ADDIS ABABA UNIVERSITY
SCHOOL OF GRADUATE STUDIES

The Prevalence of Food Borne Pathogens with Particular Emphasis on *Escherichia coli* O157:H7 in Retail Meat in Addis Ababa

A Thesis Submitted to the School of Graduate Studies of Addis Ababa University in Partial Fulfillment of the Requirements for the Degree of Masters in Biology

By Abraham Mikru

July 2004

Acknowledgements

I would like to express my gratitude to my principal advisor Prof. Mogessie Ashenafi, without whom this work would not have been a reality. He has helped me from the inception, through the work and critical review of the theses. My indebtedness also goes to my co advisor Dr. Aberra Geyid. He was very cooperative in facilitating laboratory permits during part of my work at the Ethiopian Health and Nutrition Research Institute. He also provided me with some useful reference materials.

I thank my friends Ato Mekonnen Tsegaye and Ato Yirdaw Tachbele, for sharing references and for provoking ideas during our circumstantial discussion in the laboratory.

Due thanks also go to Ato Haile Alemayehu and Ato Ephraim for their technical help and for creating a conductive laboratory environment.

I am grateful to the Institute of Pathobiology, Addis Ababa University, for providing me with laboratory space and general facilities.

Table of contents page

LIST OF TABLES

Page

List of figures

PAGE

Abstract

The primary objective of this study was to determine the incidence of some foodborne pathogens in retail beef samples, and also assess the hygienic status of the retail beef with respect to microbial load and microflora. During the period between November 2001 and July 2002, a total of 200 beef samples (500g per sample) consisting of 100 sliced and 100 minced beef samples were purchased from 20 butchers' shops in four city zones of Addis Ababa. Microbiological analysis of the beef samples based on cultural, microscopic and serological methods has revealed the following results. The mean mesophilic aerobic plate counts (MAPC) of the sliced and the minced beef samples were 8.21 log cfu/g and 8.24 log cfu/g, respectively. There was no statistically significant difference between the mean MAPC values of the sliced and the minced beef samples ($P > 0.05$, $t_{\alpha} = 0.877$, at 45 d.f). Of the 25 sliced and the 22 minced beef samples, 23 and 19 beef samples respectively, were of unacceptable quality due to high mesophilic aerobic counts levels. The mean total coliform counts of the sliced and the minced beef samples were 7.02 log cfu/g and 6.44 log cfu/g, respectively. The mean counts of *Staphylococci* in the sliced and the minced beef samples were 6.68 log cfu/g and 6.91 log cfu/g respectively. Although enterotoxigenicity of the strains was not assessed, 4 of the 25 sliced beef and 4 of the 22 minced beef samples had staphylococci counts (> 7 log cfu/g) near the threshold for production of detectable *Staphylococcus* enterotoxin (SE). In the sliced beef samples, micrococci *(40.8%),* staphylococci (29.1%), *Bacillus* spp. (13.3%) and the *Enterobacteriaceae* (10.4%), in their order, were the four top leading dominant mesophilic aerobic bacteria (MAB) whereas in the minced beef samples micrococci (41.2%), the *Enterobacteriaceae* (19.69%), other gram negative rods (OGPR) (12.73%) and, *Bacillus* spp (10.3) in their order, dominated the

mesophilic aerobic flora. Shigellae were not detected in any of the 200 beef samples. Salmonellae were detected in 2 (1%) of the 200 beef sample. The two isolated strains belonged to *Salmonella* serogroup D and were sensitive to all the ten antimicrobials tested. Of the total 200 beef samples screened, only 1 (0.5%) sliced beef sample was found to be positive for E. *coli* O 157:H7 and the isolated single strain was resistant to cephalothin, amoxicillin and ampicillin but was susceptible to the remaining seven antimicrobials used in the test. Virulence of the isolated *E. coli* O157:H7 was not determined. This work is the first report on the occurrence of the serotype in beef in Ethiopia and may serve as a pedestal in launching more comprchensive investigation to conclusively establish the occurrence of virulent strains. Considering the very poor hygienic standard of the retail beef as evidenced by the high microbial load, the count of staphylococci and the incidence of salmonellae, and *E. coli* O157:H7, the consumption of raw or undercooked beef should be discouraged. Based on tentative observations of some factors that might have contributed to the poor hygienic conditions of the beef samples, a flow chart-based potential critical points are outlined

I. INTRODUCTION

1.1 Meat as a foodstuff

Meat can be defined as the flesh of animals that is used for human consumption. In this context, the meat from bovine animals is generally known as beef. Other meat types may be identified by the name of the specific animal like, goat meat, poultry meat, horsemeat, etc (Gebre-Emanuel Teka, 1998). Specific names are also given to beef types from bovine animals of different age groups, such as veal, baby beef, beefling, heifer beef etc. (Dodsworth, 1972).

Meat is not an essential food as evidenced by the vegetarians who thrive without flesh foods, but, is a tasty, easily digested, and highly efficient source of the multifarious proteins of the animal body (Bender, 1992).

1.2 Meat Microbial Safety

The reasons for why meats are the most perishable of all important foods lie not only in their nutritional richness, but also on the presence of all the requisite nutrients and conditions for microbial growth in available form in fresh meats (Jay, 1996). In the absence of special care in handling and processing, meat can be a suitable medium for microbial growth and disease transmission.

In light of current knowledge the basic purpose of meat hygiene is to avert spoilage, and to prevent meat borne infection. Based on their mode of transmission, Bryan (1986) has classified and tabulated meat borne bacterial pathogens. Accordingly, the pathogens that are usually

transmitted to man by ingestion of raw or undercooked meat included *Bacillus anthraces, Campylobacter jejuni, Escherichia coli, Salmonella, and Yersinia.* These are primarily zoonotic and usually arise from the meat animal. Other pathogenic bacteria may also be transmitted to man by ingestion of cooked meat that has been contaminated after heat processing. Nearly all foodborne bacterial pathogens can be transmitted this way, but epidemiologically the most important ones are *Bacillus cereus, Clostridium botulinum, Cl. perfringens,* Shigellae, and staphylococci.

1.3 Global Spectrum of Meat borne Infection

The global emergence of meat borne disease parallels the ever-growing meat consumption and the increasing meat processing industries and international trade with animal products. In the USA alone there are over 2 million cases of meat and poultry-associated foodborne diseases in humans per year, at a cost approaching 1.4 billion dollars (Menning, 1988). Most of these are attributed to *Salmonella* and *Campylobacter* (Fedorka-Cray *et al.*, 1994). The situation in other parts of the world is not different (Zhao *et al.*, 2001). A review of foodborne disease surveillance in Scotland showed that during the period between 1980 and 1991, a total of 2514 episodes of foodborne disease had occurred (Sharp *et al.*, 1992). *Salmonella* and *Campylobacter* were the leading etiological agents with poultry meat and red meat being the predominant vehicles. In Finland, a total of 890 foodborne disease outbreaks involving 27,858 persons were reported during the years 1975-1990 (Hirn *et al.*, 1992). The main causative agents were *Staphylococcus aureus* (199 out breaks; 1968 affected persons), *Cl. perfringens* (187; 5224), *Salmonella* (102; 3341), and *Bacillus cereus* (73; 1842). During the last eight years of the surveillance program,

meat and meat products were responsible for 41.2% of the outbreaks. *Cl. perfringens* caused over 40% of the meat borne outbreaks. The corresponding figure for *B. cereus, Salmonella*, and *S. aureus* were about 10% each (Hirn *et al.*, 1992). Apart from these established pathogens, new meat borne pathogens such as *E. coli* O157:H7 have now assumed a global scale emergence (Mead and Griffin, 1998).

E.coli O157:H7 was first recognised as a foodborne pathogen in 1982, when it caused two outbreaks of gastroenteritis in the USA (Riley *et al.,* 1983). The two outbreaks involved at least 47 people in Oregon and Michigan who had eaten hamburgers made from contaminated beef patties. Since then, the organism has been increasingly recognised as a predominant cause of hemorrhagic colitis (HC) and hemolytic-uremic-syndrome (HUS).

The salient epidemiologic features of the infection include the gut of cattle and other animals as reservoir; transmission by a wide variety of food items, with beef being the major vehicle of infection; and a very low infectious dose, enabling high rates of attack and person-to-person spread (Nataro and Kaper, 1998).

1.4 The case of Ethiopia

In Ethiopia, as in other developing countries, adequate and reliable data on foodborne infectious diseases are lacking. The existing disease reporting system does not clearly and quantifiably indicate foodborne disease prevalence (CSA, 2002). Outpatient morbidity statistics (hospitals and health centres only) of selected foodborne illnesses indicated that, the annual incidence ranged from 3.4 to 9.3 percent with median 5.8 percent for the year 1985/86 to 1989/90 (Gebre-Emanuel Teka, 1997). The top leading causes were Q-fever, anthrax, Salmonellosis and other unspecified causes. Current estimate is unavailable. Taking the above rate and considering the current total population of Ethiopia 67.22 million (CSA, 2002), the current annual number of cases could be estimated between 2.3 million and 6.3 million. This perhaps is a great underestimation, and does not include the untold number of suffering rural majority who have limited access to the meagre health care service.

On top of the problem of underreporting, some proportions of foodborne illnesses are caused by pathogens that have not been identified and thus cannot be diagnosed. The importance of this factor cannot be overemphasized. Many of the pathogens of greatest concern in the developed countries today (e.g. *Campylobacter jejuni, E. coli* O157:H7, *Lysteria monocytogens, Cyclospora cayetanensis*) were not recognized as foodborne pathogens just until the 1980 (Mead *et al.*, 1999).

Determining the extent to which *E. coli* O157:H7 is responsible for dysentery in Africa is more than an academic concern, because syndromic management protocols for dysentery in areas where the etiologic agents cannot be readily confirmed often include empirical treatment with

antibiotics (Effler *et al.*, 2001). Antibiotics have not been clearly shown to ameliorate *E. coli* O157:H7 infection and recent data indicated that antibiotics might predispose the patient to developing serious complications such as haemolytic-Uraemia syndrome (Dundas *et al.*, 2001).

No published work is available on the incidence of *E. coli* O157:H7 in Ethiopia. Considering the worldwide occurrence of the pathogen and its close association with cattle and beef, it is highly imperative to undertake such an investigation in a country with high livestock population and a potential for meat industry.

The livestock sub sector in Ethiopia is estimated to contribute about 30% to the agricultural GDP and 15% to the foreign export earnings (LMA, 2000). In the present world animals and animal products are playing a great role in the trade and of national economic significance. Among the major livestock products that have a promising prospect for expanded trade in foreign markets, meat and meat products come to the forefront (LMA, 2000).

In order to increase the production and exporting of meat and meat products, sufficient considerations have to be given to the safety demand of customers by supplying meat and meat products of acceptable quality. This, in turn, demands strict surveillance of zoonotic pathogens such as *E. coli* O157:H7.

Apart from foreign trade and economic considerations, the assurance of safety of meat is essential to augment the supply of safe protein food to the domestic consumers. By its nature and origin, meat is not only prone to microbial spoilage but is also frequently incriminated in the spread of foodborne pathogens. Although microbiological and relevant epidemiologic data are lacking, it is

not unusual to hear in the news media, meat-associated disease outbreaks and deaths following holiday weeks in Ethiopia. Recently, Mogessie Ashenafi (2002) has reviewed the few studies done on microbial safety of meat and meat products in Ethiopia. Overall, *Salmonella* is the most commonly reported meat associated pathogen.

This work presents the general hygienic conditions of retail beef in Addis Ababa as well as the first ever report on the incidence of *E. coli* O157:H7 in beef samples from some butchers' shops in Addis Ababa, Ethiopia.

1.5 Study Objectives

1.5.1 General objective

The general objective of this investigation was to assess the hygienic status and microbial safety of retail meat (beef) in Addis Ababa with respect to:

- The occurrence of *Escherichia coli* O157:H7
- The occurrence of some commonly isolated foodborne pathogens and
- The magnitude of microbial load and the spectrum of microflora

1.5.2 Specific Aims

The specific aims of this investigation were:

- To detect and isolate *E. coli* O157:H7 in the retail beef samples and determine its incidence
- To detect and isolate *Salmonella* and *Shigella* spp. in the retail beef and determine their incidence.
- To determine the mesophilic aerobic count of the retail beef samples
- To identify the dominant mesophilic aerobic flora in the beef samples.
- To determine the total coliform count of the beef samples
- To determine the count of *Staphylococci* in the beef sample.
- To assess the susceptibility patterns of all isolates of enteric bacterial pathogens against some antimicrobial agents.

II. Literature Review

2.1 Microflora of Meat carcasses

Meat may be regarded as a source of edible tissue sandwiched between two regions of an animal that are heavily contaminated (Grau, 1986). These two layers are the external layer skin, hair, wool, feathers, and the internal intestinal tract and its contents. The first aim of the abattoir is to harvest the edible tissue from between these layers with as little contamination as possible.

The hide exterior can carry (per gram or cm^2), 10^6-10^8 aerobic mesophiles, 10^4-10^6 psychrotrophs, 10^3-10^6 *Enterobacteriaceae*, 10^1-10^5 *Eschericlia coli*, 10^5-10^6 *Bacillus* spores, and 10^3 or more yeast and molds (Grau, 1979 cited in Grau, 1986). Salmonellae have been detected in counts up to 400 per cm^2 on the hide of more than half of 200 animals at an a slaughterhouse (Grau and Smith, 1974). Large numbers and variety of microorganisms are also carried on to the slaughter floor on the hooves of animals, and this population may also include *Salmonellae* (Patterson and Gibbs, 1978 cited in Grau, 1986).

At slaughter, rumen contents may contain per gram, 10^6-10^8 aerobic mesophiles, 10^2-10^5 psychrotrophs, and 10^3-10^7 *Enterobacteriaceae* and *E.coli* (Grau, 1986). Faeces contain per gram, 10^8-10^9 aerobic mesophiles, 10^2-10^5 psychrotrophs, and 10^6-10^9 of *Entrobacteriaceae* (Howe *et al.*, 1976). In addition faeces may contain up to10^6 *Cl. perfringens* spores per gram as well as salmonellae and campylobacters (Grau, 1986).

Most of the bacterial contamination of carcasses is acquired during the removal of skin. The first incision through the heavily contaminated skin carries microorganisms on to the carcass tissue. As the knife is used to remove the skin from the tissue organisms are wiped from the knife on to the carcass. Bacterial population on the tissue are highest in the region below the initial incision through the skin and lowest in the regions furthest removed from the incision (Mulder and Krol, 1976). Further contamination of the carcasses can occur from the hide folding over and contacting the exposed tissue and from contact with the hands of the workers. During removal of the skin and feet salmonellae can be transferred from the hooves and hide to the carcasses (Grau and Smith, 1974; Stole, 1981).

The hands of workmen handling the skin and hocks become heavily contaminated. Sterile knives used for incising the skin and removing hocks can acquire on blades about 10^7 aerobic mesophiles, 10^5 *Bacillus* spores and psychrotrophs and 10^3 *Enterobacteriaceae* (Hess and Lott, 1970; cited in Grau, 1986). In another study, Smeltzer *et al.* (1980) performed counts on various equipment in an abattoir. Counts on steel ranged 0-153 salmonellae per steel; aprons from 0-14 per 100 cm^2; scabbards from 0 to greater than 3667 per scabbard and carcass bump points from 0-40 per 100 cm^2. Contamination rates were highest for the equipment of workers whose function brought them in contact with the hide (Smeltzer *et al.*, 1980).

During evisceration there is contamination of the carcass with salmonellae and *Enterobacteriaceae* without any significant increase in the number of mesophilic organisms (Grau, 1986). During removal of the viscera, some leakage of rumen fluid occasionally occurs and this may contaminate the carcass and the offal. However, most of the contamination of carcasses with microorganisms from the intestinal tract seems to occur without obvious spillage

(Grau, 1986). The operation involved in freeing the anal sphinicter and rectal end of the intestine constitute a major source of *E. coli* and *Salmonella* contamination of sheep and cattle carcasses (Grau, 1986). Part of the flora of meat arises from the lymph nodes of the animal. The gall bladder, hepatic, and mesenteric lymph nodes may be infected with salmonellae. At times there can be more than 5000 *Salmonella* cells per gram in mesenteric lymph nodes (Samuel *et al.*, 1980 cited in Grau, 1986). Frequently *Campylobacter* can also be found in the bile (Bryner *et al.*, 1972 cited in Grau, 1986).

At the completion of slaughter, dressing, and washing, cattle and sheep carcasses will have on their surfaces about 10^3-10^5 aerobic mesopiles per cm^2, the count varying with site and the animal species (Ingram and Roberts, 1976 cited in Grau, 1986). The number of psychrotrophs varies between 0.1 and 10 % of the mesophiles. While there are usually less than 10 *Enterobcteriaceae* or *E. coli* cells per cm^2, the number again depend on site and the animal species (Howe *et al.*, 1976). *Clostridium perfringens* can frequently be found in small numbers on sheep and cattle carcass (Smart *et al.*, 1979).

Subsequent procedures for handling of meat through chilling, freezing, and distribution are aimed at reducing or preventing increase in microbial load that may occur either by growth or further contamination. Butchering of carcasses adds further to the bacterial load as bacteria are transferred to the meat surface from tool, work surfaces and hands.

When several thousands of animals are slaughtered and handled in a single day in the same abattoir, there is a tendency for the external carcass biota to become normalized among carcasses, although a few days may be required (Jay, 1996). The same may happen to the microflora of the

butchery environment after handling so many carcasses over a long period of time. This may allow prediction of the type of contaminants likely to be found in retail meat.

It is apparent from the foregoing discussion that bacterial contamination of meat occurs at multiple stages in the meat chain. The relative importance of the wide variety of microorganisms that may contaminate meat during its production is dependent on the type of microorganisms and the subsequent treatment of the meat before consumption.

2.2 Escherichia *Coli* O157:H7

2.2.1 General Features

Escherichia coli represents the type species of the genus *Escherichia* which contains mostly motile gram-negative bacilli within the family *Enterobacteriaceae* and the tribe escherichieae (Edwards and Ewing, 1972). Although most strains exist as intestinal symbionts, there are many pathogenic *E. coli* strains that cause a variety of diseases in animals and humans. *E. coli* O157:H7 is one of the many pathogenic strains that belong to the pathotype enterhemorrhagic *Escherichia coli* (EHEC).

EHEC represents a subset of shigatoxin (Verocytotoxin) producing *E. coli (STEC)* and denotes a clinical connotation (Nataro and Kaper, 1998). STEC and VTEC are equivalent terms and both refer to *E. coli* strains that produce one or more of the shigatoxin (STX) family.

The serotype O157:H7 differs from the other *E. coli* serotypes in that it is a non-sorbitol fermenter (NSF) and fails to express β-glucuronidase activity. Other biochemical features are the same as that of the type species *E. coli* and the details of which can be found in Edwards and Ewing (1972).

2.2.2 Virulence factors and pathogenesis

The ability to withstand the acidity of the stomach is an essential feature for enteric pathogens. *E. coli* O157:H7 has been shown to be able to grow at pH 3.75 and survive for seven days at pH 3.3 and six hours at pH 2.8 in the presence of 3% (V/V) acetic acid (Betts, 2000). Acidic foods have also been incriminated in *E. coli* O157:H7 outbreaks (Morgan *et al.*, 1993). After surviving the acidity of the stomach the bacteria must colonize a mucosal site.

The presumed colonization sites are the colon and caecum (Phillips, *et al.*, 2000; Grauke *et al.*, 2002) and the best characterized adherence factor is a 94-to-97 KDa outer membrane protein (OMP), intimin, encoded by the *eae* gene (Louie et al., 1993; 1994; Agin and Wolf, 1997). It mediates intimate attachment of the bacterium to epithelial cells leading to attaching and effacing (A/E) lesions.

Once infection is established, the bacteria proliferate and produce one or more of the shiga toxins which is considered the principal damaging virulence factor (Paton and Paton, 1998). There are two major immunolgocially distinct groups of STX, namely, STX 1 and STX 2 (Nakao and

Takeda 2000). They are encoded by two distinct bacteriophages, which lysogenize the EHEC strains (Strockbine *et al.,* 1986; Shaikh and Tarr, 2003). STX 1 is identical to STX from *Shigella dysentriae* type 1 (Nakao and Takeda, 2000).

The toxin binds specifically to globotriaocylceramide (Gb_3) receptors on the host cell membrane (Lingwood *et al.,* 1987; Soltyk *et al.,* 2002), and is taken up by endocytosis. In the sensitive target cells it inhibits protein synthesis and leads to cell death by apoptosis (Yoshida *et al.,* 1999; Foster *et al.,* 2000; Smith *et al.,* 2003). *E. coli* O157:H7 is noninvasive (Paton and Paton 1998). In order to cause systemic complications, STX produced in the intestine must be translocated across the intestinal epithelium to the blood stream (Philpott *et al.,* 1997).

2.2.3 Clinical features

The infective dose of *E. coli* O157:H7 is unknown but there have been food poisoning cases where the implicated foods have been found to contain low levels of contamination (Tuttle *et al.,* 1999) and some estimates state as few as ten cells are required to cause illness (Betts, 2000).

The incubation period can last 1 to 8 days, 3 to 4 days being the most typical (Boyce *et al.,* 1995). The disease presents differently among individuals ranging from asymptomatic carriage, non-bloody diarrhoea, bloody diarrhoea (hemorrhagic colitis) haemolytic uremic syndrome (HUS), thrombotic thrombocytopenic purpura and may even culminate in death (Griffin *et al.,* 1988). In 2-15% of patients infected with *E. coli* O157:H7 the illness progress to HUS (Dundas *et al.,* 2002). Most patients will recover with appropriate supportive therapy, but 3 to 5 % of victims

(Especially children and elderly) will die and about 30% of survivors suffer a range of permanent disabilities including chronic renal insufficiency, hypertension, and neurological deficits (Brandet *et al.*, 1994; Tarr, 1995).

2.2.4 Epidemiology

Human infection with *E. coli* O157:H7 has been reported from over 30 countries on six continents (Mead and Griffin, 1998). Incidence rates are relatively high in North America, Europe and Japan. In the developing counties, EHEC is much less frequently isolated than other diarrheagenic *E. coli* such as ETEC and EPEC (Nataro and Kaper, 1998).

Many domestic and wild animals are known to harbour *E. coli* O157:H7 and other STEC strains (Beutin *et al.*, 1993; Chapman et al., 1993; Rabatsky-Ehr *et al.*, 2002, Renter *et al.*, 2003). However, cattle are most often implicated as the zoonotic sources of human infection (Gansheroff and O'Brien, 2000).

Beef and beef products are the primary vehicles associated with *E. coli* O157:H7 (Hart *et al.*, 1997). Contamination of crops and water supplies, with the use of manure as fertilizer, or the close proximity to cattle explain the involvement of the other variety of food vehicles or transmission routes (Gansheroff and O'Brien, 2000).

Contamination of carcasses occurs during the slaughter process by contact with hides and intestinal content of the infected cattle. In one study of abattoirs, Elder *et al.* (2000) have shown

that the total pre-harvest (faecal and hide) prevalence of *E. coli* O157:H7 correlated with the prevalence of carcass contamination within a given lot. Subsequent DNA finger printing analysis of the isolates confirmed the relationship by showing that 68.2% of the post-harvest (Carcass) isolates matched the pre-harvest (animal) isolates (Genevieve *et al.*, 2001). In an earlier study of an abattoir, Chapman (2000) reported isolation of *E. coli* O157:H7 from 4% of cattle at slaughter and on up to a third of carcasses from rectal swab positive animals.

In another study Chapman and colleagues (2000) reported isolation of 72 *E. coli* O157:H7 strains from raw beef and lamb products. The isolates were subdivided into 20 different subtypes of which 18 were identical to strains previously isolated from cattle and sheep. Eight of the 18 strains were also found to be identical to isolates from human cases of infection during the study period. Similar correlation findings and subtype matches have also been reported in a subsequent study (Chapman *et al.*, 2001).

Richards and co-workers (1998) reported that there existed, a significant tendency for carcasses present in the same abattoir on the same day to have similar results suggesting cross contamination. Thus even if just a few infected cattle are slaughtered, there exists ample opportunity for subsequent cross contamination of carcasses from uninfected cattle processed in the same abattoir.

The problem of cross-contamination is further compounded during the production of ground beef. Because the meat of many carcasses is mixed in the production of ground beef it only takes a few cattle to be infected to contaminate a large amount of meat (Flores and Tamplin, 2002).

The largest beef-associated *E. coli* O157:H7 outbreak occurred in the USA. The outbreak involved four states between November 1992 and February 1993, affecting about 800 people, 3 of whom died (Bell *et al.,* 1994). Epidemiological trace back associated the cases with eating hamburgers made from contaminated ground beef patties at restaurants of one fast food chain. Isolates of *E. coli* O157:H7 from the recalled beef patties were indistinguishable from those isolated from the victims on pulsed field gel electrophoresis (Tuttle *et al.,* 1999). Public health action removed more than 250,000 potentially contaminated hamburger patties preventing an estimated 800 cases (Bell *et al.,* 1994). Three multistate hamburger-associated *E. coli* O157:H7 outbreaks had occurred in the USA during the year 1999 (CDC, 1999). One of these led to a nation wide recall of over 170,000 pounds and another 250,000 pounds of ground beef.

Similarly during the year 2002, an ongoing epidemiological and laboratory investigation linked 28 case of *E. coli* O157:H7 human infections in Colorado and six other states to consumption of ground beef products recalled by Con-Agra beef company Following the recognition of the multistate outbreak and strain matches of isolates from victims and recalled beef products, a further in-plant inspection of the beef company was initiated. The recall of beef eventually soared to the staggering 18.6 million pounds of fresh frozen ground beef and beef trimmings (CDC, 2002).

2.2.5 Laboratory Diagnosis

Three important biochemical characteristics of *E. coli* O157:H7 are exploited in its isolation and identification. These are inability to ferment D-sorbitol and rhamnose, and lack of β–glucuronidase activity.

The culture medium most commonly used for the isolation of *E. coli* O157:H7 is the sorbitol-MacConkey (SMAC) agar (March and Ratnam, 1986; Smith and Scotland, 1993; Paton and Paton, 1998). It contains 1% sorbitol in place of lactose in the standard MacConkey agar medium as a differential component. *E coli* O157:H7 colonies are identified as colourless sorbitol-nonfermenters (SNF) on SMAC agar incubated at 37 ^{0}C for 18-24 hours.

A number of modified SMAC agar media with improved selectivity and sensitivity have been developed. The use of novobiocin-supplemented E.C broth (EC+n) as a selective enrichment step, followed by plating on cefixime-tellurite- SMAC (CT-SMAC) agar has been used with success (Paton and Paton, 1998).What ever plating and enrichment method is used, presumptive isolates must be biochemically confirmed as the biotypic *E. coli*.

Serotyping of *E. coli* is performed using antisera specific for O157:H7 either by slide or tube agglutination technique. A number of cross-reacting bacteria are known, *Escherichia hermani* being the most notable one (Smith and Scotland, 1993, Chart and Jenkins, 1999). *E. hermani* also shares many of the salient biochemical properties of *E. coli* O157:H7 but unlike O157:H7, it does not ferment cellobiose and does not grow in the presence of Potassium cyanide (KCN) (Smith

and Scotland, 1993).

Because no role for preformed STX in food is known, the presence or absence test for STX is done only on clinical specimens (diarrhoeal stool). The gold standard test for detection STX in stool is the Vero cell assay (Smith and Scotland 1993).

STX specific ELISA and DNA-based methods have also been developed; comprehensive review can be found elsewhere (Paton and Paton, 1998). The methods involve the use of labelled oligoneucleotide probes and the polymerase chain reaction (PCR) technology. Pulsed filed gel electrophoresis (PFGE) is the most extensively sued DNA-based sub typing method in outbreak settings (Barret *et al.,* 1994; CDC, 2002)

2.2.6 Control and prevention

No specific chemotherapy has been shown to be effective against *E. coli* O157:H7 infection (Nataro and Kaper, 1998). The role of antibiotic treatment is controversial. A study by Wong *et al.* (2000) has revealed that antibiotic treatment of children infected with *E. coli* O157:H7 increased the risk of haemolytic-uremic-syndrome. A similar finding was also reported by Dundas and Co-workers (2001). A promising therapeutic agent that may be helpful to prevent systemic complications of the *E. coli* O157:H7 infection is the Synsorp K. It is a Gb_3 analogue and presumed to absorb STX produced in the gut and prevents systemic complication (Paton and Paton, 1998). There are no currently available vaccines. Most vaccines on trials in animal models are based on STX (Ishikawa et al., 2003; Sheoran *et al.,* 2003). STX-based vaccines may be

useful to prevent systemic complication but cannot prevent bacterial colonization.

What enables *E. coli* O157:H7 to beat all the hurdles of modern hygienic control measures appears to be its remarkable low infectious dose. The recognition of the low infectious dose of the pathogen has now forced some developed countries to adopt zero tolerance of faecal contamination of meat carcass in slaughter plants (Heuvelink *et al.*, 2001; Reinders, *et al.*, 2001).

Despite all these unprecedented efforts, this newly emerging pathogen continues to strike one country after another challenging the biomedical community, alarming food producers and transforming the publics' perception about the safety of their food (Mead and Griffin, 1998).

2.3 General Consideration on other foodborne pathogens

2.3.1 Salmonella enterica

The genus *Salmonella* is now considered to be a single species named *Salmonella enterica*. Serotyping differentiates the strains and these are referred to by name as for example, *Salmonella enterica* serotype Typhimurium, or *Salmonella* Typhimurium (Hohman, 2001).

Salmonellae are gram-negative, motile (with a few exceptions), facultatively anaerobic bacteria that conform to the characterstics of the family *Enterobacteriaceae* and the tribe salmonellae (Edwards and Ewing, 1972). The details of their biochemical profile can be found in Edwards and Ewing (1972). They grow between 5.2 ^{0}C and 46.2 ^{0}C, and at pH of 3.7 to 9.5. Their limiting

level of water activity is about 0.94 with the maximum tolerable NaCl concentration being 8% (UNIDO, 2003).

Over 2500 *Salmonella enterica* serotypes or serovars are recognized and all are regarded pathogenic to humans. While some serotypes of *Salmonella* such as *S.* Typhi and *S.* Paratyphi A and C (in humans) and *S.* Pullorum (in poultry), have restricted host range, most serotypes infect a broad range of warm-blooded animals and are capable of causing disease in humans (Darwin and Miller, 1999).

Salmonella is capable of causing a variety of disease syndromes: enteric fever, bacteraemia, enterocolitis and focal infections. Enterocolitis is by far the most common manifestation of disease caused by *Salmonella*, but bacteraemia and focal infections can accompany or follow enterocolitis. Enteric fever (typhoid fever) is caused primarily by *S.*Typhi and *S.* Paratyphi and occasionally by other serotypes (Darwin and Miller, 1999).

The typhoidal salmonellae (human-host-adapted) are characterized by low infective dose, prolonged incubation period (10-20 days or more), septicaemia, tendency to produce permanent carriers and become endemic. They are transmitted by faecal contamination of food and water and rarely person-to-person (Silliker and Gabis, 1986).

While approximately 2000 serotypes of nontyphoidal salmonella have been associated with enterocolitis, at a given time it is a smaller set of about 10 serotypes that accounts for the majority of infections; these typically include *S.* Typhimurium, *S.* Enteritidis, and *S.* Heidelberg (Tauxe, 1996, Cited in Darwin and Miller 1999). It is estimated from volunteer studies that 10^5 to

10^{10} *Salmonella* are required to initiate an infection (Reviewed in Silliker and Gabis, 1986). The exact infective dose varies with the strain, the food vehicle and the physiological state of the host (Darwin and Miller, 1999).

The invasive journey towards illness in the host is influenced by distinct temperature differences, osmolarity, oxidation-reduction potentials, environmental iron concentrations, pH and organic and inorganic nutrient environment (Salauch *et al.,* 1997 cited in WHO/FAO, 2002). Nontyphoidal Salmonellae possessing certain adaptive features are more likely to produce foodborne disease. First they must be acid tolerant to survive the pH of the stomach. They must be able to attach to invade intestinal epithelia and Peyer's patches (Darwin and Miller, 1999).

Bacterial virulence factors include those that promote adhesion to host cells in the intestines: specific fimbriae, chromosome-coded bacterial surface adhesins, haemagglutinins and epithelial cell induction of bacterial polypeptides that can promote adhesion (Darwin and Miller, 1999).The O side chain of lipopolysaccharide (LPS), affect resistance of salmonella to lytic action of complement, invasiveness, and enterotoxin production (Murray, 1986). Siderophores are necessary for the accumulation of sufficient iron to allow growth of salmonella. Virulence plasmids have been associated with the ability to spread after colonization, invasion of intestinal wall, ability to grow in the spleen and a general suppression of the host immune response (Chin *et al.,* 1999). Other factors that aid in the damage of the host include cytotoxins and diarrhoeagenic enterotoxins. Host factors that are known to have impact on the out come of *salmonella* infection include, demographic and socioeconomic factors, genetic factors and health status (WHO/FAO, 2002).

In cases of enterocolitis, the incubation period is typically 6 to 48 h and is followed by headache, abdominal pain, diarrhoea and vomiting. The diarrhoea can contain blood, lymphocytes and mucus. Fever, malaise and muscle ache are quite common. Symptoms usually resolve within a week but *Salmonella* can be shed in the faeces for up to 20 weeks by children < 5 years of age and for 8 weeks by adults (Turnbull, 1979). Children may shed up to 10^6 to 10^7 salmonellae per gram of faeces during convalescence (Cruickshank and Humphrey, 1987; cited in WHO/FAO, 2002).

In some patients long-term sequele may occur and a variety have been identified, including arthritis, osteoarthritis, appendicitis endocarditic, pericarditis, meningitis, peritonitis and urinary tract infections. The mortality rate due to salmonelosis is estimated to be 0.04 to 0.1% (Mead *et al.,* 1999). Severe illness due to *Salmonella* infection is further exacerbated by the emergence of strains of multi-drug resistant *Salmonella* such as *S.* Typhimurium defintive phage type 104 (MR-DT 104) (Akkina *et al.,* 1999).

DT 104 carries chromosomally encoded resistant genes. It is resistant to at least 5 antimicrobials including ampicillin, chloramphenicoles, streptomycin, sulphonamides, and tetracyclines (R-type ACSSuT). It is an example of the increasing emergence of antimicrobial resistant pathogens that are causing international concern. (Threlfall, 2000)

Over the last decades this clone has caused outbreaks of infection in food animals and humans in numerouse European countries including the Irish republic (Corbett-Feeney and Riain, 1998), Denmark (Molbak *et al.*, 1999), Germany (Malorny *et al.* 1999), and France (Caslin *et al.*, 1999). The emergence of the strain in the USA has also been reported (Glynn *et al.* 1998)

An increase in virulence could result from linkage of antimicrobial resistance factors to other virulence genes and greatly alter the disease outcome. Resistant pathogens limit therapeutic options and may lead to failure of therapy and increased morbidity and mortality.The prognosis of salmonellosis caused by resistant strains is more dependent on host characteristics than that caused by sensitive strains.

Antimicrobial therapy should be initiated for only those who are severely ill and for patients with risk factors for extraintestinal spread of infection, after appropriate blood and faecal cultures are obtained (WHO/FAO, 2002). Worldwide salmonellosis is a leading cause of enteric infectious disease attributable to foods. Approximately 40,000 *Salmonella* infections are culture confirmed, serotyped and reported in the USA and 96% of these are caused by foods (Mead *et al.,* 1999). The incidence of *Salmonella* per 100,000 people during the year 1997 was estimated to be 14 in the USA, 38 in Australia, 73 in Japan, and in the European Union ranged between 16 in Netherlands and 120 in Germany (Thorns, 2000; cited in WHO/FAO, 2002).

In Ethiopia, crosssectional studies on the prevalence of *Salmonella* among diarrhoeal out patients in Addis Ababa have been reported including, 3.8% (Daniel Asrat*et al.*, 1999), and 10.7% (Birhanu Andulaem and Aberra Geyid, 2003).

There are also some studies on the incidence of *Salmonella* in some Ethiopian foods. Pegram *et al.* (1981) isolated *Salmonella* from samples obtained from farm livestock, an abattoir and a bone factory in Ethiopia. They detected 27 serotypes in 130 contaminated samples. In another study Wolde-Aregey Erku and Mogessie Ashenafi (1998) reported the isolation of *Salmonella* from bottle contents made of cow's milk and gruel from cereal blend.

Most studies on *Salmonella* in Ethiopian foods focus on beef and poultry meat. In their study of the safety of street foods from some quarters of Addis Ababa, Dereba Muleta and Mogessie Ashenafi (2001) reported the isolation of *Salmonella* from 9 of 30 kitfo samples purchased from street vendors. In another study Girma Zewdie (1999) isolated *Salmonella* from variouse parts of chicken carcass. He reported that 62% of whole carcass, 80% of wings, 60% of back, and 50% of liver samples yielded *Salmonella.* The most frequent serotypes were *S.*Hadar (47%), *S.* Enteritidis (23.5%), and *S.* Branderup (11.8%). Other species consisting of S. Heidelberg, *S.* Indiana, and *S.* Typhymurium were also detected. Bayleyegn Molla *et al.* (1999) have also isolated *Salmonella* from 19 of 85 chicken giblet samples obtained from retailc markets in Addis Ababa.

More recently Bayleyegn Molla and Coworkers (2003) reported the isolation of *Salmonella* from 80 of 378 chicken carcass and giblet (liver, gizzard, and heart) samples obtained from processing plants at Debrezeit and supermarkets in Addis Ababa. Fifty-one (63.7%) of the 80 salmonellae were resistant to one or more antimicrobials of which 42 (52.5%) displayed multiple drug resistance. S. Typhimurium var. Copenhagen (100%), S. Anatum (62.5%), S. Typhimurium (33.3), and S. Braenderup (34.3) showed multiple antimicrobial resistances.

Worldwide a variety of foods have been implicated in foodborne illness due to *S. enterica* with the principal vehicles being beef poultry and eggs (Darwin and Miller, 1999). A review of 500 human salmonellosis outbreaks showed that meat was the second most important vehicle food accounting of 13% of the outbreaks among the known food sources (Gangarosa, 1978; cited in Silliker and Gabis 1986). In another review of 265 human salmonellosis outbreaks, beef was found to be the top leading vehicle food accounting 9.7% of the outbreaks (Bean and Griffin, 1990; cited in Jay 1996). The US federal court rules that salmonellae are inherent defects in red

meat and poultry (Silliker and Gabis, 1986).

2.3.2 Shigella species

The genus *Shigella* belongs to the family *Enteobacteriaceae,* as do the salmonellae and escherichiac. Only four species are recognized: *S. dysentriae, S. flexineri, S. boydii* and *S. sonei* (Jay, 1996). Phylogenetically, they are closer to the escherichiae than the salmonellae.

They are nonmotile and produce acid from glucose fermentation but without gas formation. Biochemically, they are less active than the salmonellae and escherichiae. They grow between 6.1 ^{0}C and 47.1 ^{0}C and at pH of 4.8 to 9.3, and require a water activity of about 0.96 or more with the maximum tolerable NaCl concentration being 5.2 % (UNIDO, 2003). The details of their biochemical profile can be found in Edwards and Ewing (1972). Since the shigellae lack flagellar antigens they cannot be expected to attain the taxonomic complexity of the salmonellae.

The shigellae are primarily human intestinal pathogens, so that when a member of this genus has been identified as the cause of an epidemic it may be presumed generally that the source was the faeces of a human case or carrier of bacillary dysentery (Dolman, 1957).

All four species invade the colonic mucosa, multiply and elicit acute inflammatory infiltrates in the lamina propria (Finlay and Flakow, 1988; cited in Jay, 1996). In essence, they produce diarrhoea by penetration and destruction of colonic epithelia. Once they have been internalized in endocytic vacuoles, their escape requires a 220-Kbp plasmid, especially in *S. flexneri*. This plasmid is responsible for the inhibition of protein synthesis in infected cells (Jay, 1996).

In the USA, the incidence rate of shigellosis during the year 1996 was estimated to be 9 per 100,000 people (CDC, 1997), ranking third, next to *Salmonella* and *Campylobacter* spp. In Ethiopia, cross-sectional studies on prevalence of shigellosis among diarrhoeal out patients in Addis Ababa have been reported, including, 5.4 % (Abebe Mache *et al.,* 1997;cited in Birhanu Andualem and Aberra Geyid, 2003), 11.7% (Daniel Asrat *et al.,* 1999), and 5.8% (Birhanu Andualem and Aberra Geyid ,2003). According to an earlier report, the most prevalent species in Ethiopia are *S. flexineri* and *S. dysentriae* (Afeworki Gebre-Yohannes and Drasar, 1987).

All species of *Shigella* may be involved at least theoretically in meat borne infections (Dolman, 1957). Personal hygiene is a common factor in foodborne shigellosis. Flies often play an important part in epidemic shigellosis particularly in camps where poorly constructed latrines encourage their breeding.

2.3.3 Staphylococcus species

The genus *Staphylococcus* contains the most important foodborne pathogens in the family micrococcaceae. The members of this genus are gram-positive cocci commonly occurring in grape-like clusters. They react positively with the catalase test, which differentiates them from the *Streptococcus* spp (Sierra *et al.,* 1995).

Around 31 species are included in the genus *Staphylococcus* and 18 of these are considered to be of potential interest in foods (Jay, 1996). They synthesize one or more enterotoxins and release it into the food medium and this preformed enterotoxin is responsible for the gastroenteritis

syndrome. Although enterotoxin production is believed generally to be accociated with *S. aureus* strains that produced coagulase and thermonuclease (TNase), many species of *Staphylococcus* that produce neither coagulase nor TNase are known to produce enterotoxins (Jay, 1996).

Staphylococcus aureus grows between 7^0 C and 47.8^0 C and produces enterotoxins between 10^0 C and 46^0 C with the optimum being between 40^0 C and 45^0 C (Smith *et al.,* 1983, cited in Jay, 1996). With respect to water activity, the limiting levels for growth and enterotoxin production are 0.83 and 0.85, respectively (UNIDO, 2003). *S. aureus* grows well in culture media without NaCl; it can tolerate NaCl concentration up to a maximum of 25%. The pH ranges for growth and enterotoxin production are 4 to 10 and 4 to 9.8, respectively. Increasing the cocnentration of NaCl progressively narrows the pH range for cnterotoxin production towards neutrality (Geneigeorgis *et al.,* 1971 cited in Jay 1996).

Nine staphylococcal enterotoxins have becn identified which differ in their chemical and physical properties. In addition to the staphylococcal enterotoxins (SE) some strains also produce the toxic shock syndrome toxin (TSST) (Jay, 1996). The SEs are quite heat resistant. The biological activity of SEB was retained after heating for 16h at 60^0C and pH 7.3 (Schantz *et al.,* 1965; cited in Jay, 1996). *S. aureus* cells are by contrast considerably more sensitive. In meat products, SEA was produced with log 7.2 cells/g (Notermans and Van Otterdijk, 1985; cited in Jay, 1996). All staphylococcal enterotoxins (SE) have the same potency.

The minimum quantity of SE needed to cause illness in humans is about 20 ηg (Evenson *et al.,* 1988; cited in Jay, 1996). The SE and TSST are considered superantigens and act by eliciting cytokine production, particularly interleukin 2 *(IL-2)*. The incubation period ranges between 1

and 6h and the symptoms include nausea, vomiting, abdominal cramps, diarrhoea, sweating, headaches, prostration and sometimes hypothermia. The illness resolve within 24 to 48 h and mortality rate is very low to nil. Definitive diagnosis is established by recovering enterotoxigenic staphylococci from leftover food and from stool cultures of victims.

Staphylococci are host adapted with about one half of the known species inhabiting humans solely (e.g. *S.cohnii* sbusp. *cohnii*) or humans and other animals (eg. *S.aureus*).The two most important sources to foods are nasal carriers and individuals whose hands and arms are inflicted with boils and carbuncles who are permitted to handle food.

All raw foods of animal origin or those that are handled directly by humans are expected to contain some level of staphylococcal contamination. In all cases of staphylococcal food poisoning, the foodstuff or one of the ingredients, is contaminated with an SE-producing staphylococci strain and is exposed, at least for a while, to temperatures that allow *S. aureus* growth. Most of the time the foodstuff reaches this temperature because of a failure in the refrigeration process, or because a growth-permissive temperature is required during processing

The foods that are most often involved in staphylococcal food poisoning differ widely from one country to another. In the United Kingdom, for example, 53% of the staphylococcal food poisonings reported between 1969 and 1990 were due to meat products, meat-based dishes, and especially ham; 22% of the cases were due to poultry, and poultry-based meals, 8% were due to milk products, 7% to fish and shellfish and 3.5% to eggs (Wieneke *et al.*, 1993; cited in Loir et al., 2003).

In France, things are different. Among the staphylococcal food poisonings reported in a two-year period (1999-2000), milk products and especially cheeses were responsible for 32% of the cases, meats for 22%, sausages and pies for 15%, fish and seafood for 11%, eggs and egg products for 11% and poultry for 9.5% (Loir *et al.*, 2003).

In the United States, among the staphylococcal food poisoning cases reported between 1975 and 1982, 36% were due to red meat, 12.3% to salads, 11.3% to poultry, 5.1% to pastries and only 1.4% to milk products and seafoods. In 17.1% of the cases, the food involved was unknown (Genigeorgis, 1989; cited in Loir *et al.*, 2003). Thus, the origins of staphylococcal food poisoning differ widely among countries; this may be due to differences in the consumption and food habits in each of the countries.

Published reports on outbreak investigation of food poisoning due to stphylococci in Ethiopia are unavailable. There are limited reports on the isolation of coagulase positive staphylococci from various Ethiopian foods, but enterotxigenicity of the strains was not assessed in any of the studies. Mogessie Ashenafi (1989) isolated *S. aureus* from 4- 8 % of tomato, green pepper, eggs and fish samples. Also, Girma Tulu and Birihanu Abegaz Gashe (1992) isolated two species of staphylococci including *S. aureus* from foremilk of 120 apparently healthy lactating cows in and around Addis Ababa. In another study Mogessie Ashenafi and Yewelsew Abebe (1996) reported the isolation of staphylococci at leveles of 10^5 cfu/g from 30 samples each of kocho and bulla from Awassa open market. *S. aureus* constituted 50 – 100 % of the staphylococci.

Staphylococcus was also among the foodborne pathogens that were isolated from weaning foods (Zeleke Wolde-Tensy and Haile-Selassie Tesfaye, 1992; Wolde-Aregay Erku and Mogessie Ashenafi, 1998) and various fruits and vegetables in Ethiopia (Abera Geyid *et al.*, 1991).

III. Materials and Methods

3.1 Collection of Beef samples

Beef samples were purchased from twenty butcher shops using polyethylene or paper bags commonly used by butchers. Sterility of all containers was not checked as it was intended to indicate the microbial profile during typical handling procedures at the retail level. A total of 200 beef samples (500g per sample) consisting of 100 sliced and 100 minced beef samples were purchased during the period between November, 2001 and July 2002 form the 20 butchers'shops around four city zones in Addis Ababa. The meat cuts were random as is customary in the butchers' shops, and anatomical parts were not specified during purchasing. Samples were brought to the laboratory at ambient temperature but were stored in refrigerators (4^0C) when there were occasional delays before analysis. All meat in this work refers to beef. Unless specified all culture media powders used were from Oxoid (Basingstoke, England).

3.2 Antisera

Polyvalent O and group specific (A to E) and capsular (VI) antisera for *Salmonella* (all were BBL brand) were used. Also, O157 specific latex agglutination test kit (Oxoid), and H7 specific antiserum for *Escherichia coli* were used.

3.3 Antimicrobial susceptibility test discs

Oxoid brand discs of 10 antimicrobial agents, having the indicated concentrations were used These included, Ampicillin (Amp) 10 μg, Cephalothin (Cep) 30ug, Genetamicin (Gen) 10μg, Kanamycin(Kan) 30μg, polymixin B (pol) 300 units, Streptomycin (Str) 10μg, Tetracyclin (Tet)30μg, Amoxycilin (Amx) 2μg, Amikacin (Ak) 30μg, and Nitrofurantion (NF) 300μg,

3.4 Study design

Screening for *Escherichia coli* O157:H7 *Salmonella* and *Shigella* spp. were performed on all the 200 beef samples, whereas enumerations of the mesophilic aerobic flora were done on 47 beef samples consisting of 25 sliced and 22 minced beef sample.

3.5 Preparation of Homogenate

3.5.1 Sliced beef samples

For each sample, 25g of random excision pieces of beef were aseptically weighed directly into a stomacher bag (Seward Medical) near the flame of a Bunsen burner and homogenized by a stomacher (Seward Medical) with 225 ml of 0.1% peptone water for 2 minutes.

3.5.2 Minced beef samples

Each sample was thoroughly mixed while it was still in the polythene bags without being opened by kneading manually. From the well mixed sample, 25g of minced beef was weighed out into stomacher bags (Seward Medical) and homogenized with peptone water as in 8.1.

3.6 Enrichment and isolation

3.6.1 Presumptive *Escherichia coli* O157:H7

From each homogenate, a loopful was streaked out on a sorbitol-MacConkey (SMAC) agar plate and 0.1ml amount was pipetted into a tube of 9ml nutrient broth. The broth tube was vortexed and along with the SMAC plate, both were incubated at 37 ^{0}C for 18 to 24 hours. At the end of the incubation period a loopful was streaked from the broth culture on to a sterile SMAC agar plate and incubated as above. The nutrient broth was intended to resuscitate *E. coli* cells that might have been stressed in the beef sample. In either case the SMAC agar plates were examined for sorbitol non fermenter (SNF) colonies at the end of incubation periods. Two to three typical SNF colonies were isolated and purified by repeated sub culturing, as needed and the isolates were maintained on nutrient agar slants until biochemical and serological confirmation.

3.6.2 Presumptive *Salmonella* and *Shigella* species

A loopful of each homogenate was streaked out on to Xylose-Lysine-Desoxycholate (XLD) agar plates and 0.1 ml amount was pipetted out into 9ml of Rappaport-Vassiliadis (RV) broth (MERCK, Germany). The XLD plate was incubated at 37 ^{0}C for 18 to 24 hours whereas the RV

broth was vortexed and incubated at 42 ^{0}C for 18 to 24 hours. At the end of the incubation period a loopful from the RV broth culture was streaked on to a sterile XLD agar plate and incubated at 37 ^{0}C as above. In all cases the XLD agar plates were examined for typical colonies of *Salmonella* (black colonies with red centre) and *Shigella* (Red or colourless transparent colonies). Whenever suspect colonies seen multiple colonies were isolated and purified by repeated sub culturing. The presumptive *Salmonella* and *Shigella* isolates obtained as such were maintained on nutrient agar slants for biochemical and serological confirmation.

3.7. Enumeration of mesophilc aerobic flora

3.7.1 The mesophilic aerobic plate count (MAPC)

The 1:10 beef homogenate was further diluted six-fold serially in tubes of 0.1% peptone water. From each of the three tubes having the highest dilutions (10^{-4}, 10^{-5} and 10^{-6}), 0.1 ml amount was spread plated on to the surface of separate nutrient agar plates. All plates were incubated at 37 ^{0}C for 18 to 24 hours. At the end of the incubation period, plates having counts between 30 and 300 colonies were considered to determine the average MAPC.

3.7.2 Determination of the total coliform count (TCC)

From each of the 1:10^3, 1:10^4 and 1:10^5 dilutions prepared as in 7.1, 0.1ml amount was spread plated on to separate plates of Violet-Red-Bile Agar (VRBA). All plates were incubated at 37^0 C for 18 to 24 hours and the average TCC was determined as in 7.1 by counting the rose pink colonies.

3.7.3 Determination of the count of *Staphylococci*

The average staphylococci count was determined on Mannitol-Salt agar (MSA) plates by spread plating from the dilutions as in 7.2, and counting the yellow colonies after 48h of incubation at 37^0 C.

3.8 Analysis of the dominant mesophilic aerobic flora

From the countable plates used in MAPC (7.1) 15 random colonies were isolated by transfer into separate tubes of nutrient broth (3ml); Their purity was ascertained by repeated subculturing as needed and they were tentatively identified to the genus level by subjecting each pure isolate to the following tests.

3.8.1 Gram reaction using the KOH method

From purified plates, a colony was picked with a loop and mixed with a drop of 4% KOH on a glass slide. The presence or absence of stringy consistency was examined by rotating and mixing and raising with the loop. The isolate was then scored as KOH positive (Gram negative), if it exhibited the stringy consistency or as KOH negative (Gram positive) if it failed to do so.

3.8.2 Catalase reaction

A colony was picked from a purified plate and mixed with a drop of 30% H_2O_2 on a glass slide. Isolates that exhibited the tiny bubbles were scored as catalase positive and those that failed to show the bubbles were scored as catalase negative.

3.8.3 Oxidase test

A drop of freshly prepared oxidase reagent was was used as deseribed in Edwards and Ewing (1972)

3.8.4 Oxidation Fermentation (O/F) test

Hugh and Leifeson's medium was used as described in Edwards and Ewing (1972)

3.8.5 Microscopy

A wet mount was prepared from a colony of each purified isolate and was examined under oil immersion objective of a light microscope for the following bacterial morphological feature, *viz.* cell shape, arrangement and sporulation (in some cases),.

3.8.6 Spore staining

This was done to differentiate gram-positive rods that reacted positively for catalase but failed to show sporulation on wet mount examination. The safranin-methyl blue staining procedure was employed on a fixed smear prepared from a 3-day-old pure culture of each isolate. The catalase positive, gram positive rods were differentiated as *Bacillus* spp if they showed sporulation or other gram positive rods (OGPR) if they failed to show sporulation.

3.9 Biochemical tests for the enterics

The presumptive isolates of *E. coli* O157:H7 *Salmonella* and *Shigella* spp. that were maintained on nutrient agar slants were activated by transfer into separate tubes of nutrient broth. After overnight incubation at 37 ^{0}C, the broth cultures were used as inoculants in the standard panel of biochemical tests for *Enterobacteriaceae* (Edwards and Ewing, 1972). The test panel consisted Triple sugar iron agar, Lysine iron agar, urea agar Simon's citrate agar, Glucose broth, Mannitol broth, Malonate broth, and Indole tests.

3.10. Motility test (Semisolid medium)

This semisolid medium was stab inoculated and incubated at 37 ^{0}C for 18 to 24h. It was used to determine the motility of the test organism. Based on the motility feature and the biochemical tests, the biotype of the presumptive isolates was determined using a key chart according to Edwards and Ewing (1972).

3.11 Serological tests

3.11.1 Serogrouping of the *E. coli* isolates

Sorbitol nonfermenter (SNF) isolates that were biochemically confirmed as *E. coli* were serogrouped by latex agglutination method according to the instruction enclosed in the kit (Oxoid); briefly, part of a colony from a pure plate was mixed with a 50µl of sterile saline on the slab of paper slide to form an emulsion. The bacterial suspension was then thoroughly mixed

with the latex test spots on the slide. The presence or absence of agglutination was noted. The same was performed on the reagent control spot on the opposite end of the slide.

3.11.2 Serotyping of *E. coli* O157

The sorbitol nonfermenter (SNF) *E. coli* that were determined as belonging to the serogroup O157 were further serotyped for H7 flagellar antigen. This was done by tube agglutination method according to the procedure enclosed in the Kit (Oxoid).

Briefly, the test antigen was prepared first by passing the test culture 2 times in motility medium and then transferred to nutrient broth which was incubated overnight at 37 ^{0}C. The broth culture was inactivated by adding formalin to make 0.3% (V/V). The turbidity was adjusted to approximately the third McFarland's standard and this was used as test antigen. Meanwhile, the H7 specific antiserum was rehydrated by adding 3ml of sterile saline and after thorough solubilization, 0.1 ml amount was transferred to a clean and sterile tube containing 24.9 ml of sterile saline to make a 1: 500 dilution (according to the kit). Of this diluted antiserum, 0.5 ml was mixed with an equal volume of the test antigen in a separate clean and sterile tube which was then incubated at 50^{0} C water bath for 1 hours. Positive control (*E. coli* O157:H7 MF-1847, Howard University, USA) and negative control (*E. coli* Non-O157 strains) were also included to assist interpretation and the precipitation reaction was noted at the end of the incubation period and recorded

3.11.3 Serogrouping of *Salmonella*

The presumptive *Salmonella* isolates which exhibited the typical biochemical profile were serogrouped by slide agglutination method according to the procedure enclosed in the kit (BBL). In brief, a sweep of growth on TSI agar slant was used as test antigen which was mixed with a drop of the test antisera on a sterile and clean glass slide. This was first done with the polyvalent O antisera and then positviely agglutinatinating test cultures were tested against individual antisera.

3.12 Antimicrobial susceptibility test

Biochemically and serologically confirmed *E. coli* O157:H7 and *Salmonella* isolates were tested for susceptibility by the standard disc diffusion method (Bauer *et al.*, 1966).

In short, an overnight broth culture of the test organisms was streaked out on MacConkey agar plates and incubated at 37 ^{0}C for 18 to 24h. Four to five colonies from the MacConkey plate were transferred into a tube of TSB broth and thoroughly suspended by vortex mixing. This suspension was used as inoculum to seed a Muller-Hinton agar plate by dipping with a sterile cotton swab. The sceded agar plate was left for 3 to 5 minutes while the cartridges of the antibiotic discs were loaded. Then, the discs were dispensed on to the surface of the seeded Muller Hinton agar and gently pressed with a sterile forceps to ensure firm contact. The plate was incubated at 37 ^{0}C for 18 to 24 h. At the end of the incubation period, the inhibition zones were measured in millimetres using a calliper and measurements were interpreted as sensitive, intermediate or resistant according to a standard

IV. Results

4.1 Microbial load of the beef samples

4.1.1 The Mesophilic aerobic plate counts (MAPC)

The MAPC values for the sliced beef samples ranged between 6.7 and 9.2 log cfu/g and the average value for the same parameter was 8.21 log cfu/g (Table 1).

The MAPC values for the sliced beef samples in general were variable as evidenced by the value of the coefficient of variation (CV) which was greater than 2 (Table 1). Of the 25 the sliced beef samples 17 showed MAPC levels of less than 8 log cfu/g in which only 2 of the sliced beef samples had MAPC levels less than 7 log cfu/g; thus 23 of the 25 sliced beef samples exhibited MAPC levels greater than or equal to 7 log cfu/g (Table 2).

The minced beef samples also showed comparable MAPC levels ranging between 6.69 and 8.97 log cfu/g and an average MAPC value of 8.24 log cfu/g with standard deviation being 8.37 log units (Table 1). There was no statistically significance difference between the mean MAPC of the sliced and the minced beef samples ($P > 0.05$, $t_{\alpha} = 0.877$, at 45 d.f).

The MAPC value for the minced beef samples were less variable than those of the sliced beef samples as was evident from the CV value of 1.34 (Table 1). Of the 22 minced beef samples 11 had MAPC levels less than 8 log cfu/g in which 3 of the samples showed MAPC levels less than 7 log cfu/g, hence, 19 of the 22 minced beef samples had MAPC levels greater than or equal to 7

log cfu/g (Table 2).

4.1.2 The Total Coliform Counts (TCC)

With regard to TCC levels, the sliced beef samples exhibited a range of values between 4 and 8.26 log units and an average TCC of 7.02 log cfu/g (Table 1).

A CV value of 3.58 means, the TCC value for the sliced beef samples were extremely variable (Table 1).

Of the 25 sliced beef samples, 16 showed TCC levels less than 6 log cfu/g in which 11 had TCC levels less than 5 log cfu/g. Therefore, over half of the sliced beef samples had TCC values greater than or equal to 5 log cfu/g (Table 2).

The TCC values for the minced beef samples ranged between 4.43 and 7.13 log cfu/g and the average value was 6.44 log cfu/g (Table1). The CV value of 1.21 attests to the fact that, the minced beef samples exhibited relatively less variability in their TCC values than the sliced beef sample (Table1). There was no statistically significance difference between the mean TCC values of the sliced and the minced beef samples ($P > 0.05$, $t_{\alpha} = 0.31$, at 45 d.f).

Of the 22 minced beef samples, 11 had TCC scores less than 6 log cfu/g in which 3 of the samples had TCC scores less than 5 log cfu/g (Table2). Therefore 19 of the 22 minced beef samples had TCC values greater than or equal to 5 log cfu/g (Table2).

4.1.3 The Count of Staphylococci

The sliced beef samples had staphylococci count ranging between 4.62 and 7.45 log cfu/g and an average staphylococci count of 6.68 log cfu/g (Table 1). In general the sliced beef samples, relatively, showed a higher concordance with respect to staphylococci count than the MAPC and TCC values as evidenced by a CV value of 1.68 (Table 1).

Of the 25 sliced beef samples 11 had staphylococci count less than 6 log cfu/g, in which the number of sliced beef samples with staphylococci count values less than 5 log cfu/g was 2 (Table 2). Thus, 23 of the 25 sliced beef samples had staphylococci count values greater than or equal to 5 log cfu/g.

With regard to staphylococci count levels, the minced beef samples showed a range of values between 5.28 and 7.28 log cfu/g and an average of 6.91 log cfu/g (Table 1). Compared to the MAPC and TCC values the staphylococci count the minced beef samples had the least concordance as evidenced by the CV value of 1.8 (Table 1). There was no statisticaly significance difference between the mean staphylococci count of the sliced and the minced beef samples ($P > 0.05$, $t_{\alpha} = 0.34$, at 45 d.f).

Of the 22 minced beef samples, only 5 had staphylococci count values less than 6 log cfu/g and none of the minced beef samples had staphylococci count values less than 5 log cfu/g (Table 2).

Table 1 Mean counts (log cfu/g) of mesophilic aerobic bacteria (MAPC), total coliforms (TCC), and staphylococci in the 25 sliced and the 22 minced beef samples

Meat type	Microbial parameters	Minimum	Maximum	Mean	Standard deviation	Coefficient of variation
Sliced beef	MAPC	6.7	9.2	8.21	8.52	2.06
	TCC	4	8.26	7.02	7.57	3.58
	Staphylococci	4.62	7.45	6.68	6.91	1.68
Minced beef	MAPC	6.69	8.97	8.24	8.37	1.34
	TCC	4.43	7.13	6.44	6.52	1.21
	Staphylococci	5.28	7.82	6.91	7.17	1.8

Table 2 Grouped frequency distribution of mesophilic aerobic count, total coliform count and staphylococci count of the sliced (25) and the minced (22) beef samples

Count category (log cfu/g)	Mesophilic aerobic count		Total coliform count		Staphylococci count	
	Sliced beef	Minced beef	Sliced beef	Minced beef	Sliced beef	Minced beef
4-4.99	---	---	11	3	2	---
5-5.99	---	---	5	8	9	5
6-6.99	2	3	6	10	10	13
7-7.99	15	8	2	1	4	4
8-8.99	7	11	1	---	---	---
9	1	---	---	---	---	---

4.2 The spectrum of Mesophilic aerobic flora

4.2.1 The sliced beef flora

A total of 375 random colonies (15 colonies per sample) were analysed for tentative identification to the generus level from among the 25 sliced beef samples. They were grouped into 7 types of tentatively identified mesophilic aerobic bacteria and their distribution is presented table 3.

More than 93% of the mesophilic aerobic flora of the sliced beef samples was accounted by four types of mesophilic aerobic bacteria, including micrococci (40.8%), staphylococci (29.1%), *Bacillus* spp (13.3%), and members of the family *Enterobacteriaceae* (10.4%) in order of their abundance (Table 3).

The remaining three types of mesophilic aerobic bacteria accounted for less than 7% of the *mesophilic aerobic* flora of the sliced beef samples; and these were streptococci (2.13%), other gram positive rods (OGPR, 1.3%) , and other gram-negative rods (OGNR, 2.93%) (Table 3).

In general the mesophilic flora of the sliced beef samples exhibited a preponderance of gram positive bacteria which accounted for more than 86% and in which the dominant genera (69.9%) were *Micrococcus* and *Staphylococcus* spp. Among the gram negative flora the *Enterobacteriaceae* were predominant (Table 3).

The proportion of the individual group of mesophlic aerobic bacteria as a dominant flora per sample was variable among the 25 sliced beef samples. Micrococci and staphylococci accounted for more than 50% of the dominant flora in 5 and 7 of the 25 sliced beef samples, respectively. (Table 3).

In terms of incidence in the 25 sliced beef samples, micrococci (24/25), staphylococci (22/25), *Bacillus* spp. (20/25), and the *Enterobacteriaceae* (11/25), in their order, ranked as the most frequently encountered mesophilic aerobic bacteria (Table 5). The remaining groups were encounterd in less than 10 of the 25 sliced beef samples.

4.2.2 The Minced beef flora

A total of 330 random colonies (15 colonies per sample) were analysed for tentative identification to the generic level from among 22 minced beef samples. They were grouped into 6 tentatively identified mesophilic aerobic bacterial genera and their distribution in the 22 minced beef samples is presented in table 4.

The four top leading mesophilic aerobic bacteria were *Micrococcus* spp. (41.21%) the *Enterobacteriaceae* (19.69%), other gram-negative rods (OGNR, 12.73%), and *Bacillus spp.* (10.33%). These four mesophilic aerobic bacteria accounted for more than 80% of the mesophilic aerobic flora of the minced beef samples (Table 4). Micrococci and *Enterobacteriaceae* accounted for more than 50% of the dominant flora in 8 and 2 of the 22 miced beef samples, respectively. *Staphylococcus* spp. (6.96%) and other gram positive rods (OGPR, 9.1%) accounted

for less than 20% of the mesophilic aerobic flora (Table 4).

In general the minced beef samples showed more or less identical spectrum of mesophilic aerobic flora to that of the sliced beef samples. As in the case of the sliced beef samples, the mesophilic flora of the minced meat samples was dominated by gram-positive bacteria which accounted for more than 67% of the mesophilic aerobic flora with *Micrococcus* spp still out ranking all others. Among the gram-negative bacteria the *Enterobacteriaceae* were again the predominant in the minced meat mesophilic aerobic flora (Table 4).

Micrococcus spp (21/22), the *Enterobacteriaceae* (19/22), other gram-negative rods (OGNR, 19/22), and *Bacillus* spp (14/22), in their order, were the most dominant groups in the 22 minced beef samples. *Staphylococcus* spp. was encountered in only 10 of the 22 minced beef samples. This was much lower than the 22/25 (88%) incidence of staphylococci in the sliced beef samples. In sliced beef samples, however, OGNR and *Enterobacteriaceae* showed a higher incidence rate (Table 5).

Table 3 Distribution of the dominant mesophilic aerobic flora in the 25 sliced beef samples

Sample No.	Staphylococci	Micrococci	Streptococci	*Bacillus* spp.	OGPR	*Entero bacteriacea*	OGNR
1	3	7	4				1
2		8	1	4	1		1
3	6	8		1			
4	3	11		1			
5		14		1			
6	3	10		1		1	
7	9	6					
8	5	3		7			
9	10			2			3
10	11	4					
11	1	6		1		7	
12	5	10					
13	7	5	3				
14	2	7		2	1	3	
15	2	6		1		3	3
16	5	4		3		2	1
17	10	2		2			1
18	8	3		1		3	
19	4	2		1		8	
20	2	5		3		5	
21		6		4		5	
22	5	6		3		1	
23	6	5		3		1	
24	1	7		4	3		
25	1	8		5			1
Sum	109	153	8	50	5	39	11
Percentage (%)	29.1	40.8	2.13	13.3	1.33	10.4	2.93

OGPR=other gram-positive rods, OGNR=other gram negative rods

Table 4 Distribution of the dominant mesophilic aerobic flora in the 22 minced beef samples

Sample No.	Staphylococci	Micrococci	*Bacillus* spp.	OGPR	*Entero bacteriaceae*	OGNR
1	4	7	1		2	1
2	2	6	2	1	3	1
3	4	7	1		2	1
4		9	4			2
5		8	3		1	3
6		9	3	1	1	1
7		3			11	1
8		11			1	3
9	2	6		4		3
10	1	6	3		1	4
11		11	1	1	2	---
12	2	2			10	1
13		12		3		---
14		4		1	3	7
15	2	9		1	1	2
16		8	2	1	3	1
17		3	2		7	3
18		4		7	3	1
1`9	2		4	5	3	1
20	1	6	2		6	---
21	3	4	2	2	2	2
22		1	4	3	3	4
Sum	23	136	34	30	65	42
Percentage %	6.96	41.2	10.3	9.1	19.69	12.73

OGPR=other gram-positive rods, OGNR=other gram negative rods

Table 5.The Incidences of the prevailing mesophilic aerobic bacteria (MAB) in sliced beef (n= 25) and minced beef samples (n=22)

Types of MAB	Incidence in Sliced beef (n= 25)	Incidence in minced beef (n= 22)
Micrococcus spp	24	21
Staphylococcus spp.	22	10
Bacillus spp.	20	14
OGPR	3	12
Streptococcus spp.	3	0
Enterobacteriaceae	11	19
OGNR	7	19

OGPR= other gram-positive rods, OGNR= other gram-negative rods

4.3 The Incidence of *Shigella, Salmonella* and *E. coli O 157:H7*

Shigellae were not encountered in any of the 200 beef samples screened. However, 6 biotypic *Salmonella* Strains were isolated form 6 sliced beef samples and two of these were serogrouped as *Salmonella* spp. bioserogroup D (Table 6). The remaining 4 biotypic strains were unreactive with the available polyvalent O antisera which had the capacity to detect serogroups belonging to A, B, C, D, and E, plus the capsular VI antigen.

Of the 200 beef samples screened on SMAC agar 44 or the sliced beef and 30 of the minced beef were positive for sorbitol nonfermenter (SNF) bacteria (Table 6). From the positive samples a total of 87 SNF colonies were isolated (53 from the sliced and 34 from the minced beef samples) of which 10 showed the profile of the *E. coli* biotype. However, only one of the 10 SNF biotypic *E. coli* was found to be bioserotype O157: H7. It was initially isolated from a sliced beef sample. The remaining 9 SNF biotypic *E. coli* (7 from the sliced and 2 from the minced beef samples) were unreactive with the O157 specific latex agglutinins (Table 6). Thus the incidence of *E. coli* O157: H7 in the 200 beef samples from the butchers' shops in Addis Ababa was 0.5% where as that of *Salmonella* spp. was 1 % (Table 6).

4.4 Drug sensitivity of the E. coli O157:H7 and the Salmonella isolates

The *E. coli* O157: H7 isolate was resistant to 3 of the 10 antimicrobial agents used in the test. These three antimicorbial agents were Amoxicillin (Amx), Cephalothin (Cep), and Ampicillin (AmP) (Table 8). The seven efficacious drugs against the *E. coli* O157:H7 isolate, included, Amikacin (AK), Kananycin (Kan), Streptomycin (Str), Gentamycin (Gen), Nitrofurantion (NF)

polymyxin B (pol), and Tetracycline (Tet) (Table 7).

The antimicrobial agents used in the test against the two Salmonella isolates were identical to those used against the *E. coli* O157:H7 and all were efficacious (Table 7).

Table 6 Incidence of E. coli O157:H7, Salmonella and Shigella spp. in sliced (n= 100) and minced (n=100) beef samples from butcher's shops in Addis Ababa

Parameters	Sliced beef (n=100)	Minced beef (n=100)	Total N= 200	%
Number of samples with SNF colonies	44	30	74	37
Number of SNF isolates	53	34	87	43.5
Number of SNF biotypic *E. coli*	8	2	10	5
SNF *E. coli* bioserotype O157:H7	1	0	1	0.5
Biotypic *Salmonella.*	6	0	6	3
Salmonella bioserogroup (D)	2	0	2	1
Shigella spp.	0	0	0	0

Table 7 Antibiogram profiles of the E. coli O157:H7 and the two salmonella spp. isolates from the retail beef samples in Addis Ababa

Organism	Ak 30 µg	Amx 2µg	Kan 30 µg	Cep 30 µg	Amp 1- µg	Stra 10 µg	Gen 10 µg	NF 300µg	Pol 300 units	Tet 30µg
E. coli O157:H7 (CM45)	21(S)	7 (R)	21(S)	13(R)	10(R)	16(S)	20(S)	17(S)	12(S)	20(S)
Salmonella (Cm 49)	22(S)	19(S)	20(S)	25(S)	25(S)	16(S)	21(S)	23(S)	13(S)	20(S)
Salmonella (CM 55)	25(S)	20(S)	25(S)	21(S)	21(S)	22(S)	22(S)	22(S)	15(S)	23(S)

AK = Amikacin, Amx= Amoxoycilin, Kan= Kanamycin, Cep= cephalothin

Amp= Ampicillin, Str= Streptomycin, Gen= Gentamycin, NF= Nitrofurantion

Pol= polymyxine B, Tet= Tetracychine, (s) = sensitive, (R) = Resistant

Figures in the table are diameters of inhibition zones in mm

V. Discussion

Despite within sample variability the microbial load of the sliced and the minced beef samples were comparable. There was no statisticaly significance diference between mean mesophilic aerobic plate counts (MAPC) of the sliced (8.21 log cfu/g) and the minced (8.24 log cfu/g) beef samples ($P > 0.05$, $t_{\alpha} = 0.877$, at 45 d.f). This may appear to contradict with the generally accepted norm; however, it is within reasonable bounds.It is generally accepted that comminuted meats such as minced beef, have higher numbers of microorganisms than noncomminuted meats such as sliced beef (Jay 1996). This holds true for commercial ground meats which are produced from various cuts and trimmings and handled excessively. One heavily contaminated piece is sufficient to contaminate the entire lot and more microbes are inevitably added as the meat passes through the grinder. On top of this, the product is likely to be held for longer period at chilled storage or display units which encourage proliferation of psychrotrophs. The increased surface area in the ground meat enhances rapid proliferations of the psychrotrophs.

Unlike the situation in the commercial ground meat production, the minced beef samples in the present study were produced from limited cuts (500g/sample) and each sample was derived from a single separate carcass. The mincing was done at the point of purchase. There was little time for the bacterial population to take advantage of the increased surface area the samples were analysed within not more than one hour after they were minced and brought to the laboratory.

The mesophilic aerobic counts of both the sliced and the minced beef samples were variable and there are many possible reasons for this. The variation, in general, may be due to differences in the handling procedures as well as differences in the sanitary facilities in the different butchers'

shops. It may also be due to differences in how long the source carcasses were on display before sampling.

It is known that the microbiological quality of meat deteriorates with elapse of time when held at ambient temperature. In one study Narasimha and Sreenivasamurthy (1985) determined the shelf life and microbial spoilage of sheep carcass meat at ambient temperature. The initial mesophilic aerobic plate count (MAPC) levels of the test carcasses ranged between 5.6 and 5.8 log/cm^2. Microbial spoilage of the carcasses occurred around the 20 hours, by which time the MAPC levels had reached between 8 and 9 log cfu/cm^2. Based on these results Narasimha and Sreenivasamurthy (1985) reported a shelf life of 19h.

While the ambient temperature and consequently the shelf life may vary with the season and geographical locations, the deterioration of meat with the elapse of time at ambient temperatures remains a fact. In the present work, not all the meat samples were purchased at specified time of day. Although most of the samples were collected during the morning hours (approximately between 8:00 am and 10: am), there were occasions when some of both the sliced and the minced beef samples were purchased during the afternoon hours.

The butchers' shops receive delivery of carcasses customarily during the evening hours from the Addis Ababa Abattoir Organization which provides slaughter service for all the butchers. With extra payment some butchers may get same-day-delivery in the early evenings when they desire their animals to be slaughtered in the first batch of the day when the killing area is clean and supervision is alert (Anon. 1995).

This means that variation in the contamination level of carcasses begins in the abattoir depending on in which batch of the day they were processed. Slaughter operation may also result in the variation of contamination levels at different anatomical sites in a single carcass (Mulder and Kroll 1976, Bell 1997; Unterman et al., 1997).

This variation goes on to be compounded at the retail level by differences in hygienic handling procedures, sanitary conditions of the butchers shops and premises. At every transaction, meat carcasses are excessively handled when cutting out chunks for sale. This adds new population of contaminants and opens up new surfaces to be colonized. Meanwhile, the microbial population continues to proliferate. Thus samples are likely to have lower microbial loads if they are purchased in the early morning hours of the day than at latter hours.

The MAPC values for the minced beef samples exhibited less variability than that of the sliced beef samples, possibly because of the mincing process which redistribute the original patches and pockets of surface contamination throughout the product. This has been shown experimentally using laboratory scale meat grinders (Flores and Tamplin, 2002). Also, each minced beef sample was thoroughly mixed before weighing out the 25 g amount for analysis. This operation was not possible for the sliced beef samples.

At this point it should be recalled that each minced beef sample was derived from separate carcasses. The question arises then, as to how uniform distribution of contaminants as a result of the mincing and mixing process, explains "concordance" of MAPC values for samples derived from spatially separated source carcasses. It may be helpful to return to the basic principle of contamination of meat and the manner of distribution of microbial contaminants.

With the exception of the gastrointestinal and the upper respiratory tracts, internal tissues of healthy animals contain few microorganisms (Gill, 1979). Thus the majority of bacteria associated with meat are superficial, deposited haphazardly on the surface with the level being higher on those sites prone to hide contact faecal contamination and frequent handling (Mulder and Kroll 1976, Bell 1997; Unterman *et al.,* 1997). In general, a mosaic of patches and pockets of heavily contaminated surface sites interspersed with sites having relatively low levels of contamination can be envisaged.

If an excision sample then happens to include more of the heavily contaminated sites the microbial account will be exaggerated and if it happens to include the lesser contaminated sites the count will be underestimated. It was this kind of variability that was dampened in the case of the minced beef samples.

The log mean mesophilic aerobic plate count (MAPC) values of 8.21 and 8.24 cfu/g for the sliced and the minced beef samples, respectively are slightly higher than the 10^5 -10^8 cfu/g for raw beef samples reported from Awassa, Southern Ethiopia (Mogessie Ashenafi, 1994), and the 10^7 -10^8 cfu/g for raw kitfo samples from Addis Ababa (Mezgebe Tegegne and Mogessie Ashenafi, 1998).

Average microbial load values encountered in the literature for meat samples from countries elsewhere, are also lower than the values in the present study. These include, average mesophilic aerobic plate count (MAPC) values of 2.2x 10^6 and 7.9x10^7 cfu/g, respectively for hand-deboned and mechanically-deboned beef samples from the former Czechoslovakia (Pipova and Pleva, 1992), mean log MAPC of 2.42 cfu/cm^2 for beef carcass samples from Australia (Phillips *et al.,* 2002), and log mean MAPC values ranging between 2 and 5 cfu/cm^2 for beef carcass samples in

Canada (Gill *et al.*, 1998)

In their study of microbial contamination of beef carcasses in eight packing plants in the USA, Bacon *et al.,* (2000) determined mean mesophilic aerobic plate count (MAPC) values ranging between 8.2 and 12.5 cfu/100cm^2 for hide exterior and 3.8 and 7.1 cfu/100cm^2 for washed and decontaminated beef carcasses. It is deplorable to see that the mean log MAPC values of the beef samples in the present study lies within the range of values for the hide exterior samples reported by Bacon *et al.* (2000).

Considering the fact that variability in microbial load of meat is a rule than exception, reporting mean values only would not tell the whole story. Eight of the 25 sliced beef samples and 11 of the 22 minced beef samples in the present work had MAPC levels greater than or equal to 8 log cfu/g a microbial load close to that for the hide exterior reported by Bacon and coworkers (2000).

Levels of microbial loads can be related to the degree of meat spoilage. There is evidence that off odours in meat can be detected when the surface bacterial count is between 7 and 7.5 log cfu/cm^2 and that detectable slime develops when the surface counts reached between 7.5 and 8 log cfu/cm^2 (Ayres 1960; cited in Jay 1996).

In the present study 23 of the 25 sliced beef samples and more than 19 of the 22 minced beef samples had MAPC levels greater than or equal to 7 log cfu/g (Table 2). Although expert sensory assessment for signs of spoilage in the meat samples was not carried out, no off odour or other organoleptic alterations that would have alerted attention was noticed. It may be that because most of the contamination had occurred during late stage handling at the transaction

stages in the shops (Fig.1). Invariably in the entire butcher's shops carcass meat is displayed openly and handled with bare hands excessively when cutting out random chunks for sale.

Some of the contaminants could also have arisen from wrapping papers and balances. Frank signs of spoilage would have developed if the majority of the contaminants were the result of microbial proliferation of the meat samples from an earlier population of contaminants. Some proportions of the microbial loads may also be accounted by failure in aseptic techniques at analysis stage in the laboratory. The role of this factor is believed to have been minimized by strict exercise of the basic procedures and the fairly sufficient sample.

Raw sliced beef and minced beef can be considered ready-to-eat foods, at least in Ethiopia. According to the International Commission on Microbiological Specification of Foods (ICSMF, 1978), ready-to-consume foods with the exception of fermented ones, are regarded as unwholesome when they contain a large population of microorganisms. This remains the hygienic guideline even when the organisms are not known to be pathogens and they have not altered the character of the food noticeably.

The recommended microbiological criterion with respect to MAPC level at or above which meat is considered unacceptable is 10^7 cfu/g (Aberra Geyid 1984; ICMSF 1986 cited in Jay 1996). Adoption of this criterion would have resulted in the condemnation of 23 of the 25 sliced beef and 19 of the 22 minced beef samples. The above microbiological quality and safety specification applies to health hazard, sanitary indicator or spoilage potentials (Jay 1996).

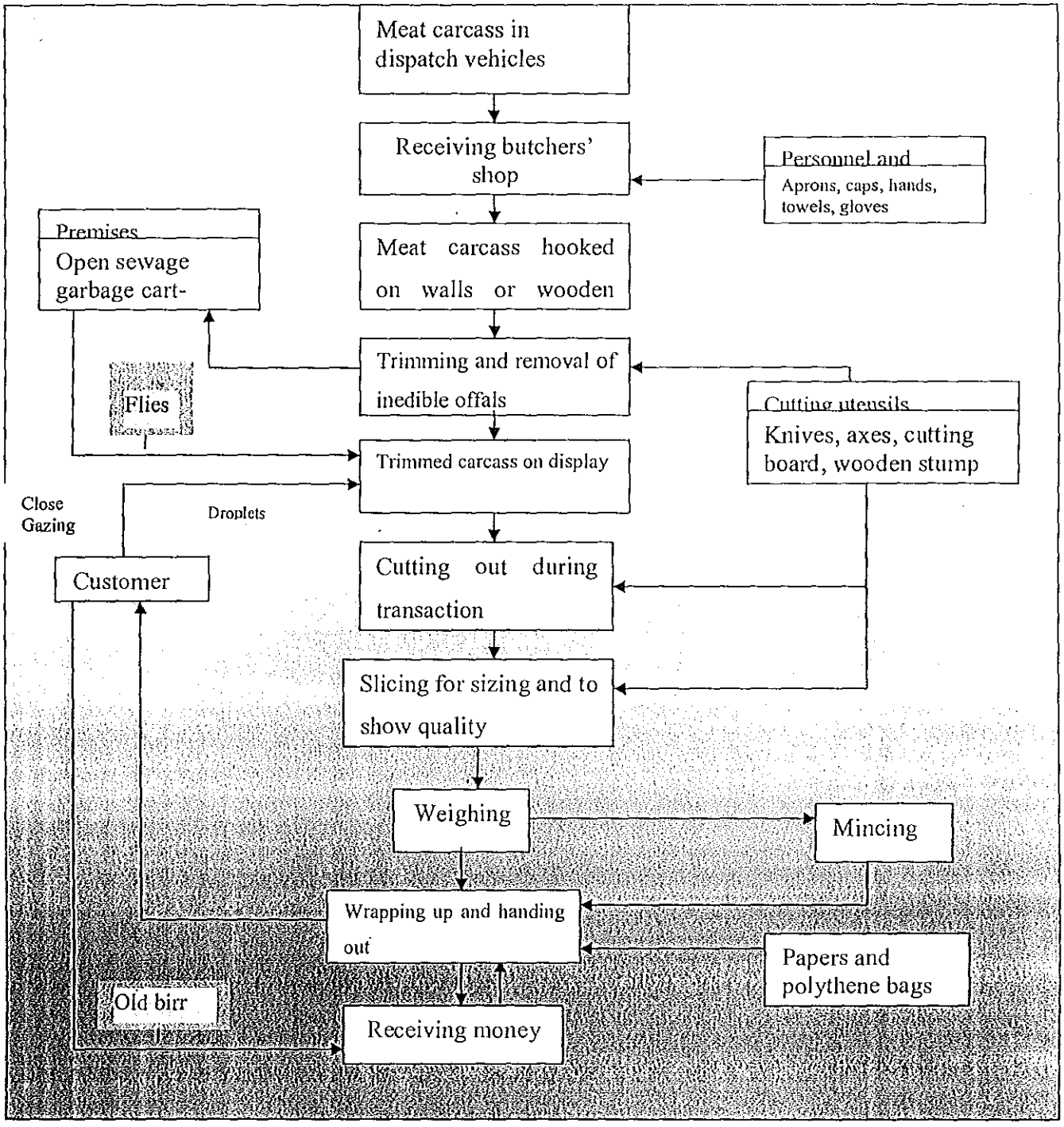

Figure 1: A flow chart of typical meat handling procedures in the butchers' shop in Addis Ababa showing potential critical control points

The mean total coliform count (TCC) of the sliced beef samples (7.02 log cfu/g) was higher than that of the minced beef samples (6.44 log cfu/g). But, this difference is not statistically significant ($P > 0.05$, $t_{\alpha} = 0.31$, at 45 d.f). This finding is reasonable since contamination is a surface phenomenon and the sliced beef samples were analyzed by random excision of surface tissue. In contrast, the analyses of the minced beef constituted a sterile deep tissue mixed with relatively small proportion of surface tissue. The size of the coliform population inside the mincers must have been very small and the proliferation of the coliform in the minced meat samples, during the brief period before analysis, must also have been too low to compensate for the effect of mincing.

The log mean total coliform counts of the beef samples in the present study are higher than all average values encountered in literature. These included mean total coliform counts of 3 to < 2 log cfu/cm^2 of beef carcass samples from Canada (Gill *et al.*, 1998), 1.5 to 3.7 log cfu/cm^2 of beef carcass sample from the USA (Bacon *et al.*,. 2000), and the 1.9 x 10^5 cfu/g and 5.4 x10^6 cfu/g, respectively of hand-deboned and mechanically-deboned beef samples from Czechoslovakia (Pipova and Pleva, 1992). Bacon *et al.* (2000) also reported mean total coliform counts (TCC) for slaughter cattle hide exteriors ranging between 6 and 7.9 log cfu/100cm^2. This range encompasses the log mean TCC levels of the beef samples in the current study. Nine of the 25 sliced beef and 11 of the 22 minced beef samples had TCC levels greater than or equal to 6 log cfu/g. The level of TCC in the human intestinal tract ranges between 8 and 9 log cfu/g of faeces (Jay, 1996) and one of the sliced beef samples in the present study had TCC level within this range.

Because of the widespread occurrence of coliforms in meat environment, the standard coliform test is not considered as suitable as the faecal coliform test for sanitary quality indication (Newton, 1979; cited in Jay, 1996). However, whether the source of the coliform contamination was the environment or human faeces, the high TCC level remains an indication of the free exposure of the beef carcasses to microbial contamination. TCC can also account for significant proportion of the mesophilic aerobic flora.

The mean count of staphylococci for the sliced beef (6.08 log cfu/g) and the minced beef (6.91 log cfu/g) samples were slightly higher than the 6 log cfu/g of raw kitfo samples from Addis Ababa (Mezgebe Tegegne and Mogessie Ashenafi, 1998) and the 5 log cfu/g of mechanically-deboned beef samples from the former Czechoslovakia (Prasovska *et al.*, 1991).

Pipova and Pleva (1992) reported mean staphylococci counts of $6.3x10^2$ cfu/g and $2x10^4$ cfu/g, respectively for hand deboned and mechanically deboned beef samples from the former Czechoslovakia. These values are also much lower than the mean staphylococci counts of the beef samples in the current study.

The minimum number of enterotoxigenic stphylococci (as determined for *S. aureus*) required to produce the minimum level of enterotoxin necessary to cause the gastroenteritis syndrome in humans (1ηg/g) appears to differ for the substrates and the particular enterotoxin. Detectable staphylococcal enterotoxin A (SEA) has been found with as few as 10^4 cfu/g (Hirooka *et al.*, 1987, cited in Jay, 1996). In meat products, SEA was produced with log 7.2 *S. aureus* cell/g (Notermans and Van Otterdijk, 1985; cited in Jay 1996).

Although enterotoxigenicity of the strains was not assessed in the current study, about 4 of the 25 sliced beef and 4 of the 22 minced beef samples had staphylococci counts greater than or equal to 7 log cfu/g; a level near the threshold for production of detectable amount of SEA.

Staphylococci ranked second as the dominant mesophilic aerobic bacteria in the 25 sliced beef samples; but it was the least dominant genera in the 22 minced beef samples. Moreover, it was encountered in 22 of the 25 sliced beef samples, but only in 10 of the 22 minced beef samples. This finding is contrary to what one would expect considering the distribution effect of mincing.

The omission of groups which occur in low number in beef samples is more highly likely when the excision method (as in sliced beef samples) is used than when the minced beef is used in the microflora analysis. Hence, the incidence rates of the different mesophilic aerobic bacteria in the sliced beef samples probably are underestimations whereas that in the minced beef samples are very close to the true incidence rates. Although the excision sampling method has been used with success for enumeration purposes it may not be suitable for assessing incidence rates (Jericho *et al.*, 1994; Dorsa *et al.*, 1996).

In light of this argument a higher incidence rate of staphylococci would have been expected in the minced beef samples than that in the sliced beef samples. It may be helpful to turn to the principle of microbial interaction to unravel this puzzle.

In general staphylococci and particularly *S aureus* do not compete well with the normal flora of most foods. At temperatures that favour *S. aureus* growth, the normal beef saprophytic biota

offers protection against staphylococcal growth through antagonism, competition for nutrients, and modification of the environment to conditions less favourable to the staphylococci. Among bacteria known to be antagonistic to *S. aureus* growth include, *Acinetobocter, Aeromonas, Bacillus, Pseudomonas*, and the *Eneterobacteriaceae* (Mossell, 1975; cited in Jay 1996). The other-gram-negative-rods (OGNR) encompass all of these gram-negative bacteria except the *Enterobacteriaceae*. The *Enterobacteriaceae* and OGNR occurred in relatively higher incidence rate in the minced beef samples than in the sliced beef samples in the present study (Table 5).

It may be then that *S. aureus* was the most dominant species among staphylococci in the minced beef samples. However, due to the occurrence of the antagonists in relatively higher frequency in the minced beef samples than in the sliced beef samples, suppression of growth of *S. aureus* must have occurred resulting in the observed lower incidence rate of the staphylococci in the minced beef samples. The distribution effect of the mincing and the mixing processes were also supported by the observed higher incidence rate of the *S. aureus* antagonists in the minced beef samples (Table 5).

Exceptions to the argument of the distribution effect and the expected higher incidence rate in the minced beef samples are the streptococci, Salmonellae and *E. coli* O157:H7. All of these three organisms were detected in the sliced beef samples, but not in any of the minced beef samples. Chance is the most probable explanation for these observations.

In general, the mesophilic aerobic flora of both the sliced and the minced beef samples were dominated by micrococci, *Bacillus* spp. and the *Enterobacteriaceae,* with staphylococci having higher significance in the sliced beef samples next to the micrococci. This finding is more or less in agreement with an earlier report by Mogessie Ashenafi (1994). Other gram-positive rods (OGPR) also accounted for significant portion of the mesophilic aerobic flora of the minced beef samples. These groups encompass the coryneforms which were also among the dominant mesophilic aerobic bacteria in fresh raw beef samples reported by Mogessie Ashenafi (1994).

However, generic level identification of the isolates designated as OGPR was not possible with the panel of tests used in the present study. The higher dominance of the *Enterobacteriaceae* in the minced beef samples may indicate their abundance in the mincers.

Among the commonly encountered enteric pathogens, *Shigella* species occurred in neither the sliced beef nor in any of the minced beef samples. The shigellae are primarily human intestinal pathogens transmitted *via* finger-borne, faecal-oral route. The absence of shigellae in the beef samples may be an indication of the absence of contact with the above factors.

Reports on the incidence of shigellae in raw beef are far and wide. In one study Ghoshal (1992) reported the isolation of 5 strains of shigellae from 3000 processed meat samples in India.

Regarding salmonellae, their incidence rate in the whole (2/200) beef samples in the current study is lower than all previously reported incidence rates encountered in the local literature. These include the incidence of *Salmonella* in 9% of fresh raw beef samples from butchers' shops in

Awassa, southern Ethiopia (Mogessie Ashenafi 1994), the 26% in meat and milk samples from Arsi region central Ethiopia (Bagni *et al.*, 1998), and the reported findings equivalent to a 42% incidence rate of *Salmonella* in raw kitfo samples from restaurants in Addis Ababa (Mezgebu Tegegne and Mogessie Ashenafi, 1998).

In their study on the occurrence of *Salmonella* in 205 retail foods from Addis Ababa, Bayleyegn Molla *et al.* (1999) reported findings equivalent to a 40% incidence rate of *Salmonella* in raw minced meat samples. Nyeleti *et al.*, (2000) determined the prevalence and distribution of *Salmonella* in Slaughter cattle, slaughter house personnel and minced beef samples in Addis Ababa. *Salmonella* was encountered in 9.8% of abdominal muscles and 11.9% of diaphragmatic muscles. About 8% of the minced meat samples from supermarkets were also positive for *Salmonella*. The *Salmonella* isolates belonged to six different serovars consisting of *S.* Dublin (54.1%) *S.* Anatum (27.6%) *S.* Saintapaul (9.2%) *S.* Meleagrids (5.1%) *S.* Roughform (3.1%) and *S.* Muenchen (1.0%).

In the present study *Salmonella* was detected in two of the sliced beef samples but in none of the minced beef samples. The four presumptive *Salmonella* isolates, which exhibited the typical biochemical profile of *Salmonella,* but uncreative with the polyvalent O antiserum, were also isolated from the sliced beef samples. Since the available polyvalent O antiserum could detect only 95% of the serogroups of *Salmonella* pathogenic to man, (according to the kit), the possibility that the four unreactive presumptive isolates may belong to serogroups other than the 95%, cannot be ruled out

With respect to findings elsewhere, both lower and higher incidence rates of *Salmonella* in meat and meat products than the incidence rate in the present study have been encountered in the literature. Among the reported lower incidence rates include, the finding of *Salmonella* on 0.2% of beef carcass samples and 0.1% of boneless beef samples in Australia (Phillips et al., 2001). In India, Ghoshal (1992) reported the isolation of *Salmonella* in 18 of 3000 samples of retail meat products; this being equivalent to an incidence rate of 0.6% and hence lower than this study.

Of the reported higher incidence rates, Sharma *et al.*, (1992) found *Salmonella* in 2 of 34 beef and pork cocktail sausages in India. A study of raw beef products from retail shops in London revealed the occurrence of *Salmonella* in 2% of beef sausages, 3% of minced beef and 3% of beef burger samples (Roberts *et al.*, 1992). In two other investigation of raw processed meat products in the United Kingdom, Little *et al.*, (1998, 1999) reported 4% and 7% incidence rates of *Salmonella*. An abattoir level study in Ireland showed the occurrence of *Salmonella* in 7.6% of beef carcass samples (McEvoy *et al.*, 2003). In the USA, the prevalence of *Salmonella* in ground beef from 25 large plants during the year 1998 was 4.8% (Anon 1999). This was a decline from the 7.5% prevalence rate in the era before the implementation of the HACCP program.

The incidence rate of *Salmonella* in the beef samples in the present study is much lower than the previously reported incidence rates in Ethiopia. Information as to any currently innovated control measures in the farm, at abattoir level or in the retail level is unavailable. It is not clear whether the observed low incidence rate of *Salmonella* in the beef samples is due to pure chance or a reflection of improved operations in the meat chain.

The methods used to recover *Salmonella* from the beef samples in the present study are comparable with those used in the earlier investigation in Ethiopia. The R.V enrichment broth

and the XLD agar are sufficiently sensitive to recover *Salmonella* from meat or other foods (Kalapothaki et al. 1983; Oxoid, 1995).

In general, considering the high microbial load and the TCC levels in the beef samples in the present study, the incidence of *Salmonella* is paradoxically too small to support the assumption of widespread occurrence in beef. In a review of microbial ecology of meat, Grau (1986) stated that the rate of detection of salmonellae varies considerably and is strongly influenced by the pre-slaughter treatment of the animals. As stated in the foregoing, information on any current changes in the control measures in the meat chain for the Ethiopian case is lacking.

On the other hand, in an older report, the working group of the codex committee on food hygienic concluded that the incidence of *Salmonella* in red meat and poultry is so high that application of microbiological criteria would be impractical (FAO/WHO, 1979; cited in Silliker and Gabis, 1986). This seems reasonable when one considers that, except the human host adapted serotypes nearly all salmonellae are zoonotic or potentially zoonotic. (Acha and Szyfres 2001 cited in WHO/FAO, 2002). However, the concept of variability in incidence rate of *Salmonella* appears more reconciling than the assumption of consistently high incidence rate. Stressing this point, Grau (1986) stated that while occasionally all carcasses in a group can be contaminated with *Salmonella*, contamination rates are normally considerably less than this. Thus the incidence rate of *Salmonella* in the beef samples in the current study as compared with earlier findings in Ethiopia appears to fit this norm.

A public health laboratory guide line by the Ethiopian Health and Nutrition Research Institute recommended that *Salmonella* should not be isolated from any of five sample units of fresh meat

in a lot (Aberra Geyid, 1984). Considering the fact that nearly all *Salmonella* serotypes are potentially pathogenic to man and consumption of raw meat is common in Ethiopia, the guideline is reasonable enough. However it is not clear whether this guideline is applicable to meat at either the domestic or export abattoir levels. There is no clear-cut public health legislation with regard to the microbial safety of meat or other foods in Ethiopia. (Gebre-Emanuel Teka, 1997). Strict adoption of the above criterion would have resulted in the condemnation of the source carcasses from which the two *Salmonella* positive sliced beef samples were derived.

Both of the two *Salmonella* isolates were sensitive to all antimicrobials tested. Because the definitive serotype of the isolates was not determined and the possible sources of contamination can only be speculated, it is not possible to comment much on this finding. Assuming that the isolates were originally in the live bovine, the absence of resistance to any of the antimicrobials may reflect the absence of prior exposure to all of the antimicrobials. Unlike the developed countries, animal husbandry in Ethiopia is largely dependent on range cattle rather than feed lot. Intensive use of antimicrobials as growth promoters in feedlot cattle in the developed countries is the main reason for the emergence of multidrug resistant strains such as *S. Typhimurium* DT 104 (Akkina *et al.*, 1999).

Escherichia coli bioserotype O157:H7 was found in only 1 (0.5%) of the 200 beef samples. Published works on the incidence of the strain in Ethiopia are unavailable. This is the first report on the isolation of *E. coli* O157:H7 from retail beef in Ethiopia. In the first published study on the prevalence of *E. coli* O157:H7 in meats, Doyle and Schoeni (1987) reprorted the occurrence of the strain in 6 (3.7%) of 164 beef, 4 (1.5%) of 264 pork, 4 (1.5%) of 263 poultry and 4 (2.0%) of 205 lamb samples. The contaminated samples were from retail outlets in Canada and the USA.

Since that work, there has been a plethora of literature on the subject and some of those encountered are tabulated to allow comparison (Table 8).

In general 20 reported incidences of *E. coli* O157:H7 in raw beef and beef products from 14 countries, in 18 published articles during the years between 1996 and 2003 are summarized. Seven of the reported incidences from six countries are lower than that in the current study. (Table 8)

The incidence of *E. coli* O157:H7 in the beef samples in the present study (0.5%) is similar to that in the mixture of minced beef and pork samples from the Netherlands (Heuvelink *et al.*, 1999).While comparison is made, to show where the result of the present study may fit in the context of some findings in the literature, the methods used to recover *E. coli* O157:H7 are not comparable. Particularly the absence of enrichment procedures in the current study is suspected to have resulted in diminished sensitivity..

In the absence of selective enrichment steps, the high microbial load of the beef samples would be expected to compromise the growth of E. coli O157:H7. The competitive inhibition effect of high levels of background flora has been shown using ground beef models (Vold *et al.*, 2000; Berry and Koohmaraie, 2001).

Table 8 Some Reported Incidences of Escherichia coli O157:H7 in raw beef and beef products from different countries as compared with that in the present study

Type of sample	Sample size	Incidence (%)	Country	Reference
Retail cut beef	114	0(0)	Croatia	Uhitil *et al.*,2001
Ground beef	1400	0(0)	USA	Tarr *et al.*, 1999
Frozen beef	990	0(0)	Australia	Phillips *et al.*,2001
Beef carcass	1275	2(0.1)	"	" "
Minced beef	3450	4(0.12)	France	Vernozy-Rozand *et al.*, 2002
Beef patties	1190	2(0.16)	UK	Coia *et al.*, 2001
Beef carcass	4067	19(0.47)	UK	Richards *et al.*, 1998
Minced+sliced beef	200	1(0.5)	Ethiopia	This study
Minced beef-pork	402	2(0.5)	Netherlands	Heuvelinke *et al.*, 1999
Minced beef	571	6(1.1)	"	" "
Beef product	3216	36(1.1)	UK	Chapman *et al.*, 2000
Beef carcase	1500	21(1.4)	UK	Chapman *et al.*, 2001
Beef patties	2840	73(2.73)	USA	Pruett *et al.*, 2002
Beef carcass	330	12(3.6)	Turkey	Gun *et al.*, 2003
Ground beef	160	6(3.8)	Argentina	Chinen *et al.*, 2001
Retail cut beef	95	4(4.21)	Thailand	Vuddhakul *et al.*, 2000
Minced beef	58	3(5)	Spain	Blanco *et al.*, 1996
Retail cut beef	40	2(5)	China	Zhou *et al.*, 2002
Ground beef	50	3(6)	Egypt	Abdul-Rauf *et al.*, 1996
Beef carcass	100	12(12)	Italy	Bonardi *et al.*, 2001
Retail cut beef	25	9(36)	Malaysia	Radu *et al.*, 1998

With regard to susceptibility to antimicrobial agents, the isolated strain of *E. coli* O157:H7 was resistant to amoxycilin cephalothin and ampicillin but was susceptible to the rest of seven antimicrobials among the ten agents used in the test (Table 7). In Malaysia, Radu *et al.* (1998) tested 12 strains of *E. coli* O157:H7 isolated from retail beef samples and of these seven strains were resistant to cephalothin of which two were also resistant to amoxicillin. Another strain was found to be resistant to both cephalothin and ampicillin. Resistance per strain to cephalothin amoxicillin and ampicillin was observed in none of the isolates (Radu *et al.*, 1998).

In another study Schroeder and co workers (2002) investigated the prevalence of antimicrobial resistance among a total of 361 *E. coli* O157:H7 recovered form humans, Cattle, swine and food in the USA. Based on broth micro dilution results, 61(17%) of the isolates were resistant to cephalothin and 48 (13%) were resistant to ampicillin. Multiple resistance to amoxicillin, cephalothin and ampicillin was not encountered.

The resistance of the *E. coli* O157:H7 strain to cephalothin, ampicillin and amoxycillin could portend an increased prevalence of this pathogen in bovine animals that receive these antibiotics. However, data on the extent of veterinary usage of antimicrobial agents in Ethiopia is unavailable. Selection pressure imposed by the use of antimicrobial, whether therapeutically in human and veterinary medicine or as prophylaxis in the animals production environment, is a key driving force in the selection of antimicrobial resistance in *E. coli* O157:H7 (Schroeder *et al.*, 2002).

VI. Conclusions and Recommendations

Within the limits of the samples, the methodologies used and based on the results summarized and the arguments expounded in the discussions, the following conclusions and recommendations may be forwarded.

The hygienic status of the retail beef samples was very poor. This was evidenced by the very high microbial load of the majority of the beef samples. The microflora was largely dominated by microccoci, *Bacillus* spp., *Enterobacteriaceae*, and staphylococci. Based on tentative observation of some factors that might have contributed to the high microbial load, a flow chart-based potential critical control points are outlined (Fig. 1). Although at low incidence rates, *Salmonella* and *E. coli* O157:H7 were also encountered. Thus the consumption of raw or undercooked beef can be hazardous and should be discouraged.

- The use of random excision method tends to exaggerate the variability in microbial load suggesting a mosaic of surface contamination of beef carcasses. Mincing dampens this variability. Future investigations should consider this when choosing methods.

- Late stage handling practices in the butchers' shops should be considered potential critical control points. Owners and operating staff of butcheries should be made aware of this and their educational status and knowledge of the basic hygienic guidelines should be considered during issuance and renewal of permits.

- This is the first ever report on the occurrence of *E. coli* O157:H7 in beef in Ethiopia. However the virulence properties of the isolated strain were not determined. Future investigations should focus on virulence trait-oriented assessment.

- Initiation should also be taken to launch more comprehensive investigation on the prevalence of *E. coli* O157:H7 in clinical settings as well as the whole meat chain using more sensitive methods in Ethiopia.

- While conclusive proof on the occurrence of virulent *E. coli* O157:H7 in Ethiopia remains to be determined, the public health defence team of the country should be on guard.

- Since there is no clear cut public health legislation with regard to meat safety or other foods in Ethiopia, future drafting of such laws should consider the potential domino effect of the occurrence of *E. coli* O157:H7 in meat in Ethiopia and should address the problem accordingly.

- The ripple of this finding should be felt from export-oriented abattoirs and meat processing industries to livestock marketing and the tourism industries.

Reference

Abdul-Rauf, U.M., Ammar, M.S., Beuchat, L.R., (1996). Isolation of *Escherichia coli* O157:H7 from some Egyptian Foods *Int. J.Food.Microbiol.* **29:** 423-426.

Aberra Geyid, (1984). *Manual for the bacteriological analysis of food and beverages.* CLRI publication, number 18, Central laboratory and Research institute and Ababa.

Aberra Geyid, Frew Tekabe, Asmamaw Tigre, Mulu Girma and Sisaynesh Assefa (1991). A preliminary study of the microflora level of some fruits and vegetables: Pre- apost-preservation. *Ethiop. J. Health Dev.* **5**: 57-65

Afeworki Gebre-Yohannes and Drasar, B.S., (1987) *Shigella dysentriae* and *Shigella flexineri*: serotypes prevalence and seasonal distribution in Addis Ababa, Ethiopia (1978-85). *Ethiop. J. Hlth. Dev.* **2**: 51-8

Agin, T.S., Wolf, M.K., (1997). Identifieation of a family of Intimins common to *Escherichia coli* causing Attaching Effacing Lesions in Rabbits, Humans, and Swine. *Infect. Immun.* **65**: 320-326.

Akkina, J.E., Hogue, A.T, Anguo, F.J.,Johnson, R. Peterson,K.E., Saini, P.K., Fedorka Cray, P.J., and Schlosser, W.D., (1999). Epidemiologic aspects, control and importance of multiple drug resistant Salmonella Typhimurium DT104 in the United Staes. *JAVMA.* **214**: 790-98.

Anonymous (1995). Addis Ababa Abattoir orgnaization (AAAO) Ethiopia, Addis *Ababa meat plant feasibility studies phase 1, Final report June* 1995. Danagro adviser a/s. Agricultural consultants and planners and Carl/Bro foods

Anonymous (1999). Prevalence of *Salmonella* in ground beef: post HACCP implementation results from large plats 1998-1999. *www/A:/ HACCP Implementation First year Salmonella: Test Results htm.*

Bacon, R.T., Belk, K.E., Sofos, J.N., Clayton, R.O., Reagan, J.O., and Smith, G.C., (2000). micobial populations on animal hides and beef carcasses at different stages of slaughter in plants employing multiple sequential interventions for decontaminations. *J.food. Prot.* **63**: 1080-1086.

Bagni, M., Cuteri, V., and Mekonnen, M., (1998) *Salmonella* contamination of food of animal origin in Ethiopia. *Ob. Doc. Vet.* **19**: 55-58.

Barrett, T.G., Lior, H., Green, J.H., Khakhria R. Wells, J.G., Bell, B.)., Greene, K.D., Lewis, J., and Griffin, P.M., (1997) Laboraotry investigation of a multistate food borne outbreak of *Escherichia coli* O157:H7 by using pulsed-field gel electrophoresis and phage typing. *J. Clin. Microbiol.* **32**: 3013-3017.

Bauer, A.W., Kirby, W.M.M., Sherris, J.C. and Turk, M. (1966). Antibiotic susceptibility testing by standardized single disc method. *Amer. J. Clin. Path.* **45**:493-501

Bayleyegn Molla, Kleer,J. and Sinell,H.J. (1999). Occurrence , distribution and level of *Salmonella* in food items in Addis Ababa (Ethiopia). *Fleischwirtsch. Int.* **4**:37 – 39

Bayleyegn Molla, Arthuro Mesfin, and Daniel Alemayehu (2003. Multiple antimicrobial-resistant *Salmonella* serotypes isolated from chicken carcass and giblets in Debrezeit and Addis Ababa, Ethiopia. *Ethiop.J. Health Dev.* **17**:131-149

Bell, B.P., Goldft, M., Griffin, P.M., Davis, M.A., Gordon, D.C., Tarr, P.I., Bartleson, C.A., Lewis, J.H., Barrett, T.J., Wells, J.G., et al (1994). A multistate outbreak of *Escherichia coli* O157:H7 associated bloody diarrhea and hemolytic uremic syndrome from hamburgers. The Washington experience. *J. Am. Med. ASo.* **272**: 1349-1353.

Bell, R.G. (1997). Distribution and sources of microbial contamination of beef carcasses. *J. Appl. Microbial.* **82**: 292-300.

Bender, A. (1992). Meat and meat products in human nutrition in developing countries. *FAO.*

Nutr. Paper. **53:** 42-43.

Berry, E.D., and Koohmaraie, M., (2001). Effect of different levels of beef bacterial microflora on the growth and survival of *Escherichia coli* O157:H7 on beef carcass tissue *J. Food.Prot.* **64**: 1138-1144.

Betts, G.D., (2000). controlling *Escherichia coli* O157. *Nutr.Food.Sci.* **30**: 183-186.

Beutin, L., Geier, D., Sterinruck, H., Zimmermann, S., and Scheutz, F.,(1993). Prevalence and some properties of Verotoxin (Shiga Like toxin) producing Escherichia coli in seven different species of Healthy Domestic animals. *J. Clin. Microbiol.* **31**: 2483-2488.

Birhanu Adulaem and Aberra Geyid, (2003). The prevalence of *Yersinia enterocolitica* isolates in comparison to those of the commonly encountered enteropathegnes causing diarrhoea among Ethiopian patients in Addis Ababa. *Focus chronicle* 3: 298-307.

Blanco, J.E., Blanco, M., Mora, A., prado, C., Rio, M., Fernandez, L., Fernandez, M.J., Sainz, V., and Blanco, J., (1996) Detection of enterohemorrhagic *Escherichia coli* O157:H7 in minced beef using immunomagnetic separation. *Microbiologia* **12**: 385-394.

Bonardi, S., Maggi, E., Pizzin, G., Morabito, S. and Caprioli, A., (2001). Fecal carriage of verocytotoxin producing *Enchiridia coli* O157 and carcass contamination in cattle at slaughter in northern Italy. *Int. J. Food. Microbiol.* **66:** 47-53.

Boyce, T.G., Swerdlow, D.L., and Griffin, P.M., (1995). Current concepts: *Escherichia coli* O157:H7 and the hemolytic uremic syndrome. N. *Eng. J. Med.* **333:** 364-368.

Brandt, J.R., Fouser, L.S., Watkins, S.L., Zelikavic, I., Tarr, P.I., Nazar-Stewart, V., Avner, E.D., (1994) *Escherichia coli* O157:H7 associated hemolytic syndrome after ingestion of contaminated hamburgers *J. pediatr.* **125**: 519-526.

Bryan, F.L., (1986). Miscellaneous pathogenic Bacteria in meat and poultry produots. In: Pearson, A.M., and Dutson, T.R., (Eds). *Advances in Meat reaserch*. 241-275

Caslin, I., Breuil, J., Brisabois, A., Moury, F., Grimont,F., and Cllatz, E., (1999). Multidrug resistant human and animal *Salmonella Typhimurium* isolates in France belong predominantly to a DT 104 clone with the chromosome and integrone-encoded β-lactamase PSE-1. *J. Infect. Dis.* **179**: 1173-82

CDC (1999). Summary of outbreaks of Esehericlia coli O157 and other shiga toxin producing *Escherichia. coli* reported to CDC 1999. *http://www. cdc. gov/ncidod/dbmd/ diseaseinf /files/ecoli 99 summary. pdf.*

CDC (2002). Multistate outbreak of *Escherichia coli* O157:H7 infections associated with eating ground beef; United States June-July 2002. *Morbid.Mortal. Wkly Rep.***51**: 637-639.

Chapman, P.A., Cerdan Malo, A.T., Ellin, M., and Harkin, A.R. (2001). *Escherichia coli* O157 in cattle and sheep at slaughter on beef and lamb carcasses and in raw beef and lamb products in south Yorkshire, U.K. *Int. J. Food Microbiol.* **64**: 139-150.

Chapman, P.A., Siddons, C.A., Cerdan Malo, A.T., and Harkin, M.A.S., (2000). A one year study of *Escherichia coli* O157:H7 in raw beef and lamb products. *Epidemiol. Infect.* **124**: 207-213.

Chapman, P.A., Siddons, C.A., Wright, D.J., Norman, P., Fox, J., and Crick, E., (1993) cattle as a possible source of veocytotxin producing *Escherichia coli* O157 infections in man. *Epidemiol. Infect.* **111**: 439-447.

Chapman, P.A., Siddons, C.A.S Zadik, P.M., and Jewes L. (1991). An improved selective medium for the isolation of *Escherichia coli* O157 J. *Med.Microbiol.* **35**: 107-110.

Chart, H., and Jenkins, C., (1999).The serodiagnosis of infections caused by verocytotxin producing *Escherichia coli. J. Appl. Microbiol.* **86** 731-740.

Chin, C.H., Lin, T.Y., and Ou, J.T., '91999) prevalence of the virulence plasmids of non typohodial salmonella in the serovars isolated from humans and their association with

bacteriaemia. *Micrbiol. Immunol.* **43**: 899-903.

Chinen, I., Tanaro, J.D., Miliwebsky, E., Lound, L.H., Chillemi, G., Ledri, S., Bashkier, A., Scarpin, M., Manfredi, E., and Rivas, M., (2001) Isolation and characterization of *Escherichia Coli* O 157: H7 from retail meats in Argentina *J. Food. Prot.* **64**: 1346-1351.

Coia, J.E., Johnston, Y.,Steers, N.J. and Hanson, M.F., (2001) A survey of the prevalence of Escherichia coli O 157 in raw meats, raw cow's milk and raw milk cheeses in South east Scotland. *Int. J. Food Microbiol.* **66**: 63-69.

CSA (2002). F.D.R. Ethiopia central statistical Authority (CSA). *Statistical Abstracts*

Daniel Asrat, Hathaway, A., Ekwall, E., (1999). Studies on enteric campaylobactriosis in Tikur Anbessa Hospital Addis Ababa. *Ethiop. Med.* J. **37**: 71-83.

Darwin, K.H., and Miller, V.L. (1999). Molecular basis of the Interaction of Salmonella with the intestinal mucosa. *Clin. Microbiol. Rev.* **12**: 405-428.

Dodsworth, T.L., (1972). *Beef production.* Pergamon press.pp 87

Dolman, C.E., (1957). The Epidemiology of Meat borne diseases *in Meat Hygiene* FAO/.WHO joint publication No 34.

Dontorou, C., Papadopoulou, C., Filioussis, G., Economou, V., A postolou, I., Zakkas, G., Salamoura, A., Kansouzidous, A., and Levidiotous, S., (2003). Isolation of *Escherichia coli* O157:H7 from foods in Greece. *Int. J. Food.Microbiol.* **82**: 273-279.

Dorsa, W.J., Cutter, C.N., and Siragusa, G.R., (1996). Evaluation of six sampling method for recovery of bacteria from beef carcass surfaces. *Lett. Appl. Microbiol.* **22**: 39-41.

Doyle, M.P., Schoeni, J.L., (1987). Isolation of *Escherichia coli* O 157:H7 from retail fresh meats and poultry. *Appl. Environ. Microbiol.* **53**: 2394-2396.

Dundas, S., Todd, W.T.A., Stwart, A.I., Murdoch, P.S., Chaudhuri, A.K.R., and Hatchinson, S.S., (2001). The central Scotland *Escherichia coli* O157:H7 outbreak: Risk factors for the

Hemolytic uremic syndrome and death among hospitalized patients. *Clin. Infect. Dis.* **33**: 923-931.

Edwards, P.R. and Ewing W.H., (1972). Identification of Enterobacteriaceae. 3rd edition. Burgess publishing company Minneapolis. Minne.

Effler, P., Isaacson, M., Arntzen, L., Heenan, R., canter, P., Barrett, T., Lee, L., Mambo, C., Levine, W., Zaidi, A., and Griffin, P.M. (2001). Factors contributing to the Emergence of *Escherichia coli* O 157 in Africa. *Emerg. Infect. Dis.* 7: 812-818.

Elder, R.O., Kim, J.E., Siragusa, G.R., Barkocy Gallagher,G.A., Koohmaraie, M., and Laegredi, W.W., (2002). Correlation of enterohemorrhagic *Escherichia coli* O 157 prevalence in feces and carcasses of beef cattle during processing. *Proc. Natl. Acad. Sci. USA.***97**: 2999-3003.

Fedorka-Cray, P.T., Whipp, S.C., Isaacson, R.E., Nord, N., and Lager, K., (1994). Transmission of *Salmonella typhymurium* to swine. *Vet. Microbiol.* 41:333-344

Flores, R.A., and Tamplin, M.L., (2002). Distribution patterns of *Escherichia coli* O157:H7 in ground beef produced by a laboratory scale grinder. *J.Food Prot.* **65**: 1894-1902.

Gansheroff, L.J., and O' Brien, A.D., (2000). *Escherichia coli* O157:H7 in beef cattle presented for slaughter in the U.S.: higher prevalence rates than previously estimated. *Proc. Natl Acad. Sci. USA*, 97: 2959-2961.

Gebre-Emanuel Teka, (1997). Food Hygien: Principles and methods of foodborne disease control with special reference to Ethiopia. Faculty of Medicine, Deparment of community Health, Addis Ababa University

Genevieve, A.Gallagher, B., Arther, T.M.,Siragusa, G.R., Keen, J.E Elder, R.O., Laegreid, W.W., and Koohmaraie, M., (2001). Genotypic analysis of Escherichia coli O157:H7 and O157: nonmotle isolates recovered from beef cattle and carcasses at processing plants in

the mid western states of the United sates *Appl. Environ. Microbiol.* **67**: 3810-3818.

Ghoshal, S.P., (1992). Bacteriological studies on processed meat food products with special reference to *Salmonella* and *Shigella* serotypes isolated from meat and meat products. In *proceedings of the third world congress on foodborne infection and intoxication*. I: 343-344.

Gill C. O., Deslandes, B., Rahn, K., Houde, A., and Bryat, J., (1998). Evaluation of Hygienic performance of the processes for beef carcass dressing at 120 pacing plants *J. appl. Microbiol.* **84:** 1050-1058.

Gill, C.O., (1979). Intrinsic bacteria in meat. *J. Appl. Bacteirol.* **47**: 367-378.

Girma Tullu and Birhanu Abegaz Gashe (1992). Prevalence of mastitic streptococci and staphylococci in the foremilk of lactating cows in the vicinity of Addis Ababa. *Ethiop. J. Agri. Sci.* **13**: 75-81

Girma Zewdie (1999)Productivity of three brands of modified semi-solid Rappaport – Vassiliadis (MSRV) medium and isolation of Salmonella from chicken carcass and chicken pieces. *Fleischwirtsch.* **79**:71-73

Glynn, M.K., Bopp, C., Dewitt, W., Dabney, P., Mokhtar, M., and Angulo, F., (1998) Emergence of multiresistant *Salmonella enterca* serotype Typhimurium DT104 infections in the United States. *N. Eng. J. Med.* **338:** 1333-8

Grau, F.H., (1986). Microbiology of meat and poultry. *Advances in Meat Research.* **2:**1-47.

Grau, F.H., and Smith, M.G., (1974). Salmonella contamination of sheep and mutton carcasses related to pre-slaughter holding conditions. J. Appl. Microbiol. 37:111

Grauke, L.J., Kudva, I.T., Yoon, J.W., Hunt, C.W., Williams, C.J. and Hovde, C.J., (2002). Gastrointestinal location of *Escherichia coli* O157:H7 in ruminants. *Appl. environ. Microbiol.* **68**: 2269-2277.

Griffin, P.M., Ostroff, S.M., Tauxe, R.V., Greene, K.D., Wells, J.G., Lewis, J.H., and Blake, P., A., (1988). Illnesses associated with *Escherichia coli* O157:H7 infections: A broad spectrum. *Ann. Interna. med.* **109**: 705-712.

Gun, H., Yilmaz, A., Turker, S., Tanlasi, A., and Yilamaz, H. (2003). Contamination of bovine carcasses and abattoir environment by *Escherichia coli* O 157:H7 in Istanbul *Int. J. Food Microbiol.* **84**: 339-344.

Hart, C.A., Trees, M.A.J., and Duerdin, B.J., (1997). Zoonoses. *J.Med. Microbiol.* **46**: 4-33.

Heuvelink, A.E., Roessink, G.L., Bossboom, K., and de Boer, E., (2001) Zero-tolerance for fecal contamination of carcasses as a tool in the control O157 VTEC infections. *Int. J. Food Microbiol.* **66**: 13-20.

Heuvelink, A.E., Zwartkruis-Nahuis, J.T., Beumer, R.R., and deBoer, E. (1999). Occurrence and survival of verocytotoxin producing *Escherichia coli* O 157 in meats obtained from retail out lets in the Netherlands. *J. Food. Prot.* **62**: 1115-1122.

Hirn, J., Maijala, R., and Johansson, T., (1992). Foodborne disease outbreaks in Finland 1975-1990. In : *Proceedings of the 3rd World congress on foodborne infection and intoxication.* **I** : 33-36

Hohmann, E.L., (2001). Nontyphoidal salmonellosis. *Clin. Infect.Dis* **32:** 263-269.

Howe, K., Linton, A.H., and Osborne, A.D., (1976). An investigation of calf carcass contamination by Escherchia coli from the gut contents at slaughter. *J. appl. Bacteriol.* **41**: 37

ICMSF, (1978). *Micro organisms in foods: their significance and methods of enumeration.* Second Edn. University of Toronto press.

ICMSF, (1980). Microbioal Ecology of foods. Vol. II Acadmeic press. New York.

Ishikawa, S., Kawahara, K.,Kagami, Y., Ishiki, Y., Kaneko,A; Matsui, H., Okada, N., and

Danbara, H., (2003). protection against shiga toxin 1 challenge by immunization of mice with purified mutant shiga toxin 1 . *Infect.Immun.* **71:** 3235-3239.

Jay, J.M., (1996).*Modern Food Microbiology* Fifth Edn. Chapman and Hall.

Kalapothaki, V., Mavromat, C.H., Vassiliadis, P., Trichopoulos, D., and Serie, C.H., (1983). Comparison of the original Rappaport medium (R 30) and the the Rappaport-Vassiliadis (RV) medium in the isolation of Salmonellae. In: Kiss, I., Dea, T., and Incze, K., (eds) *Microbial association and interactions in food. Proceed. 12th International IUMS-ICFMM symposium.* Budapest, Hungary. Pp. 161-64

Lahti, E., Ruoho, O., Rantala, L., Hanninen, M.L., and Bazalski, T.H., (2003). Longitudnal study of *Escherichia coli* O 157 in a cattle finishing *unit Appl. Environm. Microbiol.* **69:**554-61.

Leung, P.H., Yam, W.C., Ng, W.W., and Peiris, J.S. (2001). The prevalence and characterization of verotoxin producing Escherichia coli Isolated from cattle and pigs in an abattoir in Hong Kong. *Epidemiol Infect.* **126**: 173-179.

Lingwood, C.A., Law, H., Richardson, S., Petric, M., Brunton, J.L., De Grandis, S., and Marmali, M., (1987). Glycolipoid binding of natural and cloned *Escherichia coli* produced verotoxin *in vitro J. biol. Chem.* **262**: 8834-8839.

Litle, C., L.A., and Delouvois, J., (1998). The microbiological examination of butchery products and butchers premises in the United Kingdom. *J. Appl. Micrbiol.* **82**: 177-186.

Little C.L., Gillespie, I., de Louvois, J., and Mitchell, R., (1999). Microbiological investigation of Hall butchery products and buthcers' premises. *Commun. Dis pulbic Health.* **2**: 114- 118.

LMA (2000). F.D.R. Ethiopia Livestock marketing Authority *Guidelines on the establishment of export abattoir: Draft descriptive guidelines.* July 2000 Addis Ababa.

Loir, Y.L., Baron, F.,and Gauter, M., (2003). *Staphylococcus aureus* and food poisoning. *Genet. Mol. Res.* **2:** 63-76

Louie, M. De Azavedo,J., Clarke, R., Borczyk, A., Lior, H., Richter, M., and Brunton,J., (1994) sequence heterogeneity of the *eae* A gene and detection of verotoxin producing Escherichia coli using sertype specific primers. *Epidemiol. Infect.* **112**: 449-461.

Louie, M., DeAzavedo, J.C.,S., Handelsman, M.Y.C., Clark, C.G., Ally, B., Dytoc, M., Sherman, P., and Brunton, J., (1993) Expression and characterization of the *eae* A gene product of *Escherichia Coli* serotype O157:H7. *Infect. Immun* **61**: 4985-4092.

Malorny, M., Schroeter, A., and Helmuth, R., (1999) Incidence of quinolone resistance over the 13-year period 1986-1998 invetrinary Salmonella isolates from Germany. *Antimicrob. Agents and chemothr.* **43**: 2278-82

March, S.B., and Ratnam, S., (1986) Sorbitol MacConkey (SMAC) medium for detection of *Escherichia coli* O157:H7 associated with hemorrhagic colitis. *J. Clin. Microbiol.* **23**: 869-872.

McEvoy, J.M., Doherty, A.M., Sheridan, J.J., Blair, I.S., and McDowell, D.A., (2003) The prevalence of salmonella spp in bovine fecal, rumen and carcass samples of a commercial abattoir. *J. Appl.Microbiol.* **94**: 693-700.

McNally, A., Roe, A.J., Simpson, S., Thommson- carter, F., Hoey,D.E.E., Currie, C., Chakraborty, T., Smith, D.G., E., and Gally,D.L., (2001) Differences in levels of secreted locus of enterocyte effacement proteins between human Disease associated and bovine *Escherichia coli* O 157. *Infect. Immun.* 69: 5107-5114.

Mead, P.S., and Griffin, P.M., (1998) *Escherichia coli* O157:H7. *Lancet* **352**: 1207-1212.

Mead, P.S., Slutsker, L., Dietz, V., McCaig, L.F., Bresee, J.S., Shapiro, C., Griffin, P.M., and Tauxe, R.V., (1999). Food related illness and death in the United State. *Emerg. Infect.* Dis **5**: 697-625.

Menning, E.L., (1988). Danger lurks in your super market meat cases. *J.Am. Vet. Med. Assoc.*

192: 494-497

Mezgebe Tegegne and Mogessie Ashenafi, (1998). microbial load and incidence of *Salmonella* spp. in kitfo, a traditional Ethiopian spiced, minced meat dish. *Ethiop. J. Health. Deve.* 12: 135-140.

Moak, J.L., (2002). Thrombotic microangiopathies. *New. Eng. J.Med.* 347: 589-600.

Molbak, K., Baggesen, D.L., Aarestrup, F., Ebbesen, J.M., Engberg, J., Frydendhl, K. *et al.*, (1999). An outbreak of multi-drug resistant, quinolone resistant *Salmonella* enterica serotype Typhimurium DT 104. *N. Eng, J. Med.* 341: 1420-5

Mogessie Ashenafi, (1994). Microbial flora and incidence of some foodborne patogens on fresh raw beef from butchers' shops in Awassa, Ethiopia. Bull. *Anima. Helth prod. Afr.* 42: 273-277.

Mogessie Ashenafi, (2002). The microbiology of Ethiopian Foods and Beverages: A review. *SINET: Ethiop. J.Sci.* 25: 97-140.

Mogessie Ashenafi (1989). Microbial load, incidence and antibiograms of some disease causing microorganisms on raw food items consumed in Ethiopia. *MIRCEN J. Appl. Microbiol. Biotechnol.* 5:313-319

Morgan, D., Newman, C.P., Hutchinson, D.N., Waker, A.M., Rowe, B. and Majid, F. (1993). Verotoxin producing Escherichia coli O157 infections associated with the consumption of yoghurt. *Epidemiol. Infect* 111: 181-187.

Mulder, S.J. and Krol, B., (1976) A study on the bacteriological aspect of fresh beef. I. Effect of the slaughter of cattle *Tijdscher Diergeneeskd.*101: 587-593. (Article in Dutch).

Murray, M.J. (1986) Salmonella: Virulence factors and enteric salmonellosis *J. Am. Med. Assoc.* 189: 145-147.

Nakao, H., and Takeda, T., (2000). Escherichia coli shiga toxin. *J. Nat. Toxins.* 9: 299-313.

Narasimha, R.D. and Sreenivasamurthy, V., (1985) A note in microbial spoil spoilage of sheep meat at ambient temperature *J. Appl. Bacterial.* **58**: 457-459.

Nataro, J.P., and Kaper, J.B., (1998) Diarrheagenic *Escherichia coli. Clin. Microbiol. Rev.* **11**: 142-201.

Nyeleti, C., Bayleyegn Molla, Goetaz, H and Kleer, J. (2000) Prevalence and distribution of salmonellae in slaughter house personnel and minced beef in Addis Ababa (Ethiopia). *Bull.Anim. Health prod. Afr.* **48**: 19-24.

Paton, J.C., and Paton, A.W., (1998). Pathogenesis and diagnosis of shiga toxin producing *Escherichia coli* infections. *Clin. Microbiol. Reve.* **11**: 450-479.

Pegram, R.G., Roeder, P.L., Hall, M.L.M., and Rowe, B., (1981). *Salmonella* in livestock and animal products in Ethiopia. *Trop. Anim. Health Prod.* **13**: 203-207

Phillips, A.D., Navabpour S., Hicks, S., Dougan, G., Wallis,T., and Frankel,G., (2000) Enterohemorrhagic *Escherichia coli* target peyer's patches in humans and cause attaching and effacing lesions in both human and bovine intestine. Gut **47:** 377-381.

Phillips, D., Sumner, J., Alexander, J.F., and Dutton K.M., (2001) Microbiological quality of Austrialian beef. *J. Food prot* **64:** 692-696.

Philpott, D.J., Ackerley, C.A., Kiliaan, A.J., Karmali, M.A., Perdue, M.H., and Sherman, P.M., (1997) Translocation of VT-1 across, T84 Monolayers : mechanism of bacterial toxin penetration of epithelium. *Am. J. physiol. Gassrintest. Liver. Physiol.* **273**: G1349-g1358.

Pipova, M. and Pleva, J., (1992). Microbiological quality of mechanically-deboned meat (MDM) in *Proceedings of the third world congress on foodborne infection and intoxication* I: 216-219.

Prasovska, M., Pleva, J., Gorzova, A., (1991). Microbiological quality of mechanically deboned beef. *Zentralble mikrobiol.* **146:** 149-156.

Pruett, W.P., Biela, T., Lattuada, C.P., Mrozinski, P/M., Barbour, W.M., Flowers, R.S., Osborned, W., Reagan, J/O, Theno, D., Cook, V., McNamara, A.M., and Rose, B., (2002) Incidence of *Escherichia coli* O157:H7 in frozen beef patties produced over an 8 hour shift. *J. Food. Prot.* **65:** 1363-1370.

Rabatsky-Ehr, T., Dingman, D., Marcus, R., Howard, R. Kinney, A., and Mashar, P., (2002) Deer Meat as the source for sporadic case of *Escherichia coli* O157:H7 infection, Connecticut. *Emerg. Infect. Dis.* **8**: 525-527.

Radu, S., Mutalib, S.A., Rusual, G., Ahmad, Z., Morigak, T., Asai, N., Kim, Y.B., Okuda, J., and Nishibuchi, M., (1998). Detection of *Escherichia coli* O157:H7 in the beef marketed in Malaysia. *Appl. Environ. Microbiol.* **64**: 1153-1156.

Reinders, R.D., Weber, M.F., Lipman, L.J., Verhoeff and Bijker, P.G., (2001) control of VTE C in Dutch livestock and meat production *Int. J. Food microbiol* **66**: 79-83.

Renter, D.G., Sargement, J.M., Oberst, R.D., and Samadpour, M., (2003) Divensity, frequency and persistence of *Escherichia. coli* O157 strains from range cattle environments *Appl. Environ. Microbiol.* **61**: 542-547.

Richards, M.S., Corkish, J.D., Sayers, A.R., McLaren, I.M., Evans, S.J., and Wray, C. (1998) Studies of the presence of verotoxigenic *Escherichia coli* O157: in bovine feces submitted for diagnostic purposes in England and Wales and on beef carcasses in abattoirs in the United Kingdom. *Epidemiol. Infect.* **120**: 187-192.

Riley, L.W., Remis, R.S., Helgerson, S.D., McGee, H.B., Wells, J.G., Davis, B.R., Hebert, R.J., Olcott, E.S., Johnson, L.M,, Hargrett, N.T., Blake, P.A., and Cohen, M.L., (1983) Hemorrhagic colitis associated with a rare *Escherichia coli* serotype. *N. Eng. J. Med* **308**: 681-685.

Schroeder, C.M., Zhao, C., DebRoy, C., Torcolini, J., Zhao, S., White,D.G., Wagner, D.D.,

McDermott, P.F., Walker, R.D., and Meng, J., (2002) Antimicrobial resistance of *Escherichia coli* O 157 isolated from humans, cattle, swine and food. *Appl. Environ. Microbiol.* **68:** 576-581.

Shaikh, N., Tarr, P., (2003) *Escherichia coli* O157:H7 shigatoxin encoding Bacteriophages: Integrations, Excisions, Truncations, and Evolutionary implications. *J. Bacteriol.* **185:** 3596-3605.

Sharma, N.K.; Gill, J.P.S., Joshi, D.V., and Kwatra, M.S., (1992) Bacteriological analysis of chicken and meat products in India. *In proceedings of the third world congress on foodbrone infection and intoxiciation* I: 248-251.

Sharp, J.C.M., Collier, P.W., and Reilly, W.J., (1992). The WHO food bore surveillance prkogrammc on Europe- The first 12 years' experience in Scotland. In: *Proceedings of the 3rd World congress on foodborne infection and intoxication.* I :27-30

Sheoran, A.S., Chapman, S., Singh, P., Donohue Rolfe, A., and Tzipori, S., (2003) STX 2 specific human monoclonal antibodies protect mice against lethal infection with *Escherichia coli* expressing stx 2 variants. *Infect. Immun.* 71: 3125-3130.

Sierra, M., Gonzalez Fandos, M.E., Garcia, M.C., Garcia, M.L., Moreno, B., (1995) Numerical taxonomy f an atypical population of gram positive cocci isolated from freshly dressed lamb carcasses. *Int. J. Food microbiol.* **24**: 363-373.

Silliker,J.H., and Gabis, D.A., 1986) *Salmonella* Advances in Meat Research **2**: 209-229.

Smart, J.L., Roberts, T.A., Stringer, M.F., and Shah, N., (1979). The incidence and serotypes of *Clostridium perfringens* on beef, pork and lamb carcass. J. Appl. Bacteriol. 46: 377

Smeltzer, T., Thomas, R., Collins, G., (1980). The role of equipment having accidental or indirect contact with the carcass in the spread of Salmonella in an abattoir. *Aust. Vet. J.* 56: 14-17

Smith, H.R., and Scotland, S.M., (1993) Isolation and identification methods for *Escherichia coli*

industries. Integrated programme for private sector development, enhanced competitiveness and environmentally friendly production in Ethiopia. UNIDO Food safety project. October 2003 Addis Ababa.

Untermann, F., Stephan, R., Dura, U., Hofer, M., Heimann, P., (1997) Reliability and practicability of bacteriological monitoring of beef carcass contamination and their rating within a hygiene quality control programme of abattoirs *Int. J. Food. Microbiol.* **34**: 67-77.

Vernozy-Rozand, C., Mazuy, C., Ray-Gueniot, S., Boutrand- Loei, S., Meyrand, A., and Richard, Y., (1997). Detection of *Escherichia Coli* O 157 in French food samples using an immunomagnetic separation method and the VIDAS *E. coli* O157. Lett. *Appl. Microbiol.* **25**: 442-446.

Vold, L., Holck, A., Wasteson, Y., and Nissen, H., (2000). High levels of background flora inhibits growth of *Escherichia coli* O157:H7 in ground beef. *Int. J. Food Microbiol.* **56**: 219-225.

Vuddhakul, V., Patararungrong, N., Pungrasamee, P., Jitsuround, S., Morigaki, T., Asai, N., and Nishbuchi, M., (2002). Isolation and characterization of *Escherichia coli* O157 from retail beef and bovine feces in Thailand. *FEMS Micorbiol. Lett.* **182**: 343-347.

Wang, G., Clark, C.G., and Rodgers, F.G., (2002). Detection in Escherichia coli of the genes encoding the major virulence factors, the genes defining the O157:H7 serotype, and components of the type-2 shigatoxin family. *J. Clin Microbiol.* **40**: 3631-3619.

WHO/FAO, (2002). Risk assessments of salmonella in eggs and broliler chickens *Microbiological Risk. Assessment series* **2**: 7-15.

Wolde-Aregay Erku and Mogessie Ashenafi (1998). Prevalence of some of some food-borne pathogens and growth potential of *Salmonella* spp. inweaning foodsfrom Addis Ababa,

Ethiopia. *East Afr. Med. J.* **75**: 215-218

Wong, C.S., Jelacic, S., Habeeb, R.L., Watkins, S.L., and Tarr, P.I., (2000). The risk of the hemolytic uremic syndrome after antibiotic treatment of *Escherichia coli* O157:H7 *infections new Eng. J. Med.* **342**: 1930-1936.

Yoshida, T., Fukada, M., Koide, N., Ikeda, H., Sugiyama, T., Kato, Y., Ishikawa, N., and Yokochi, T., (1999) primary cultures of human endothelial cells are susceptible to low doses of shiga toxin and undergo apoptosis. *J. Infect. Dis*. 180:2048-2052.

Zadik, P.M., Chapman, P.A., and Siddons, C.A., (1993) use of tellurite for the selection of verocytotoxigenic *Escherichia coli* O157. *J.Med. Microbiol.* **39**: 155-158.

Zeleke Wolde-Tensay and Haile-Silassie Tesfaye (1992). Islation of enteric pathogens and coliform bacteria from infant feeding bottle content in Addis Ababa, Ethiopia. *Ethiop. J. Health Dev.* **6**:1-4

Zhao, C., Ge, B., De Villena, J., Sadler, R., Yeh, E., Zhao, S., White, D.G., Wagner, D., andMeng, J., (2001). Prevalence of Campylobacter spp., Escherchia coi and Salmonella serovars in retail chicken, Turkey, pork, and beef from the greater Washington, D.C., area. *Appl. Environ. Microbiol.* 67: 531-36

Zhou, Z., Nishikawa, Y., Zhu, P., Hong, S., Hase, A., Cheasty, T., Smith, H.R., Zheng, M., and Haruki, K. (2002) Isolation and characterization of shigatoxin producing Escherichia coli O157: H7 form beef, pork and cattle fecal samples in Changchun, China. *J. Vet. Med. Sci.* **64:** 1041-1044.