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Load and types of Bacteria in Meat during retail display at Khartoum Locality

By:

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Dedication

This research is dedicated to all health workers

To my family,

And to my colleagues

Khalid

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Acknowledgement

After all thanks to ALLAH for everything.

Special thanks to the supervisor Dr. Elrasheed A. Ali.,

Thank to all staff in the department of food hygiene and safety.

Thank you all, for your precious time and may ALLAH bless your steps.

Abstract

Background:

Meat nationally and internationally is the most nourishing and biggest food commodity, yet could easily spoil or carry very serious diseases to human, through many contaminating hazards especially microorganisms. *Enterobacteriacae* (*Escherichia, Salmonella, Klebsiella, Shigella*) and *Staphylococci* other than being pathogenic are usually considered by health authorities as critical indicators to food hygiene.

Study design:

Cross-sectional analytical study

Objectives and setting:

To identify the levels of meat contamination by determining the total viable counts on meat.

To determine the percent of market meat contaminated by *Staphylococci* generally and *Staphylococcus aureus* specifically.

To investigate the levels of carcass contamination by *Enterobacteriaceae* (*Escherichia*, *Salmonella*, *Klebsiella*, *Shigella*).

Methodology:

Hundred beef carcasses displayed at different retail butchers shops at Khartoum locality were randomly nominated for this study (Samples were collected during 1/2/2009 – 28/4/2009). 10cm^2 swabs area per carcass were hygienically collected and transferred to faculty laboratory for microbiological investigations. Each swab tested for total viable count. Isolation and identification of specific microorganisms - four Species of *Enterobacteraceae*

(*Escherichia, Salmonella, Klebsiella, Shigella*) and *Staphylococcus aureus*. (Cheesbrough, 1999).

Results:

The total counts revealed very high contamination levels (medium number in all carcasses was between 1x 10^5 to 2.89x10^7 , *Staphylococcus aureus* percent of carcasses contamination was 18%, *Salmonella* were 8%, *Escherichia coli* 7% *Shigella* 3% and *Klebsiella* were 3%.

Conclusion:

The study concluded that meat hygiene at Khartoum locality is really questionable and needs extensive hygienic efforts, research implementation of hygienic standards, use of appropriate and recommended technology as in Hazard Analyses Critical Control Point (HACCP) during transportation and display of meat. Training of meat handlers on hygienic measures and keen application of meat legislation.

المستخلص

خلفية:

محليا وعالميا اللحوم هي أجود المواد المغذية واكبر سلعة غذائية ماديا ومع ذلك أسهل المواد الغذائية قابلية للتلف وتنقل أمراض خطرة على صحة الإنسان وذلك من خلال قابلية تلوثها بعدة مخاطر أهمها الجراثيم. عائلة (*Enterobatenacae* متمثلة في *Escherichia, Salmonella, Klebsiella, Shigella,*) وعائلة ال *Staphylococci* غير أنها من الجراثيم الممرضة تؤخذان كمؤشرات حرجة لدرجات التلوث الجرثومي في الأغذية.

تصميم الدراسة:-

دراسة جرثومية تحليلية (Cheesbrough, 1999) .

أهداف الدراسة :-

تحديد مستوى التلوث الجرثومي في اللحوم المعروضة في أسواق محلية الخرطوم وذلك بمعرفة العد الجرثومي الحي الملوث للحوم .

تحديد النسب المئوية للحوم الملوثة بجراثيم *Staphylococci* عامة وجرثومة *Staphylococcus. aureus* خاصة .

دراسة مدى انتشار تلوث اللحوم بجراثيم من عائلة *Enterbacteria (Escherichia, Salmonella, Klebsiella, Shigella*)

طريقة البحث:

أختيرت مئة عينة عشوائية من أجسام الذبائح البقرية المعروضة في جزارات محلية الخرطوم بغرض الدراسة (جمعت العينات خلال 2009/2/1 الى 2009/4/28م)، اخزت مسحات من سطح الذبائح بمساحة 10سم2 وتم حفظها و ترحيلها بطريقة صحية إلى معمل كلية الصحة للتحليل الجرثومي .

كل عينة تم تحديد العد الجرثومي الحي الكلي لها كما تم التعرف على أعداد وأنواع جراثيم مختارة مسبقا من عوائل *Enteroteabacteria* هي *Escherichia, Salmonella Klebsiella, Shigella*,)كذلك فحصت كل عينة لمعرفة مدى تواجد جرثومة *Staphylococci* مع التركيز على جرثومة

Staphylococcus aureus خاصة . اتبعت الطرق الصحية الحديثة المحددة لعزل والتعرف على الجراثيم (Cheesbrough, 1999).

النتائج : -

دلت الدراسة على مستويات عالية من التلوث الجرثومي على اللحوم المعروضة في أسواق محلية الخرطوم حيث بلغ بين ($1x\ 10^5$ to $2.89x10^7$).

نسبة الذبائح الملوثة بجراثيم *Staphylococci* بلغت 18%) كما كانت نسب التلوث بجرثومة *Salmonella* (8%) وال *Escherichia* (7%) وال *Shigella* (3%) و *Klebsiella* (3%)

الخاتمة : -

أوضحت الدراسة إن صحة اللحوم المعروضة بمحلية الخرطوم مشكوك في صلاحيتها وتحتاج لجهود صحية كبيرة كالأبحاث وإتباع الطرق الصحية الموصى بها لسلامة اللحوم . إتباع النظم الحديثة لعرض وترحيل اللحوم الموصى بها في نظام (تحليل المخاطر والتحكم في النقاط الحرجة (HACCP) العالمي .

كما أوصت الدراسة بتدريب العاملين في مجال صحة اللحوم وتثقيفهم صحيا مع مراعاة تطبيق القوانين واللوائح الصحية الموضوعة في مجال صحة اللحوم .

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Chapter One

- Introduction
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Introduction:

Red meat is primarily defined as the voluntary striated skeletal muscular tissue of red meat animals. The muscle is made up of contractile myfibrillar proteins. Soluble sarcoplasmic proteins. (Roberts, et al; 1998).

Meat is the parts of a hygienically slaughtered animal intended for human consumption (Elrasheed, 2008).

Red meat is derived from a number of animal species (e.g. cattle, sheep, goat, camel, deer,). Total world production of red meats was estimated to be approximately 120 million tones carcass weight in 1991. Red meat is important in international trade with about 11 million tones per year being exported.

Red meat has the potential of carrying harmful organisms to consumers. The food-poisoning organisms which can constitute a hazard in at least some meat products include *Salmonella* spp., Enterohaemorrhagic_*Escherichia coli* (e.g. serotype 0157), some serovars of *Yersinia enterocolitica, Campylobacter jejuni, Staphylococcus aureus, Listeria monocytogenes, Clostridium perfringens, ,* and *Bacillus cereus.* Meats are also subject to microbial spoilage by a range of microorganisms which include *Pseudomonas spp*. *Enterobacteriaceae, lactic acid bacteria*, yeasts and moulds (Roberts, et al; 1998).

Red meat animals can be infected by or carry a wide range of microorganisms, which are potentially pathogenic to man. Causing zoonotic diseases, like Salmonellosis and tuberculosis. And pathogenic serotypes of *Escherichia coli*, such as O157:H7.

Justification:

Global meat trade and meat consumption patterns is changing drastically due to change in lifestyle, incomes and health concerns.

Meat like other foods can transmit certain diseases caused by microorganisms. In Sudan there are so many factors that contribute to meat contamination, which are obviously seen from the poor handling; during processing, distribution and display of meat to the consumers.

Thorough research in meat hygiene in Sudan is badly needed to correct the actual situation by improving hygiene, legislations, adopting international codes of practice, e.g. Hazard Analyses Critical Control Point (HACCP) and Good Manufacturing Practices (G.M.P).

Objectives

General objective:

To identify and quantify the types of bacteria in raw red meat at Khartoum locality.

Specific objectives:

- To identify the levels of meat contamination by determining the total viable counts on meat.
- To determine the percent of market meat contaminated by *Staphylococcus* generally and *Staphylococcus aureus* specifically.
- To investigate the levels of carcass contamination by *Enterobacteriaceae* (*Escherichia, Salmonella, Klebsiella, Shigella*).

Properties of Meat:

Meat has a high water (75%) and protein (19%) content, low in carbohydrate (1%) and contains a number of low- molecular weight soluble constituent, the vitamin content(Ug/g) of muscle is approximately; thiamine 1, riboflavin 2,niacin 45, folic acid 0.3, pantothenic acid 10,B_3& B_{12} 0.02 ,and biotin 0.04 . The concentration of vitamins varies with species, age and muscle. Pork muscle has five to ten times more thiamine than in beef or sheep muscle. Vitamins tend be higher in certain organs (e.g. Liver and kidney).

Meat is a highly nutritious substrate with a_w (0.92-0.95) suitable for the growth of most microorganisms. Growth primarily at the expense of low-molecular weight material (carbohydrate (lactates) and amino acids). Microbial proteolysis of structural proteins occurs at a very late stage of spoilage (Dainty et al, 1975). Since muscle is relatively strongly buffered and the content of utilizable carbohydrate in post rigor muscle is usually low; microbial growth does not lower the pH significantly.

At the death of an animal when the oxygen supply to the muscle is cut off anaerobic glycolsis of stored glycogen to lactic acid lower the pH. Post- mortem glycoysis continues as long as glycogen is available until a pH is reached which inhibits the glycoltic enzymes. In typical muscles especially in pork the pH 5.55. In some muscle (e.g. Beef sternocephalicus muscle) glycolysis ceases at a pH near 6, even though considerable glycogen remains. The ultimate pH varies between muscles of the same animal and between animals, and is determined by the amount of glycogen. The pH of post rifor muscle can vary from 5.4- 5.5 (lactate content close to 1%) to pH 7.0 (very little lactate present). The lactate content of muscle is inversely proportional to its pH. On the surfaces of beef and sheep carcasses, the availability of oxygen permits aerobic metabolism to continue and much of the exposed surface tissue has pH above 6 (Carse and Locker, 1974).

In the live animal, the glycogen concentration of muscle averages 1%, but varies considerably. Glycogen in pig muscle is depleted relatively readily by starvation and moderate exercise while in the muscles of cattle glycogen is more resistant to starvation and exercise. In both species, pre- slaughter stress (e.g. excitement, cold) deplete muscle glycogen. Glycogen is more concentrated in liver (2-10%) than in muscle and its content is also affected by pre- slaughter conditions. A low concentration of glycogen in muscles results in a high ultimate pH which gives rise to (dark- cutting) beef, or dark, firm and dry meat (DFD).

The amount of glucose in post – rigor muscle with PH being virtually absent in muscle of pH > 6.44. In normal pH (5.5-5.8) muscle, glucose is present at a bout 100-400 Ug/g. (Gill, 1976). Liver has high glucose content (3-6 mg/g) which appears to be independent of pH (Gill, 1998).

By the time the ultimate pH is reached, adenosine triphosphate (ATP) has largely broken down to inosine monophoshate (IMP). During the storage of meat, IMP and inosine continue to degrade to hypoxanthine ribose and ribose phosphate. Ribose, inosine and IMP can be used as energy sources by a number of fermentative Gram- negative bacteria,and ribose can be used by Broc. thermosphacta and a number of lactic acid bacteria.

Fatty tissue has lower water content than muscle, a pH near neutrality with little lactate, and contains low- molecular weight components (glucose and amino acids) from serum (Gill and Penney, 1986).

Bacteria in meat:

Salmonella:

Salmonella is a genus of rod-shaped Gram-negative enterobacteria that causes typhoid fever, paratyphoid fever, and foodborne illness (Ryan and Ray,

2004), representing a more serious form of *Salmonella* infection than the other *salmonellae* (Jay .*et. al*,1997).

They are motile in nature and produce hydrogen sulphide (Giannella, 1996).

The incidence of *salmonellae* on beef and sheep carcasses varies widely. Sometimes *salmonellae* are rarely found (Biemuller *et al,* 1973). Sometimes they can be found on close to half of the carcasses (Oosterom and Notermans, 1983), and at other times on all carcasses from a herd. In a large survey in the USA, *salmonellae* were found on 1%of excised 25 g samples of brisket from 3075 chilled carcasses of steers, heifers, bulls and cows and on 5% of samples from 39.7 calves (Hogue *et al,* 1993).

The extent of carcass contamination is strongly influenced by the incidence and numbers of *salmonellae* in the intestinal tract and, for sheep and cattle, by the contamination from fleece and hide. It is also influenced by the care taken during slaughter and dressing. The *Salmonellae* status animals at slaughter are determined by contamination acquired at the farm and by holding conditions before slaughter. *Salmonellae* can be found in the internal tissues liver and spleen from apparently normal animals ·

Normally, only low numbers of *Salmonella* are on carcass or offal meats. However, inadequate chilling, storage or transport, at temperatures above about $7C^{O}$, can permit growth. Outbreaks of salmonellosis can follow from inadequate cooking, mishandling and recontamination. Raw meat can act as a source of cross-contamination to cooked meats, or other foods, in the kitchen or in meat processing plants.

The numbers of *Salmonellae* on beef and sheep carcasses varies widely. Sometimes are rarely found while can be found on close to half of the carcass and times on all carcasses (Grau and Smith.1974)

In a large survey in USA, *Salmonellae* were found on 1% of 3075 chilled carcasses of cattle and 5% of sample from 397 calves (Hogue and et al; 1993).

Contamination rates for animals in Egyptian abattoirs were: Buffalos 1-2%. Camels up to 44 (Elmoula; 1978); sheep 3-4% and wide range of serotypes were found.

Normally only small numbers of *Salmonellae* are on carcass or offal meat ,but inadequate chilling ,storage or transport ,at temperature above $7C^{o}$ can permit growth and usually influenced by numbers in the intestinal tract and contamination of fleece and hides. Outbreaks of Salmonellosis can follow inadequate cooking mishandling and recontamination .Raw meat can act as a source of cross-contamination of cooked meats or other foods in the kitchen and refrigerators.

Escherichia coli:

Certain strains of *E. coli*, such as O157:H7, O121 and O104:H21, produce toxins. Food poisoning caused by *E. coli* are usually associated with eating unwashed vegetables and meat contaminated post-slaughter. O157:H7 is further notorious for causing serious and even life-threatening complications like hemolytic-uremic syndrome (HUS). This particular strain is linked to the 2006 United States E. coli outbreak of fresh spinach. Severity of the illness varies considerably; it can be fatal, particularly to young children, the elderly or the immunocompromised, but is more often mild. *E. coli* can harbor both heat-stable and heat-labile enterotoxins. The latter, termed LT, contains one 'A' subunit and five 'B' subunits arranged into one holotoxin, and is highly similar in structure and function to Cholera toxins. The B subunits assist in adherence and entry of the toxin into host intestinal cells, while the A subunit is cleaved and prevents cells from absorbing water, causing diarrhea. LT is secreted by the Type 2 secretion pathway. (Evans, et al. 2007).

A low percentage of cattle may carry - enterohaemorrhagic *E. coli 0157:H7* in the intestinal tract at slaughter .Care taken during evisceration and hide removal can limit, but not entirely prevent, contamination of the carcass. Growth of the organism can occur if chilling, storage or transport conditions of the carcass are inadequate (temperature above about $7C^{o}$).

Inadequately cooked ground beef, contaminated with *E. coli OI57:H7*, has caused a number of outbreaks of bloody diarrhoea (haemorrhagic colitis) and haemolytic uraemic syndrome (Doyle, 1991).

Cooking hamburgers to an internal temperature of 68°C has been recommended (Meng *et al, 1994).*

Other verotoxin-producing serotypes (e.g. O26:Hll. O103:H2. O111: NM, O113:H21, 0157:NM) associated with human bloody diarrhoea and haemolytic uraemic syndrome have also been isolated from sheep, calf and cattle faeces (Wells *et aI,* 1991).

Shigella:

Shigella is a genus of the bacterial family *Enterobacteriaceae.* Shigellae are Gram-negative, nonmotile, non-spore forming, rod-shaped bacteria, very closely related to *Escherichia coli.* (Ewing, 1986)

Shigellosis is an infectious disease caused by various species of *Shigella.* People infected with *Shigella* develop diarrhea, fever, and stomach cramps starting a day or two after they are exposed to the bacterium. The diarrhea is often bloody. Shigellosis usually resolves in 5 to 7 days, but in some persons, especially young children and the elderly, the diarrhea can be so severe that the patient needs to be hospitalized. A severe infection with high fever may also be associated with seizures in children less than 2 years old. Some persons who are infected may

have no symptoms at all, but may still transmit the *Shigella* bacteria to others. (Brenner, 1984)

Shigella were discovered over 100 years ago by a Japanese microbiologist named Shiga, for whom the genus are named. There are four species of *Shigella: boydii, dysenteriae, flexneri, and sonnei. Shigella sonnei,* also known as "Group D" *Shigella,* accounts for over two-thirds of the shigellosis in the United States. *Shigella flexneri,* or "group B" *Shigella,* accounts for almost all of the rest. Other types of *Shigella* are rare in this country, although they are important causes of disease in the developing world. One type, *Shigella dysenteriae* type 1, causes deadly epidemics in many developing regions and nations. (Brenner, 1984)

Determining that *Shigella* is the cause of the illness depends on laboratory tests that identify the bacteria in the stool of an infected person. Some of the tests may not be performed routinely, so the bacteriology laboratory should be instructed to look for the organism. The laboratory can also do tests to determine which type of *Shigella* is involved, and which antibiotics, if any, would be best for treatment. (Ewing, 1986)

Shigella are transmitted from an infected person to another who become infected. *Shigella* are present in the diarrheal stools of infected persons while they are sick and for a week or two afterwards. Most *Shigella* infections are the result of the bacterium passing from stools or soiled fingers of one person to the mouth of another person. This happens when basic hygiene and handwashing habits are inadequate. It is particularly likely to occur among toddlers who are not fully toilet-trained. Family members and playmates of such children are at high risk of becoming infected. The spread of *Shigella* from an infected person to other persons can be stopped by frequent and careful handwashing with soap, a practice that is important among all age groups. (Brenner, 1984)

Part of the reason for the efficiency of transmission is because a very small inoculum (10 to 200 organisms) is sufficient to cause infection. As a result, spread can easily occur by the fecal-oral route and occurs in areas where hygiene is poor. Epidemics may be foodborne or waterborne. *Shigella* can also be transmitted by flies. (Ewing, 1986)

Shigella infections may be acquired from eating food that has become contaminated by infected food handlers. Vegetables can become contaminated if they are harvested from a field with contaminated sewage or wherein infected field workers defecate. Flies can breed in infected feces and then contaminate food. *Shigella* infections can also be acquired by drinking or swimming in contaminated water. Water may become contaminated if sewage runs into it, or even if someone with shigellosis swims or bathes or, much less, defecates, in it. (Brenner, 1984)

Klebsiella:

The most distinctive bacteriologic features of the genus *Klebsiella* are the absence of motility and the presence of a polysaccharide capsule. This gives colonies a glistening, mucoid character and forms the basis of a serotyping system. Over 70 capsular types have been defined, including some that cross-react with those of other encapsulated pathogens, such as *Streptococcus pneumoniae* and *Haemophilus influenzae*. Limited studies suggest that the capsule interferes with complement activation in a way similar to the other encapsulated pathogens. Several types of pili are also present on the surface and probably aid in adherence to respiratory and urinary epithelium. (Ryan& Ray, 2004).

Klebsiella pneumonia:

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

the most important member of the *Klebsiella* genus of *Enterobacteriaceae*; it is closely related to *K. oxytoca* from which it is distinguished by being indole-negative. It naturally occurs in the soil and about 30% of strains can fix nitrogen in anaerobic condition (Postgate, 1998).

K. pneumoniae, the most common species, is able to cause classic lobar pneumonia, a characteristic of other encapsulated bacteria. Most *Klebsiella pneumonias* are indistinguishable from those produced by other members of the Enterobacteriaceae. Of all the *Enterobacteriaceae, Klebsiella* species are now among the most resistant to antimicrobics. (Ryan& Ray, 2004).

Campylobacter jejuni:

After slaughter, C. *jejuni* have been found on from 19 to 70% of sheep carcasses, from 2 to 32% of adult cattle carcasses, and from 20 to 97% of calf carcasses.

Campylobacters (mostly C. *coli)* have been found on 20-60% of pork carcasses. In a Canadian survey. campylobacters were found on 12% of pork. 15% of beef and 35% of beef neck samples taken from carcasses before chilling. During carcass chilling. there is a significant reduction in the incidence and number of viable campylobacters, and. even on originally relatively heavily contaminated carcasses, numbers are usually less than l/cm^2.Additional drying that occurs during storage and transport will further reduce campylobacters. (Wells et al ,1991).

Contamination rates on offal meats at retail sale are higher than on carcass meats. This is probably due to higher initial contamination rates at slaughter and better survival on offals which do not undergo as much drying during chilling.

Epidemiological evidence indicates that carcass meat of cattle. sheep and pigs are relatively .minor causes of human campylobacter infection (Lammerding *et ai.,* 1988).

A small percentage of cattle carry Enterohaemorrhagic E. coli in intestinal tract. Care during evisceration and hide removal is important.

Growth occurs if chilling, transport and storage conditions are inadequate (temperature above 7C^{o}).

Other verotoxin-prducing serotypes (e.g.026:H11,0103:H2, 0111:NM,0157H7) associated with human bloody diarrhea and heamolytic ureamic syndrome have also been isolated from sheep ,calf and cattle faeces (Wells et al ,1991).

Staphylococcus aureus:

During slaughter and dressing, carcasses of ruminants become contaminated from the skin of the animal, the equipment used (e.g. mesh protective gloves and aprons), and the hands of the workers (Peel *et al,* 1975). Chilling storage or transport at temperatures below 7°C will prevent growth. Even at high temperatures, on raw unprocessed meat the organism is a poor competitor and is outgrown by other flora. For sufficient enterotoxin to be produced to cause food poisoning, *Staph. aureus* has to grow to a population of at least 10^6/g. In addition, strains of *Staph. aureus* from animal sources appear less likely than those of human origin to produce the enterotoxins (e.g. enterotoxin A) that are commonly implicated in food poisoning. Staphylococcal food poisoning is not caused by raw unprocessed meat (Devriese, 1990).

Listeria monocytogenes:

This, and other *Listeria* spp., can contaminate carcasses from the hide or fleece of cattle and sheep, and from surfaces in the slaughter and dressing area. There are opportunities for additional contamination as carcasses are moved through chillers, storage cold-rooms and transport to retail sale. Some growth can also occur even under adequate chilling conditions ($\leq 5^{o}$C). Thorough cooking will

destroy the organism. Raw meat can be one of the sources for contamination of ready-to-eat processed meats (Gobat and Jemmi, 1991).

Clostridium botulinum:

Most of the clostridia that occur in raw meats are harmless putrefactive mesophiles. However, from time to time *CI. botulinum* occurs. Estimates of contamination range from <0.1 spore to 7 spores per kg (Lucke and Roberts, 1993).The incidence appears to be lower in beef and lamb than in pork. There are no means available to guarantee the absence of *C.botulinum* from raw meat. Most cases of meat-borne botulism have been from improperly preserved, home-produced processed meats that have been eaten without prior cooking (Tompkin. 1980).

However ,consumption of raw meat from marine mammals handled under conditions in which psychrotrophic non- proteolytic *Cl. Botulinum* strains have grown , has caused outbreaks of botulism in the inuit population of Northern Canada and Alaska . Apart from this ,no cases of botulism from consumption of fresh meat has been reported (Lucke and Robert ,1993)

Clostridium perfringens:

This is a common surface contaminant of beef, and sheep carcasses at slaughter. It occurs in low numbers (<200/100 cm2) and mainly as vegetative cells.

Contamination is from faecal material and from soil and dust on the skin of the animal. The internal tissue of offal (e.g. liver) may contain small numbers of *cl. perfringens* fresh meat is stored at temperatures (<15°C) that are too low to allow growth.

Viable vegetative cells will tend to decrease in numbers during chill storage and will be destroyed in thorough cooking. Food poisoning results from the

survival of spores in cooked meats and considerable growth (to $>10^5$ cfu/g) during inadequate cooling of the cooked product (holding for some hours 4 between 15and 50°C) (Bauer *et at.,* 1981).

Yersinia enterocolitica

The incidence of pathogenic serotypes of *Y. enterocolitica* on carcasses can range from low, none detected on 210 carcasses (De Boer and Nouws, 1991), to 2.5% to very high 31% or even 63% (Nesbakken ,1988) . Care taken during evisceration has a marked influence on the extent of contamination with *Y. enterocolitica* careful removal of the tongue and tonsils is also needed to prevent carcass contamination.

Epidemiological studies have associated human yersiniosis with the consumption of minced raw pork (Belgium ; Tauxe et al; 1987), of undercooked pork and ,in the US , of chitterlings, Household preparations of chitterling has also been implicated with an outbreak in children (Lee et al ; 1990). There is also a strong geographic correlation between the serotypes of strains isolated from pigs and from humans. Similarly, biochemical, phagetyping and DNA analysis have been unable to distinguish between. However, though pathogenic serotypes can grow at 1^0C,growth is slow relative to that of spoilage microorganisms ,and pathogenic strains are not good competitors . Consequently ,the extent of growth in normal minced meat is restricted in the presence of the background flora at temperature up to at least 15^0C. A major problem may be the use of head-meat, particularly when remnants of tonsils and oral mucosa are present ,in the production of minced raw pork. (Nesbakken ,1988).

Primary sources and routes of microorganisms to fresh meats:

It is generally agreed that the internal tissues of healthy slaughtered animals are free of bacteria at the time of slaughter, assuming that the animals are not of

exhaust, but meat fresh examines and poultry at the retail level, varying numbers and types of microorganisms are found. (James, et al; 2005).

The following are the -primary sources and routes of microorganisms to fresh meats with particular emphasis on red meats:

The stick knife:

After being stunned and hoisted by the hind legs, animals such as steers are exsanguinated by slitting the jugular veins with what is referred to as a "stick knife." If the knife is not sterile, organisms are swept through the bloodstream, where they may be deposited throughout Lie carcass.

Animal hide:

Organisms from the hide are among those that enter the carcass via the stick knife. Others from the hide may be deposited onto the dehaired carcass or onto freshly cut surfaces. Some hide biota becomes airborne and can contaminate dressed out carcasses.

Gastrointestinal tract:

By way of punctures, intestinal contents along with the usual heavy load of microorganisms may be deposited onto the surface of carcasses, especially important in this regard is the rumen and intestine of ruminant animals, which typically contains 1010see again bacteria per gram.

Hands of handlers:

This is a big source of contaminant to fresh meat. Even when gloves are worn, organisms from one carcass can be passed on to other carcasses.

Containers:

Meat cuts that are placed on nonsterile containers may be expected to become contaminated with the organisms from containers.

Handling and storage environment:

Circulating air is not an insignificant source of organisms to the surfaces of all slaughtered animals.

Lymph nodes:

In the case of red meats, lymph nodes that are usually embedded in fat often contain large numbers of organisms, especially bacteria. (James, et al; 2005).

Contamination of red meat:

Meat can easily be contaminated with microorganisms and support their growth if not properly handled, processed and preserved. Extensive contamination, or abusive conditions of handling and storage that allow microbial proliferation, increase the potential for presence of pathogenic bacteria and formation of toxins, and may lead to product spoilage and public health problems (Sofos et al., 1999).

A variety of sources, including air, water, soil, feces, feed, hides, intestines, lymph nodes, processing equipment, utensils and humans, contribute to the microbial contamination of the sterile muscles of healthy animals during slaughter, fabrication, and further processing and handling (Bell, 1997).

The types and extent of contamination depend on sanitation procedures, hygienic practices, product handling and processing, application of decontamination interventions, and conditions of storage and distribution. Contamination with spoilage microorganisms may lead to product and economic losses, while presence of pathogens or their toxins may be the cause of foodborne disease that may lead to loss of human life (Sofos, 1994).

Highly publicized outbreaks of foodborne disease caused by pathogens, such as *Escherichia coli* O157:H7 and *Listeria monocytogenes*, have increased consumer concerns and interest in food safety (Sofos and Smith, 1993).

Sources and Extent of Contamination:

Animal contamination:

Live animals are often highly contaminated, or are asymptomatic carriers of pathogenic bacteria (Fedorca-Cray et al., 1998), and can serve as sources of subsequent meat contamination. Animal cleanliness is influenced by climate, geographic location, method of transportation and holding conditions. For example, animals raised on pastures may carry more bacteria of soil origin, while microorganisms of intestinal origin may be more common on carcasses from animals finished in feedlots (Sofos, 1994).

Carcass contamination:

In general, the muscles of live healthy animals are sterile, while lymph nodes, some organs, and, especially, surfaces exposed to the environment, such as external hide, pelt, or fleece, the mouth and the gastrointestinal tract carry extensive contamination (Sofos et al., 1999).

These are major sources of plant, carcass and meat contamination during slaughtering and processing.

Edible offal contamination:

Variety meats (edible offal) may carry a higher level of microbiological contamination than other meat animal tissues, either by nature and origin, or due to poor hygienic and chilling conditions (Gill, 1998).

Processes to Reduce Contamination:

Animal cleaning:

One, seemingly obvious, approach that may contribute to the reduction of external animal contamination, and subsequently, carcass contamination is to clean or wash the hide of the animals before slaughter and dressing. Pre-slaughter washing of sheep has been practiced in New Zealand, while, partial or complete, washing of cattle before slaughter has been used by some plants in the United States. Individual operations have evaluated, or applied interventions, such as removal (by cutting or shearing) of hair and fecal tags from the exterior of the animals or washing of animals before slaughter, but in many instances the results are generally less than promising (Gill, 1998). In general, animal washing before slaughter has variable influence on carcass contamination. Furthermore, application of the procedure may be limited by climate, type of animal, and availability of facilities (Sofos and Smith, 1998a).

Safety and meat quality:

Application of decontaminating processes may have an influence on product and worker safety and product quality, and, therefore, these criteria should be considered in treatment selection. Acceptable decontaminating processes should not have adverse toxicological or other health, effects on workers during their application or on consumers as a result of their use. Decontamination technologies based on heat are not associated with potential health concerns or with product safety, provided that the water meets drinking standards. Use of chemical solutions, however, depends on their toxicological properties, as well as on their effects on product quality and acceptability, and on the potential for environmental pollution problems associated with their use. Application of any decontamination technology should be in compliance with worker safety guidelines. Potentially undesirable effects of thermal and chemical

decontaminating processes may be associated with color/appearance and flavor/odor changes.

Therefore, their concentration, intensity and length of application should be selected based on antimicrobial as well as quality criteria (Gill and Badoni, 1997).

Even if spray-washing or other types of decontaminating technologies are effective on carcasses, the microbial status of the resulting meat will be affected by subsequent handling, exposure to additional contamination, and application of further decontamination or preservation treatments.

It is logical to expect however, that carcass decontamination, if proper and effective, should reduce incidence of pathogens of fecal origin that are mostly introduced in the plant, and originating on or in the animals. Carcass decontamination coupled with proper subsequent sanitation and handling of the resulting meat, should reduce levels of pathogens that need to be controlled or inactivated before consumption (Sofos and Smith, 1998a).

Carcass cooling:

Carcasses may be exposed to additional contamination and microbiological proliferation during chilling or cooling, which follows slaughter, dressing and decontamination. These problems can be minimized by sanitary and hygienic practices and facilities, and proper chilling of carcasses to temperatures that do not allow, or greatly reduce, microbial growth (Schmidt et al., 1998).

Fabrication and storage:

Carcass chilling is usually followed by fabrication into primal and sub primal cuts, and trimmings, which are packaged in pouches or bags, placed in boxes or in combo bins, and shipped elsewhere for further fabrication or processing before retailing. These processes should also be performed in sanitary

and hygienic environments and equipment, under good manufacturing practice principles.

All the gains in reduction of contamination achieved by decontaminating processes during slaughter, dressing and chilling may be compromised during fresh meat fabrication, handling and distribution. Proper plant and equipment cleaning and sanitation practices to eliminate organic matter residues, microbial contamination and biofilm formation, as well as personnel training in hygienic practices are important prerequisites, along with appropriately chilled carcasses, properly cooled fabrication environment, and rapid product throughput to avoid contamination problems during fabrication. Fresh meat should be stored at low temperatures to prevent or reduce microbial growth. Temperatures below 5-7^{o}C inhibit growth of mesophilic microorganisms, while psychrotrophs may grow, but at a reduced rate. Frozen storage below -5 to -10^{o}C will inhibit growth of all microorganisms of concern in foods.

Another important consideration is whether carcass decontamination has any lasting effect on the microbial quality of the resulting meat (Sofos and Smith, 1998).

Chapter Two

Material and Methods

Material and Methods

Material and Methods:

Hundred samples of raw meat were collected randomly from carcasses at Khartoum markets; (Samples were collected during 1/2/2009 – 28/4/2009) the samples 10cm^2 swabs were collected in a sterile icebox and transported hygienically to the faculty laboratory for microbiological analysis.

Equipments & Materials:

1. Autoclave
2. Ice box
3. Refrigerator
4. Microscope slides
5. Sterile plastic bags
6. Microscope
7. Sterile swabs
8. Set of gram's stain
9. Water bath
10. Colony counter.
11. Incubator, 37ºC.
12. Loops, racks, markers, filter papers, cotton and soap.
13. Sensitive balance.
14. Volumetric flasks, test tubes, Petri dishes (glass and plastic), slices, Durham's tubes, sterile pipettes, sterile bottles for samples handling.

Plate Count:

Viable PCs is determined using plate count agar, plates were incubated at 37C^O for 24 - 48 hours before colonies were counted. 1 ml from each 10cm^2 swab

sample was added to 9 ml peptone water tube and the process was repeated to the 5^{th} dilution.

Biochemical methods used for identification of isolated bacteria:

Indole test:

Testing for indole production is important in the identification of enterobacteria , most strain such as *Escherichia coli* break down the amino acid triptophan with the release of indole.

Requirements:

1- Peptone water medium 5g, sodium chloride 1.25g , distilled water
2- Kovac's reagent.

Method:

1- 4 ml of sterile peptone water were poured into test tube ;
2- The organism was removed by using a sterile wire and inoculated in the test tube ;
3- Incubated at 37ºC for 24 hr (overnight).
4- Examined for indole production by adding 1 ml of Kovac's reagent to the culture.
5- A positive reaction was indicated by a red colour in the reagent layer (red circle).

Methyl red test (MR):

This test is used to assist in the differentiation of enterobacteria.

Requirements:

- (Glucose 0.5, peptone 0.5, distilled water 100ml, di-potassium hydrogen phosphate 0.5 (glucose phosphate peptone water).
- Methyl red solution.

Method:

- Two ml of sterile glucose phosphate peptone water was poured into test tube and inoculate the test organism with a sterile wire; incubated at 37ºC for 24 hours.
- A drop of methyl red was added after overnight incubation, and then shaken gently.
- A red colour indication showed positive reaction, orange colour indicated a positive or negative reaction, yellow colour indicated a negative reaction.

Citrate test:

This test is used to differentiate between Enterobacteriace that utilize citrate as a sole source of Carbon.

Requirements:

- Simmon's citrate media (slant position) 2g.
- Distilled water 100 ml.

Method:

- 3 ml of sterile Simmon's medium was poured into test tube and with a sterile straight wire inoculate the test organism ;
- Incubate at 37ºC for up to 4 days (the growth had been checked daily).
- Blue color indicates positive reaction, while negative reaction shows green.

Catalase test:

This test is used to differentiate those bacteria that produce the enzyme catalase such as staphylococci – from non – catalase producing bacteria.

- Requirements: Hydrogen peroxide (H_2O_2) reagent 3%.
- Method:

- Pour 2 ml of H_2O_2 solution into a test tube.

- The test organism will be removed using a sterile wooden stick and immersed into the hydrogen peroxide solution.
- Positive result production of gas bubbles, negative no gas bubbles.

Coagulase test:

This test is used to identify *Staphylococcus aureus*, and differentiate between it and other staphylococci, which doesn't produce coagulase.

Requirements:

- Undiluted plasma.
- Slide, distilled water, sterile applicator stick.

Method:

- Place a drop of distilled water on a slide.
- A colony is emulsified in the drop to make a thick suspension.
- A drop of plasma is added to the suspension and gently mixed.
- A positive reaction is indicated by coagulant plasma.

Oxidation – Fermentation Test:

Test to differentiate between aerobic and anaerobic breakdown carbohydrate.

Requirements:

- O-F media, bromothymol blue solution , paraffin oil .
- Glucose 10g , distilled water , tubes .
- Weighted ingredients of the medium is dissolved with 500ml of distilled water then the pH is measured (7.1) before adding the indicator (bromothymol blue).
- The medium is sterilized by autoclaving for 15 minutes at 121^{o}C.
- 10 gms of glucose is dissolved in 100ml distilled water and sterilized by steaming in an autoclave for 10 minutes.

- The carbohydrate added to the sterile medium, then poured into sterile test tubes about 5ml (2 tubes were used for each test).
- Duplicate tubes of medium were inoculated by test organism (used sterile straight wire).
- One tube is covered with a layer of sterile paraffin oil to a depth of 10mm and both incubated at 37^{o}C for 14 days.
- Fermented organisms produced acid reaction through out the medium in both covered and opened tube, while the oxidized organisms produced acid reaction only in the opened tube.
- On the other hand, organisms that can not breakdown the carbohydrate show no changes in the medium.

Kligler iron agar (KIA) to detect H_2S:

This medium is suitable for detecting H_2S production by *Enterobacteria*. H_2S is detected by the ferric citrate contained in the medium, KIA is a differential slope medium used to assist in the identification of *Salmonella, Shigella*, and other enteric bacteria.

Salmonella and *Shigella* produce a pink-red slope and yellow butt indicating the fermentation of glucose but not lactose.

Fermentation of sugars:

Requirements:

- Peptone water media 3.75gms , distilled water 500 mls
- Carbohydrate media (lactose broth, xylose, glucose, maltose, mannitol).
- Bromothymol blue solution. (adjust the pH 7.2-7.3)
- Durham's tubes.
- Test tubes.

Method:

- Dissolve 3.75g m of peptone in 250 ml of distilled water , then add 3.12ml of bromothymol blue solution (adjust the pH 7.2-7.3).

- Then pour into test tubes contained Durham's tubes (inverted), and sterilized by autoclaving for 15 minuets at 121^{o}C.
- Prepare sugars by dissolving 0.62gms from each sugar into 6.25 ml of distilled water (in screw cap bottle) sterilized by autoclaving (steaming) for 10 minutes;
- Add each sugar to each sterilized medium, all tubes will be inoculated with the growth from surface of the culture media and incubated at 37^{o}C for 24 – 48 hours.
- Positive reaction indicated by changing color to yellow and gas production (showed in Durham's tube).

Motility test:

Requirements:

- Semi solid media .(0.4% of agar mixed with 3.25g nutrient broth powder)
- Tubes.
- 250 ml distilled water.

Method:

- Isolate by inoculated stabbing tube containing semi solid media (about half the depth) using straight wire, and then incubated at 37oC for 24 hours.
- A motile bacterium showed diffuse and hazy growth spread throughout the medium (Cheesbrough, 1999).

Chapter Three

Results

Results

Standard microbiological analytical method study was conducted by take on Sample of one hundred beef Carcasses at Elsog Elmarkazi Elkhartoum as well as taking 10 cm^2 by Swab in the carcasses.

Table (1) illustrated the percentage of bacteria in each Carcasses by the total count of bacteria average ($1x10^5$ to $2.89x10^7$)

Table (2) explained the difference between the Cocci bacteria as a result of catalase and coagulase test

Table (3) distinguishes between the *Enterobacteriaceae* families using the lMVIC test.

Figure (1) Revealed microorganism rode (36.2%) and cocci (63.8%)

Figure (2) Shows Staphylococcus aurues in the carcasses (18%), the *Staphylococcus* (8%), other cocci (12%), *Salmonella.spp* (8%), *Escherichia.spp* (7%), *Shigella.spp* (3%), *Klebsiella.spp* (3%), and other microorganisms (39%).

Table (1) shows the numbers of Bacteria /cm^2 of samples.

Sample No	Number of colonies	Number of Bacteria/cm^2
1	237	$2.37x10^7$
2	197	$1.97x10^7$
3	170	$1.70x10^7$
4	210	$2.10x10^7$
5	91	$9.1x10^6$
6	177	$1.77x10^7$
7	26	$2.6x10^6$
8	53	$5.3x10^6$
9	113	$1.13x10^7$
10	126	$1.26x10^7$
11	231	$2.31x10^7$
12	289	$2.89x10^7$
13	74	$7.4x10^6$
14	71	$7.1x10^6$
15	97	$9.7x10^6$
16	177	$1.77x10^7$
17	97	$9.7x10^6$
18	85	$8.5x10^6$
19	52	$5.\ 2x10^6$
20	126	$1.29x10^7$
21	89	$8.9x10^6$
22	52	$5.2x10^6$
23	112	$1.12x10^7$
24	136	$1.36x10^7$
25	102	$1.02x10^7$
26	91	$9.1x10^6$
27	72	$7.2x10^6$
28	156	$1.56x10^7$
29	139	$1.\ 39x10^7$
30	29	$2.9x10^6$
31	176	$1.76x10^7$
32	104	$1.04x10^7$
33	14	$1.4x10^6$

34	2	$2x10^5$
35	41	$4.1x10^6$
36	42	$4.2x10^6$
37	2	$2x10^5$
38	4	$4x10^5$
39	121	$1.\ 21x10^7$
40	48	$4.8x10^6$
41	283	$2.83x10^7$
42	276	$2.76x10^7$
43	220	$2.20x10^7$
44	234	$2.34x10^7$
45	163	$1.63x10^7$
46	22	$2.2x10^6$
47	3	$3x10^5$
48	7	$7x10^5$
49	No growth	No growth
50	218	$2.18x10^7$
51	No growth	No growth
52	14	$1.4x10^6$
53	54	$5.4x10^6$
54	1	$1x10^5$
55	4	$4x10^5$
56	5	$5x10^5$
57	11	$1.1x10^5$
58	43	$4.3x10^6$
59	5	$5x10^5$
60	95	$9.5x10^6$
61	113	$1.13x10^7$
62	87	$8.77x10^7$
63	78	$7.8x10^6$
64	102	$2.01x10^7$
65	93	$9.3x10^6$
66	56	$5.6x10^6$
67	43	$4.3x10^6$
68	27	$2.7x10^6$
69	35	$3.5x10^6$

70	68	$6.8x10^6$
71	142	$1.42x10^7$
72	206	$2.06x10^7$
73	193	$1.93x10^7$
74	187	$1.87x10^7$
75	183	$1.83x10^7$
76	243	$2.43x10^7$
77	119	$1.19x10^7$
78	68	$6.8x10^6$
79	113	$1.13x10^7$
80	159	$1.59x10^7$
81	177	$1.77x10^7$
82	68	$6.8x10^6$
83	67	$6.7x10^6$
84	63	$6.3x10^6$
85	84	$8.4x10^6$
86	84	$8.4x10^6$
87	76	$7.6x10^6$
88	47	$4.7x10^6$
89	40	$4.0x10^6$
90	46	$4.6x10^6$
91	73	$7.3x10^6$
92	98	$9.8x10^6$
93	103	$1.03x10^7$
94	18	$1.8x10^6$
95	49	$4.9x10^6$
96	133	$1.33x10^7$
97	201	$2.01x10^7$
98	55	$5.5x10^6$
99	97	$9.7x10^6$
100	23	$2.3x10^6$

Table (2) shows the sample numbers of the different cocci in meat (according to testing by Coagulase and Catalase).

Sample No (positive cocci)	**Catalas test**	**Coagulase test**	
2	Negative	Negative	-
4	Negative	Positive	-
5	Negative	Negative	-
7	Positive	Positive	*Staph. aureus*
8	Positive	Positive	*Staph. aureus*
10	Negative	Negative	-
11	Positive	Positive	*Staph. aureus*
12	Positive	Negative	*Staphylococcus*
13	Positive	Positive	*Staph. aureus*
14	Positive	Positive	*Staph. aureus*
15	Negative	Negative	-
16	Positive	Positive	*Staph. aureus*
17	Negative	Negative	-
18	Positive	Positive	*Staph. aureus*
19	Positive	Negative	*Staphylococcus*
20	Positive	Positive	*Staph. aureus*
61	Positive	Positive	*Staph. aureus*
65	Positive	Positive	*Staph. aureus*
67	Positive	Negative	*Staphylococcus*
70	Positive	Positive	*Staph. aureus*
73	Negative	Negative	-
74	Positive	Negative	*Staphylococcus*
75	Positive	Positive	*Staph. aureus*

76	Negative	Negative	*Staph. aureus*
79	Positive	Negative	*Staphylococcus*
73	Negative	Negative	-
81	Positive	Positive	*Staph. aureus*
83	Negative	Negative	-
84	Negative	Negative	-
85	Positive	Negative	*Staphylococcus*
87	Positive	Positive	*Staph. aureus*
89	Positive	Positive	*Staph. aureus*
90	Positive	Negative	*Staphylococcus*
92	Positive	Positive	*Staph. aureus*
95	Negative	Negative	-
98	Positive	Negative	*Staphylococcus*
99	Positive	Positive	*Staph. aureus*
100	Negative	Negative	-

Table (3) shows the types of *Enterobactriaceae* isolated from the samples depending on the biochemical (IMVIC) tests.

Sample No	Tests									Types of Bacteria
	Indole	M.R	V.P	Citrate	Lactose	Glucose	Sucrose	Motility	H_2S	
6	-	+	-	-	-	+	-	+	+	*Salmonella.spp*
9	+	+	-	-	+	+	-	+	-	*Escherichia. coli*
21	-	+	-	-	-	+	-	+	+	*Salmonella.spp*
22	-	-	-	+	-	+	-	-	-	*Shigella. spp*
32	+	+	-	-	+	+	-	+	-	*Escherichia. coli*
41	+	+	-	-	+	+	-	+	-	*Escherichia. coli*
42	-	-	+	+	+	+	+	-	-	*klebsiella.spp*
50	-	+	-	-	-	+	-	+	+	*Salmonella.spp*
53	-	+	-	-	-	+	-	+	+	*Salmonella.spp*
56	+	+	-	-	+	+	+	+	-	*Escherichia. coli*
60	-	+	-	+	-	+	-	+	+	*Salmonella. spp*
62	-	-	+	+	+	+	+	-	-	*klebsiella.spp*
66	+	+	-	-	+	+	-	+	-	*Escherichia. coli*
68	-	+	-	-	-	+	-	+	+	*Salmonella.spp*
69	+	+	-	-	+	+	+	+	-	*Escherichia. coli*
71	+	+	-	-	+	+	-	+	-	*Escherichia. coli*
72	-	-	+	+	+	+	+	-	-	*klebsiella.spp*
77	-	+	-	+	-	+	-	+	+	*Salmonella. spp*
78	-	+	-	-	-	+	-	-	-	*Shigella.spp*
82	-	+	-	+	-	+	-	+	+	*Salmonella. spp*
86	+	+	-	-	+	+	-	+	-	*Escherichia. coli*
88	-	+	-	-	-	+	-	-	-	*Shigella.spp*

Figure (1) shows the percentages of types of microorganisms (gram negative rods and gram positive cocci) of the (58) meat samples.

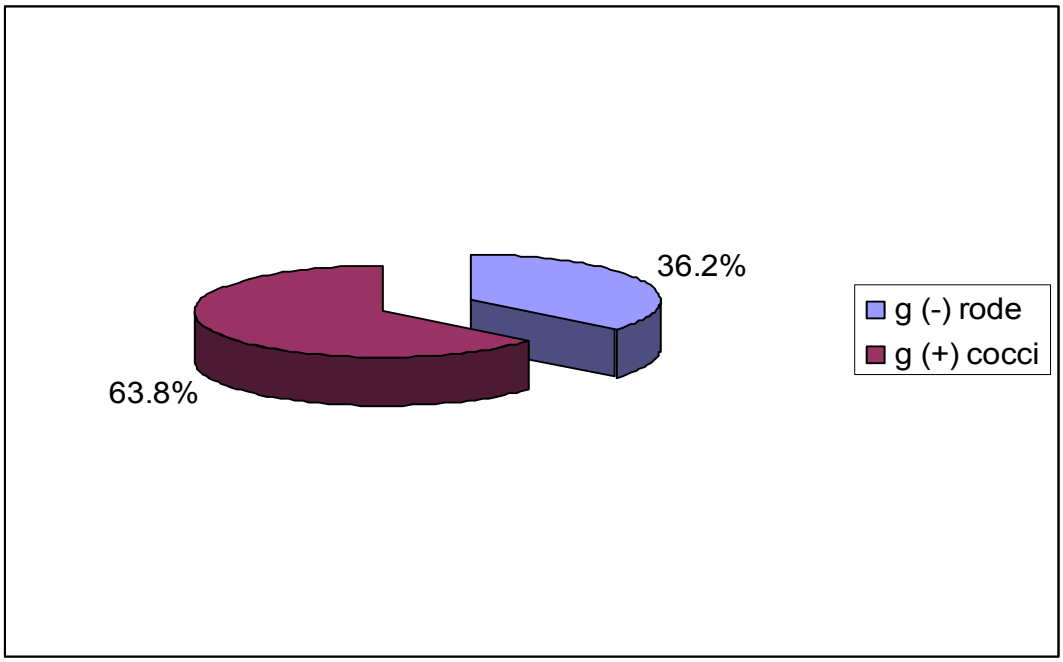
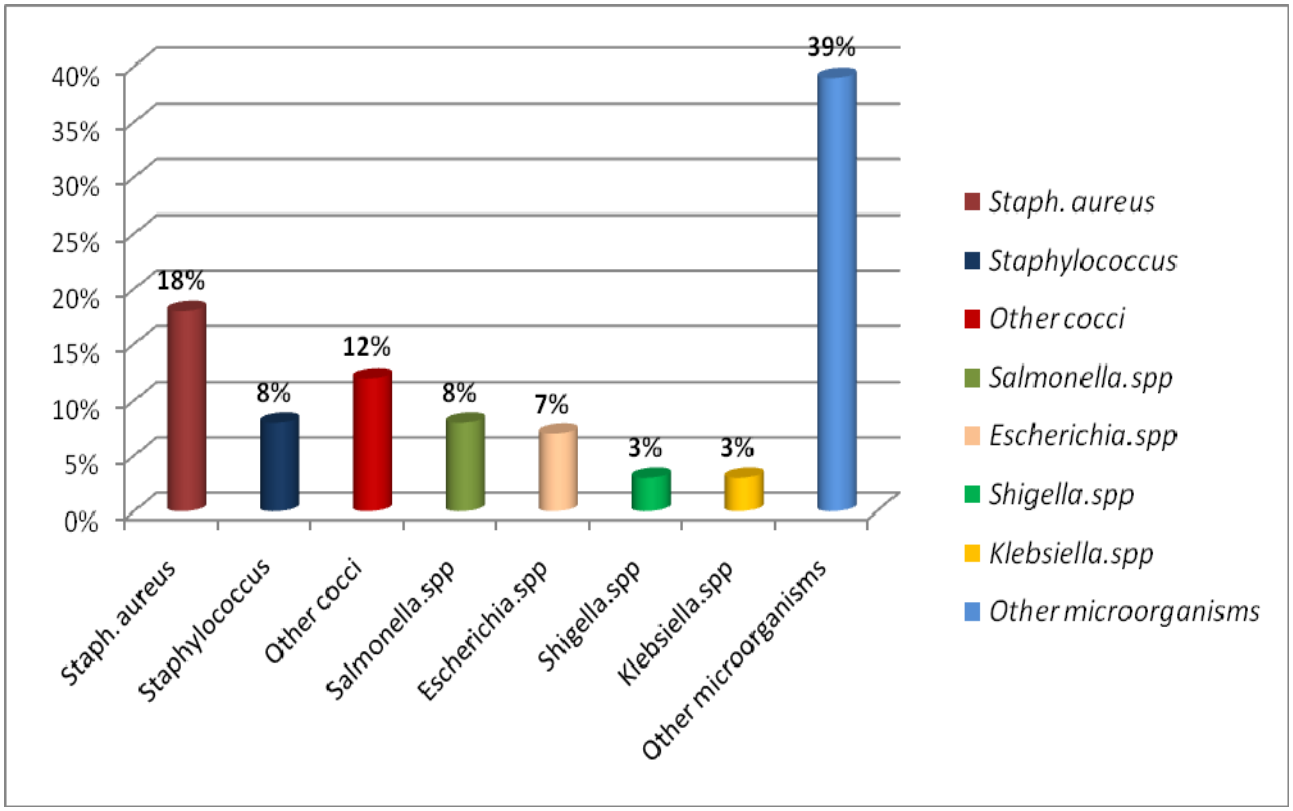


Figure (2) the percent incidence of microorganisms recovered by analyzing 98 samples of meat.



Chapter Four

- Discussion
- Conclusion
- Recommendation
- Reference
- Appendix

Discussion

Samples of meat were taken from 100 carcasses at Elsog Elmarkazi to determine the microbial contamination and identify the types of bacteria found in this meat as well as the prevalence of their existence.

By the total count of bacteria, the bacterial load on carcasses has been identified.

With the use of biochemical test and growth in special culture media types of bacteria were identified.

Catalase and Coagulase tests were used in identifying the different type of cocci.

The percentage of carcasses contaminated with *Staphylococci. aureus* was found to be (18%).

IMVIC test was used to differentiate between *Enterobactriaceae* group, the percentage of carcasses contamination with *Salmonella.spp* (8%), *Escherichia.spp* (7%), *Shigella.spp* (3%), *Klebsiella.spp* (3%).

Table (1) illustrate large numbers of bacteria contaminating carcasses average ($2.89x\ 10^7$ to $1x10^5$.) this was as attributed to the contamination caused by environmental factors in which the processing and marketing of meat was carried. (Safos , el al 1999).

Table (2) figure (2) shows the percent of carcasses contaminated with *Staphylococcus. aureus* was (18%). *Staphylococcus* contamination usually occurs during operations and slaughter, transport and display and by contaminating tools and contact with dirty surfaces and hands of workers and consumers at butcher's shops are the most common cause of *Staphylococcal* contamination as published by (peel, 1975-basch, et al 1948).

Table (3) and figure (2) shows the percent of carcasses contaminated with *Salmonella.spp* (8%), *Escherichia.spp* (7%), *Shigella.spp* (3%) and *Klebsiella.spp* (3%) of the *Enterobactriaceae.* Contamination and growth of these bacteria occurs during storage and cooling in the case of application of non-sound method and modern ways and technique during processing, transport and handling as stated (Meng, et al. 1994).

The presence of these types of bacteria and at these numbers found in this research indicates non-hygienic procedures during processing, transport and marketing.

Following the hazard analysis and critical control point (HACCP) during these different stages, will improve the hygienic situation of meat.

Training and health education also might give a hand in this situation.

Conclusion and Recommendation

The study concluded that meat hygiene at Khartoum locality is really questionable and need's extensive hygienic efforts, the total counts revealed very high contamination levels (medium number in all carcasses was between 1x 10^5 to 2.89x10^7, *Staphylococcus aureus* percent of carcasses contamination was 18%, *Salmonella were* 8%, *Escherichia coli* 7%, *Shigella* 3% *and Klebsiella* were 3% and all these levels are well above standard recommended levels for a healthy hygienic meat.

Recommendations:

- The use of modern technology and safety measures during the processes of slaughter.
- Decontamination of carcasses by water at high contact pressure and temperature ($\leq$ 70 ^{O}C) with the use of a solution of Lactic Acid 2% concentration.
- Use of healthy procedures in meat processing specifically the following:

  1. The use of refrigerators in particular, by offering meat temperatures ranging between (2- 7C^{O})

  2. The commitment of workers to wear special and clean clothing at work.

  3. Meat should be displayed separately from other products such as vegetables, fruits and others.
- Training of meat handlers on hygienic measures and application of meat legislation.

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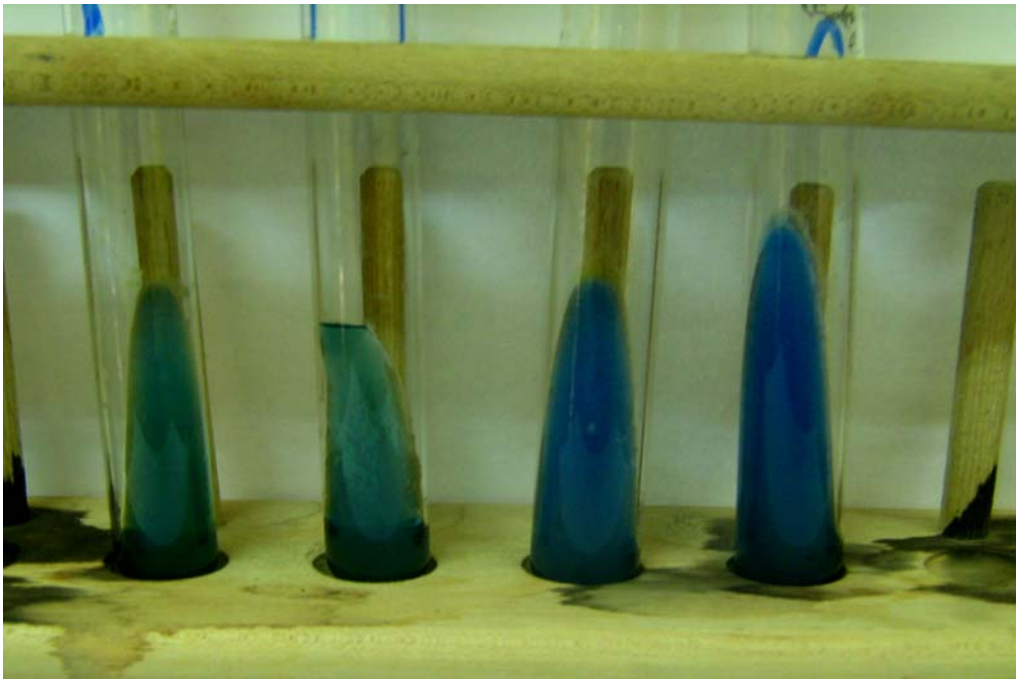
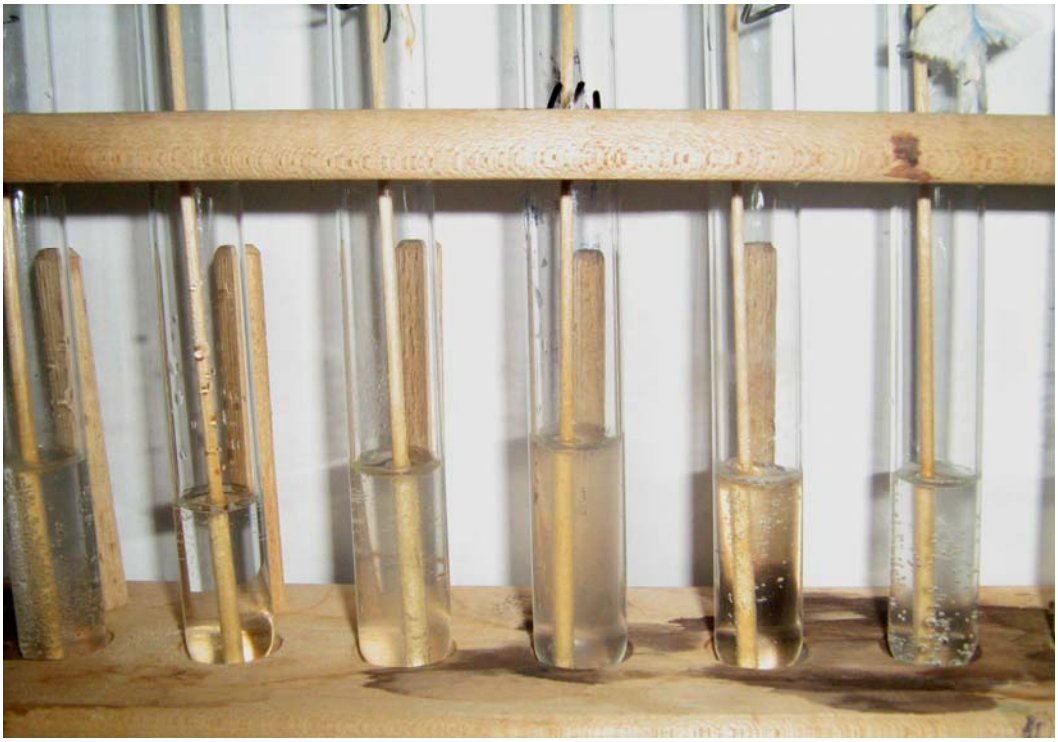
Appendix

Picture 1: shows the color change which result from growth of *Enterobacteriacea* in the Citrate media.



Picture 2: Shows the reaction between the *Staphylococcus aureus* and Catalase media.



Picture 3: Shows the red color produced from growing of *Enterobacteriacea* in the Indole media

picture 4: Shows the color change which results from growth of *Enterobacteriacea* in the Methyl read (MR) media.