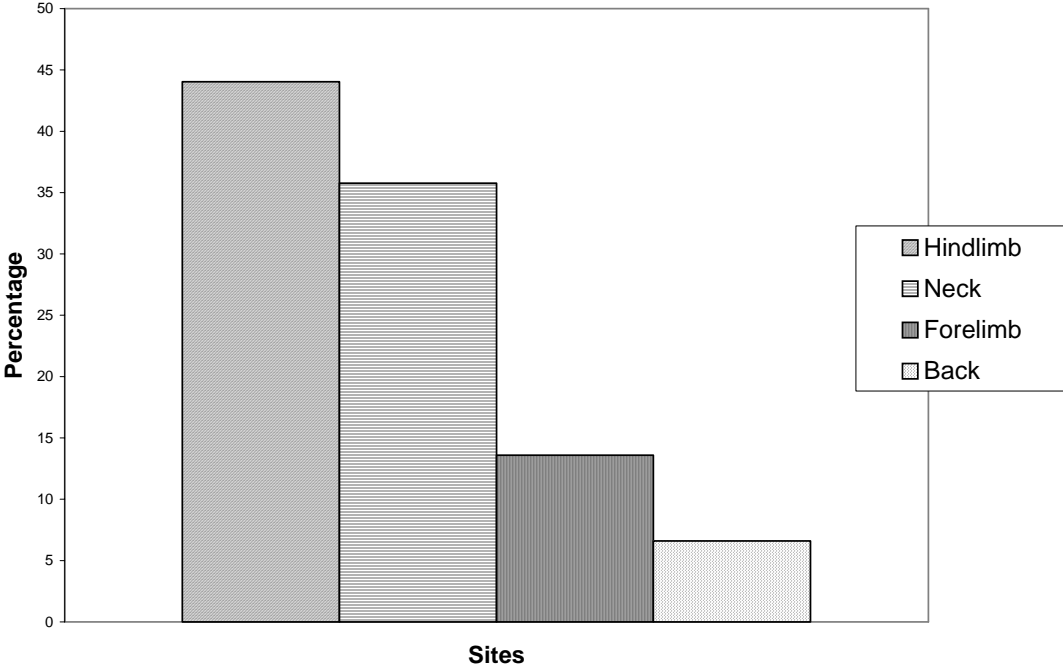
Table (7): The frequency of the isolation of the different genera of bacteria:

Type of isolate	Frequency of the isolation
<i>Micrococcus spp</i>	74.9
<i>Citrobacter spp</i>	25.1

Table (8): The frequency of isolation from different sites of carcass:

Sites of carcass	Percentage
Hind limb	44.03
Neck	35.77
Forelimb	13.60
Back	06.60

Figur no.(1): The frequency of contamination in different sites of the carcass:



CHAPTER FOUR

DISCUSSION

Most of the meat contamination is caused by aerobes. These organisms may gain access to meat from the system of living animal or as a result of slaughter contamination (Lawrie, 1979).

Meat contamination is of economic importance because it in-verse the meat quality.

Poor meat hygiene practices in the slaughterhouses before and after slaughter would lead to meat contamination.

FAO/ WHO (1962) and Thornton (1968), emphasized that meat hygiene should be observed at all stage of meat production till it reaches the consumer as fresh, sound, wholesome and safe meat.

The aerobic bacterial species in the present study were *Micrococcus* spp. and *Citrobacter* spp. These finding are in agree-ment with finding of Brahmhalt and Anjaria (1993). Who iso-lated *Escherichia coli*, *Bacillus cereus*, *Staphylo coccus aures* and *Micrococcus* spp. From raw meat. Mean while Brahamhalt and anjaria (1993) isolated *Staphylococcus epidermidis*, *Citrobacter freundii*, *streptococci faccalis*, *Entrobacter aerogenes*, *proteus mirabilis*, *Bacillus subtilis*, *Aeromonas liquifaciens*, *proteus vul-garis*, *Klebsiella pneumonias* and *pseudomonas aeruginosa*.

These finding are also in agreement with Amanie (2000) who isolated *Micrococcus* spp, *Staphylococcus leutus*, *Staphylococcus*

auricularis and *Escherichia coli* from meat at stages of processing she also isolated *Bacillus firmus*, *Bacillus pantothenicus*, *Bacillus thuringiensis*, *Baccillus anyaligufaciens*, *Aerococcus spp.* *Proteus mirabilis*, *psendomoas psendolcaligenes*, *Shewanella Putrefaciens*, *Acinetobacter lowff* and *Acinefobacter calcoacetus*, which I failed to identify in this study.

The present studies revealed that, the gram-poitive aerobes are the most frequently isolated bacteria. This observation is in agreement with Hussein (1987). Who reported that gram-positive was most frequently isolated from both fresh and refrigerated beef at different intervals of time. But this observation dose not agree with imwidihaya et al (1987). Who found that the fresh meat samples were contaminated mainly by gram-negative bacteria.

Micrococcus spp which were isolated have no importance in public health and their isolation from meat is a normal phenomenon. This bacteria may originate from environment and exposure of meat to more handling by the workers. This information was in agreemen with Omer (1990) who noticed that *Micrococcus* is a harmless suprophytic bacteria.

According to Barrow and Feltham (1993). These organisms (*Micrococcus spp*) were comonly encountered in routine laboratories either as environmental or as commensal from normal skin and only occasionally from infections.

Jay (1986) reported that Micrococci were widely distributed on skin of man and hides of animal as well as in dust, water soil and many foods.

Ajit et al. (1990) isolates from muscle, included *Escherichia coli*, *proteius*, *Pseudomonas*, *Klebsiella* and *Citrobacter* This agree with my isolate specially *Citrobacter* spp which gram-negative genera.

Also the present studies agree with Salih (1971) who reported heavy contamination of fresh meat in Khartoum state with spoilage bacteria genera like *Micrococcus*, *Streptococci*, *Bacilli*, *Pseudomonas* and *Aerogenes*.

Thronton (1952) reported that the types of bacteria expected in the slaughter house were *Staphylococcus*, *Micrococcus*, *Bacillus*, *Pseudomonas*, *Achramobacterium*, *Aerobacter* and *Coliforms*.

The most frequently isolated bacteria in this study from both fresh and chilled mutton were *Micrococcus* spp.

The higher bacterial counts obtained during this work may be due to surface contamination of meat, which came from different sources, mainly hides, hoofs, air, water, equipments, intestinal contents and slaughtering floor (Haines, 1993; Empey and Scott, 1939; String, Bilskie and Nauman, 1969).

Psychrotrophic bacteria which include potential spoilage organisms of chilled meat were common in soil, water and vegetation (Strockes and Remond, 1966; Durce and Thomas, 1970).

In this study the surface region of neck and hind limb had the highest rate of contamination compared to all parts of the carcass. This was due to the handling and contamination by intestinal contents.

In this study the hind limb had higher rate of contamination. This was due to handelling, washing water and air.

Also in this study we reported that the hind limb and the back had high contamination by Gram-positive bacteria compared with Gram-negative bacteria. This was due to of the processing of the carcass in the slaughterhouse, which means that they were a way from contamination by intestinal contents. As we reported that the neck and the forelimb had high contamination by Gram-negative bacteria, and this was due to contamination by intestinal contents.

Conclusion and recommendation

Conclusion:

- 1- It was found that contaminant bacteria were Gram-positive and Gram-negative bacteria.
- 2- Gram-positive were the more dominate than gram-negative bacteria.
- 3- The contamination was high in the condition before chilling than after chilling.
- 4- The high contaminated site of the carcass was hind limb, and the neck was also high contaminated but less than hind limb and more than forelimb and back.
- 5- The sources of contamination of mutton intended for export were: water, air, intestinal contents and the workers whom handled the meat during the processing of meat.

Recommendation:

- 1- The system of working in slaughterhouses should be contain the sanitation and training for workers and adopted them to use clean clothes and gloves.
- 2- The system of washing in the slaughterhouse must be used pure and healthy w
- 3- Cleaning and sterilization of knives and machines must be used in slaughterhouses so as to reduce the contamination.

CHAPTER FIVE

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