

**ADDIS ABABA UNIVERSITY
FACULTY OF VETERINARY MEDICINE**

**PREVALENCE, DISTRIBUTION AND ANTIMICROBIAL RESISTANCE
PROFILE OF *SALMONELLA* ISOLATED FROM FOOD ITEMS AND
PERSONNEL IN ADDIS ABABA, ETHIOPIA**

**By
ENDRIAS ZEWDU GEBREMEDHIN**

**JUNE, 2004
DEBRE ZEIT, ETHIOPIA**

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**A thesis submitted to the Faculty of Veterinary Medicine, Addis Ababa University in partial
fulfillment of the requirements for the Degree of Master of Science in Tropical Veterinary
Medicine**

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By

ENDRIAS ZEWDU GEBREMEDHIN

Board of Examiners

Signature

1. Prof. ph. Dorchies

2. Prof. Feseha Gebreab

3. Dr. Wondwossen Abebe Gebreyes

4. Dr. Giles Innocent

5. Dr. Andy Catley

6. Dr. David Barrett

Academic Advisors

1. Dr. Bayleyegn Molla

2. Prof. Mogessie Ashenafi

DECLARATION

“I declare that the information presented herein my thesis is my original work, has not been presented for a degree in any other university and that all sources of material used for the thesis have been duly acknowledged”

Name: Endrias Zewdu Gebremedhin

Signature: _____

Date of Submission _____

This thesis has been submitted for examination with our approval as University advisors.

Supervisor / advisor:

1. Dr. Bayleyegn Molla

Signature _____

2. Professor Mogessie Ashenafi

Signature _____

DEDICATION

This work is dedicated to my beloved wife Tsehay Assefa, children's (Betel, Tensay and Semere), my mother Ayelech Seyoum and my uncle Gashaw Seyoum.

ACKNOWLEDGEMENTS

The work incorporated in this thesis was undertaken using the research grant allocated by Faculty of Veterinary Medicine (FVM), Addis Ababa University (AAU). I am grateful to the University, in particular, and the government of Ethiopia, in general, for providing me the research fund. Ambo College of Agriculture, my mother institute, is also highly acknowledged for its financial, material and moral support throughout the study period.

I take this opportunity to pass my special gratitude to my academic advisor, Dr. Bayleyegn Molla, Associate Professor and Associate Dean for Research and Graduate Programs of the Faculty of Veterinary Medicine, Addis Ababa University, for his constant supervision throughout the work, provision of necessary materials and patiently and skillfully going through the manuscript. My thanks also go to Professor Mogessie Ashenafi, Institute of Pathobiology, for his excellent comments and suggestions on the scientific and editorial aspect of the thesis.

I would like to acknowledge my indebtedness to a number of people who have, in various direct or indirect ways, contributed to the success of my work, particularly Dr. Wondwossen Abebe, Assistant Professor, North Carolina State University, USA, for his literature support and Dr. Wondwossen Tsegaye, from Shola Regional Veterinary Laboratory, for supplying me with reagents whenever I was in short. Dr. Daniel Alemayehu, Dr. Getahun Ejeta and Dr. Ermias G/Michael deserve my appreciation for providing me relevant reference materials.

I wish to extend my thanks to Addis Ababa supermarket owners and employees for their cooperation during the process of sample purchase and also provision of stool samples.

I am extremely grateful to Dr. Anne Muckle, head of Laboratory for Foodborne Zoonoses, Health Canada, and Dr. Cornelius Poppe and Ms. Linda Cole at the Office International des Epizooties (OIE), Reference Laboratory for Salmonellosis, in Guelph, Ontario, Canada, for their excellent endeavors in serotyping, phage typing and antimicrobial susceptibility testing of *Salmonella* isolates.

I owe special thanks to my wife, tsehay asseffa, who gave all what she can, especially in looking after our 3 children in my absence so that i would be able to do my study quite successfully, and to betel, tensay and semere, who could not tolerate and wait for me to finish so that they could have their father back.

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LIST OF ABBREVIATIONS AND SYMBOLS

AACA	Addis Ababa City Administration
AAU	Addis Ababa University
A_w	Water activity
BPLS agar	Brilliant-Green - Phenol-red Lactose Sucrose agar
BPW	Buffered Peptone Water
CI	Confidence Interval
df	degree of freedom
DNA	Deoxyribonucleic acid
DT 104	Definitive Type 104
ELISA	Enzyme Linked Immunosorbent Assay
FVM	Faculty of Veterinary Medicine
GAP	Good Agricultural Practices
GMP	Good Manufacturing Practice
H ₂ S	Hydrogen Sulphide
HACCP	Hazard Analysis Critical Control Point
HIV/AIDS	Human Immunodeficiency Virus/ Acquired Immunodeficiency Syndrome
ICMSF	International Commission on Microbiological Specification for Foods
IFT	Institute of Food Technologist
ILCA	International Livestock Center for Africa
LPS	Lipopolysaccharide
m.a.s.l	meters above sea level
MDR	Multi-Drug Resistant
MH	Muller Hilton
NCCLS	National Committee for Clinical Laboratory Standards
NMSA	National Meteorological Service Agency
OIE	Office International des Èpizootics
PCR	Polymerase Chain Reaction
PT	Phage type
r	Correlation coefficient
RV	Rappaport Vassiliadis broth

SC	Selinite Cystine broth
Shoats	Sheep and Goats
Spp.	Species
Subsp.	Subspecies
TSI	Triple Sugar Iron agar
USDA	United States Department of Agriculture
XLD agar	Xylose Lysine Desoxycholate agar
µg	Microgram

ABSTRACT

A cross-sectional study to determine the prevalence, distribution and antimicrobial profile of *Salmonella* serotypes isolated from food items and apparently healthy supermarket butchery workers was undertaken from September 2003 to February 2004. A total of 1200 food items consisting of chicken carcass (208), pork (194), mutton (212), minced beef (142), cottage cheese (190), *Tilapia* fish meat (128) and ice cream (126) were purchased in Addis Ababa. Additionally sixty-eight stool samples were analyzed. Chicken carcass, pork, mutton and minced beef samples were collected from 32 randomly selected supermarkets while cottage cheese, fish and ice cream samples were collected from open markets (3), fish shops (6) and pastry shops (17) in Addis Ababa, respectively.

Out of the food items, 7.8% were positive for *Salmonella* and of sixty-eight stool samples five gave positive result (7.4%). About 14% of chicken carcass, 11.3% of pork, 10.8% of mutton, 8.5% of minced beef, 2.1% of cottage cheese, 2.3% of fish and none of the ice cream yielded *Salmonella*. *Salmonella* was recovered from samples taken from 21 of the 32 supermarkets considered in the study. On the other hand one open market out of three, two fish shops out of six and none of the 17 pastry shops gave *Salmonella* positive results.

A total of 14 different serotypes out of 98 *Salmonella* isolates were identified. *Salmonella* Newport (41.8%) was the most prevalent serotype, followed by *S. Braenderup* (12.2%), *S. Hadar* (8.2%), *S. Typhimurium* (7.1%), *S. Dublin* (6.1%) and *S. Haifa* (6.1%). Less commonly isolated *Salmonella* serotypes included: *S. Infantis*, *S. Kentucky*, *S. Bovismorbificans*, *S. Anatum*, *S. Zanzibar*, *S. Kottbus*, *S. Saintpaul* and *S. 1: 9, 12:-*. *Salmonella* Newport and *S. Kentucky* were reported for the first time in Ethiopia. *Salmonella* Newport was isolated from all sample types except ice cream, while *S. Braenderup*, *S. Kottbus*, *S. Saintpaul* were detected only from chicken carcass, pork and minced beef samples, respectively.

There was a statistically significant difference in the rate of *Salmonella* isolation between meats (chicken carcass, pork, mutton and minced beef) and the rest of the samples (cottage cheese, fish and ice cream) (Pearson's $X^2 = 37.569$, $df = 1$, $p\text{-value} = 0.000$). The level of antimicrobial

resistance was significantly higher for chicken carcass and pork isolates as compared to other samples ($p = 0.003$).

Assay of antimicrobial resistance revealed that 32.7% of *Salmonella* isolates were resistant to one or more of the 24 antimicrobials tested. Generally resistance for 13 different antimicrobial drugs was recognized. The most common resistance was to streptomycin (24/32, 75%), ampicillin (19/32, 59.4%), tetracycline (15/32, 46.9%), spectinomycin (13/32, 40.6%) and sulfisoxazole (13/32, 40.6%). All the three *Salmonella* Kentucky isolates showed resistance to at least 8 antimicrobials, which includes: ampicillin, amoxicillin/clavulanic acid, ciprofloxacin, nalidixic acid, spectinomycin, streptomycin, sulfisoxazole and tetracycline. Out of the 12 *Salmonella* Braenderup isolates, 10 (83.3%) showed multidrug resistance to ampicillin, spectinomycin, streptomycin, sulfisoxazole, sulfamethoxazole/trimethoprim, amoxicillin/clavulanic acid and trimethoprim. Among the 8 *S. Hadar* isolates 7 (86.5%) showed antimicrobial resistance of which three isolates showed resistance to streptomycin and tetracycline, two isolate showed resistance to tetracycline and the other two for streptomycin. All the 6 *S. Dublin* isolates were resistant to carbadox (100 %) while one was additionally resistant to tetracycline. All the 6 *S. Haifa* strain isolated were resistant for at least ampicillin, streptomycin and tetracycline. Up to ten different antimicrobial resistances pattern was observed. Antimicrobial resistance was most common among *Salmonella* isolated from chicken carcass (18/29, 62.1%) followed by pork (5/22, 22.7%). Multiple antimicrobial drug resistance was observed in 23 *Salmonella* isolates (23.5 %). The detection of 7.4% *Salmonella* carriers' supermarket workers shedding *S. Newport*, the most prevalent serotype, suggests possible linkage and potential source of infection.

The findings of the present study ascertain that *Salmonella* serotypes were widely distributed particularly in supermarket meat samples and significant proportion have developed resistance for routinely prescribed antimicrobial drugs both in veterinary and public health sectors. This poses considerable health hazards to the consumers unless prudent antimicrobial usage, adequate heat treatment, improvement of standards of hygiene and development and enforcement of suitable legislation, which safeguard consumers, are urgently instituted.

Key words: Prevalence, *Salmonella*, serotype, antimicrobial resistance, food items, supermarket, personnel, Addis Ababa.

1. INTRODUCTION

1.1. Foodborne salmonellosis

Foodborne diseases are among the most widespread global public health problems of recent times, and their implication for health and economy is increasingly recognized (Van der Venter, 1999; Gomez *et al.*, 1997). These diseases are attributable to a wide range of pathogens and toxins. *Salmonella* is a leading cause of foodborne illnesses (White *et al.*, 2001; D'Aoust, 1997; D'Aoust, 1991a; WHO, 1988). According to the World Health Organization reports of 1995, 88% of all foodborne diseases were caused by *Salmonella* (Gabert *et al.*, 1999). Salmonellosis, the disease caused by infection with *Salmonella*, is a common intestinal illness caused by numerous *Salmonella* serovars and manifested clinically in animals (Radostits *et al.*, 1994) and humans (Hohmann, 2001) as an acute enteritis and chronic enteritis, an acute septicaemic disease or as subclinical infections (Acha and Szyfres, 2001).

The epidemiology of foodborne problems like salmonellosis is complex and expected to vary with change in the pathogens themselves, industrialization, urbanization and change of lifestyles, knowledge, belief and practices of food handlers and consumers, demographic changes (increased susceptible population), international travel and migration, international trade in food, animal feed and in animals, and poverty and lack of safe food preparation facilities (Van der Venter, 1999; Altekruze *et al.*, 1998; WHO, 1988).

Foodborne illnesses, including salmonellosis, are widespread and have an impact on communities in both the developing and developed world (Van der Venter, 1999; Leegaard *et al.*, 1996). In industrialized countries the incidence of salmonellosis is on the rise due to the emergence and increase of *S. Enteritidis* and *S. Typhimurium* DT 104 (Wray and Davies, 2000; Gomez *et al.*, 1999; Van der Venter, 1999). Hundreds of millions of people worldwide suffer from communicable and non-communicable diseases caused by contaminated food. These diseases take a heavy toll in human life and sufferings, particularly among infants and children, the elderly and other susceptible persons. They also create an enormous social, cultural and economic burden on communities and their health system (Van der Venter, 1999). Interest in *Salmonella*

has heightened in recent years due to the increased susceptibility of AIDS patients to salmonellosis, the devastating effects of *S. Enteritidis* in the poultry industry, and the globalization of agricultural trade (Clarke and Gyles, 1993). Persistent and severe salmonellosis has also been recognized as a problem among patients with AIDS (Gerald, 1991).

In developing countries a rapidly growing industry of intensive animal production is accompanying the process of urbanization with all its environmental and behavioral changes favorable for *Salmonella* to prevail (WHO, 1988). Most food industries in developing countries are not well aware of food safety issues, and knowledge of modern technologies, Good Manufacturing Practices (GMP), hygiene, Hazard Analysis Critical Control Point (HACCP) system, and quality control is often limited or absent. Cold storage facilities are inadequate and quality of water used for food processing may not be suitable. The vast numbers of laborers that handle food in factories, as well as on farms, are illiterate and untrained. In developing countries including Ethiopia, foodborne illnesses are perceived as mild and self-limiting diseases. Their severe and chronic health consequence is often overlooked, as are their consequences on trade and the economy. In such countries lack of information leads to lack of appreciation of the health significance of unsafe food and this, in turn, leads to low priority and sometimes no resources assigned to food safety (Van der Venter, 1999).

Outbreaks of salmonellosis have been reported for decades, but within the past 25 years the disease has increased in incidence in many continents. The disease appears to be most prevalent in areas of intensive animal husbandry, especially of poultry or pigs and dairy cattle reared in confinement (OIE, 2000; D'Aoust, 1989). There are pandemics of *S. Enteritidis* and *S. Typhimurium* DT 104 which resulted in enacting regulations in many countries to control the prevalence of salmonellosis in farm animals in order to prevent foodborne infection (Wray and Davies, 2000).

The clinically normal carrier animal is a serious problem in all host species. Foods of animal origin, particularly meat, poultry, and, in some instances, unpasteurized egg products are considered to be the primary sources of human salmonellosis (Acha and Szyfres, 2001; White *et al.*, 2001; Wray and Davies, 2000; Gomez *et al.*, 1997; D'Aoust, 1997; Nielsen *et al.*, 1995; Tauxe, 1991; WHO, 1988; Koulikovskii, 1982). Most of these food products, e.g. beef, mutton and poultry, become contaminated during slaughter and processing, from the gut contents of

healthy excreting animals. In the same way, all food that is produced or processed in a contaminated environment may become contaminated with salmonellae and be responsible for outbreaks or separate cases of disease as a result of faults in transport, storage, or preparation (D'Aoust, 1997; Koulikovskii, 1982). Food producers, processors, and distributors need to be reminded that even low levels of salmonellae in a finished food product can lead to serious public health consequences and undermine the reputation and economic viability of the incriminated food manufacturer (D'Aoust, 1989).

Salmonellae are infrequent cause of mastitis in dairy cows but several species of *Salmonella* have been documented to colonize udders and shed at levels of up to 2000 organisms /ml (Fontaine *et al.*, 1980). According to Jayarao and Henning (2001) *Salmonella* was isolated from 6.1% of bulk tank milk samples from dairy herds in Eastern South Dakota and Western Minnesota. Cheese made from pasteurized milk can be considered safe; however, raw milk cheese has been implicated in several outbreaks (D'Aoust, 1994; Bean and Griffin, 1990; Fontaine *et al.*, 1980). The low dose of *Salmonella* potentially required to result in infection leading to variety of clinical conditions and septicemias and even deaths suggests that raw milk cheeses could pose a public health hazard, especially for people with compromised immune systems. Cottage cheese, although fermented, cannot be totally excluded from being source of infection for humans.

Studies show that antimicrobial-resistant salmonellae are increasing due to the use of antimicrobial agents in food animals, which are subsequently transmitted to humans usually through the food supply (White *et al.*, 2001; Angulo *et al.*, 2000; Fey *et al.*, 2000; Mølbak *et al.*, 1999; Tollefson *et al.*, 1998; D'Aoust 1989). If the frequency of drug resistance increases, the choice of antimicrobials for treatment of systemic salmonellosis in humans becomes more limited.

Globally, the three main causes of antimicrobial resistance have been identified as use of antimicrobial agents in agriculture, over-prescribing by physicians, and misuse by patients (IFT, 2003). *Salmonella* Typhimurium DT 104 has a broad host reservoir and is usually resistant to five antibiotics (ampicillin, chloramphenicol, streptomycin, sulphonamides, and tetracycline) and can be resistant to others (e.g., fluoroquinolones) (IFT, 2003; Glynn *et al.*, 1998). Routine assessment of patterns of emerging antibiotic resistant *Salmonella* strains is of paramount importance because such information channeled to physicians and veterinarians help to timely redirect drug

use so as to diminish the development and spread of resistance. It also helps to know the extent and temporal trends of antimicrobial susceptibility. The ultimate outcome will be to prolong the efficacy of existing and new antimicrobial agents which are desperately needed to control both human and animal diseases and to minimize the spread of resistant zoonotic pathogens to humans (Tollefson *et al.*, 1998). As serovars and phage types and, with them, antibiotic sensitivity patterns can vary annually (Van Dujkeren *et al.*, 1994b); the choice of the drug for treatment of salmonellosis should always be based on sensitivity testing of the causative strain. However, since it takes two or three days before the result is available, blind therapy has to be started in severely ill animals. Therefore, susceptibility testing combined with knowledge of the pharmacokinetic and toxicologic data of the drug are essential in choosing an effective drug for antimicrobial therapy (Prescott and Baggot, 1993).

Different reports from different African countries showed that *Salmonella* is widespread and important health problem. Multitudes of serotypes are prevalent in Egyptian slaughtered animals (Youssef *et al.*, 1982) and dairy products (Nassib *et al.*, 2003), in animals and man from Sudan (Quddus Khan, 1962), in Zambian animals (Sharma *et al.*, 1996) and chicken carcass (Hang'ombe *et al.*, 1999), in domestic animals, birds and man of Zimbabwe (Chambers, 1977), in animals of Tanzania (Hummel, 1979), in slaughter cattle, slaughter areas and effluents of Nigeria (Adesiyun and Oni, 1989), in apparently healthy animals of Botswana (Miller, 1971), in blood of septicaemic patients of Kenya and Malawi (Leegaard *et al.*, 1996) and pediatrics population in Liberia (Hadfield and Monson, 1985).

As no sufficient data are available on salmonellosis and other foodborne illnesses and on the economic effect of unsafe food in Ethiopia, the policy makers would be forced to give the subject low priority. Foodborne problems have their origin in the methods of farming. Many farmers are uneducated and follow methods of production that are centuries old. They live in very close contact with their animals, often under poor hygienic conditions, thereby increasing the likelihood of foodborne zoonoses. However, considerable proportion of patients may not visit health centers unless symptoms are serious due to shortage of resources and lack of awareness. Earlier investigations made by different worker in Ethiopia have demonstrated the presence of *Salmonella* in apparently healthy slaughtered cattle (Molla *et al.*, 2003a; Alemayehu *et al.*, 2002; Nyeleti *et al.*, 2000), minced beef (Ejeta *et al.*, 2004; Nyeleti *et al.*, 2000; Molla *et al.*, 1999a), poultry farms (Molomo, 1998), poultry meat and offals (Tibaijuka *et al.*, 2002; Molla and

Mesfin, 2003), selected food items (Molla *et al.*, 1999b; Molla *et al.*, 1999a), farm livestock, an abattoir and bone factory (Pegram *et al.*, 1981), raw “kitfo” samples (Tegegne and Ashenafi, 1998), samples from butchers’ shop (Ashenafi, 1994), and human beings (Mache, 2002; Nyeleti *et al.*, 2000; Mache *et al.*, 1997; Ashenafi and Gedebeu, 1985; Gedebeu and Tassew, 1981). With regard to the serotype identified from apparently healthy slaughtered cattle, sheep and goats, camel, and poultry, foods and human beings so far different researchers have indicated the existence of diversified serotypes in Ethiopia (Ejeta *et al.*, 2004; Weldemariam, 2003; Tibaijuka *et al.*, 2003; Molla *et al.*, 2003b; Alemayehu *et al.*, 2002; Nyeleti *et al.*, 2000; Molla *et al.*, 1999a; Molomo, 1998).

Despite some attempts to study prevalence of *Salmonella* in Ethiopia, mainly in poultry and beef, the status of the problem in fish, ice cream and milk products is still very much unknown. However, studies made elsewhere indicated that fish and milk products are important sources of *Salmonella* particularly among those raw consumers (Jay, 2000; WHO, 1988; Silliker, 1982; Fontaine *et al.*, 1980). Considering the relative unhygienic conditions prevailing at farm level, slaughterhouses and food handlers, it is likely that food items sold in Addis Ababa markets harbor *Salmonella*. This, coupled with preference of Ethiopians to raw or under cooked meat, milk and milk products and even eggs constitute a real health hazard to consumers. Intensity of *Salmonella* contamination of food items sold on market, personnel handling of these food items and antimicrobial profile of the *Salmonella* isolates has not been adequately studied in Ethiopia. Insufficient information at hand in this regard is one factor, which hindered efforts to prevent and control the disease. Based on the ubiquitous nature of *Salmonella* and previous studies made in Ethiopia, it is hypothesized that food items sold in Addis Ababa markets are sources of *Salmonella* to consumers and considerable proportion of them might have developed resistance to antimicrobials that are commonly used both in the veterinary and public health sectors.

1.2. Research goal

The ultimate goal of the current research work is to partly contribute towards the development of national food safety strategies, which aim to protect the consumer from foodborne diseases. This is through provision of basic data on the situation of *Salmonella* in food items and personnel in Addis Ababa.

1.3. Objectives

1.3.1. General objectives

The general objective of this research undertaking is microbiological study of *Salmonella* from foods of animal origin and its potential implications as health hazard to consumers.

1.3.2. Specific objectives

1. to determine the prevalence and distribution of *Salmonella* serotypes in food items sold in the Addis Ababa markets,
2. to determine the prevalence of *Salmonella* serotypes among food handler (personnel) and association of the isolates with that of food items, and
3. to investigate the susceptibility of *Salmonella* isolates to antimicrobial agents used in veterinary and human medicine for the treatment of bacterial diseases including salmonellosis.

2. LITERATURE REVIEW

2.1. Taxonomy

The genus *Salmonella* obtained its name from the American veterinarian Daniel Elmer Salmon, who first isolated *Salmonella enterica* serotype Choleraesuis from pigs in 1885 (Rabsch *et al.* 2003). Salmonellae are facultatively anaerobic, Gram-negative rods belonging to the family Enterobacteriaceae. Although members of this genus are motile by peritrichuous flagella, non-flagellated variants, such as *Salmonella Pullorum* and *Salmonella Gallinarum* and non-motile strains resulting from dysfunctional flagella do occur (D'Aoust, 1997). The naming system for *Salmonella*, which has been in use, so far combines several nomenclatural systems that inconsistently divide the genus into species, subspecies, subgenera, group, subgroups, and serotypes (serovars), and thus causes confusion (Brenner *et al.*, 2000). Recently, efforts have been made to simplify the nomenclature of *Salmonella* (IFT, 2003) and keep uniformity in nomenclature for ease of communication between scientists, health officials, and the public (Brenner *et al.*, 2000). Instead of using serotype designations incorrectly as species designations, most *Salmonella* species are now classified as *Salmonella enterica* and then further identified by serovar (e.g., *Salmonella typhimurium* becomes *Salmonella enterica* Serovar Typhimurium (Appendix 1). For convenience, the species (*enterica*) designation is frequently eliminated, leaving *Salmonella* Typhimurium (IFT, 2003; Brenner *et al.*, 2000). The genus name is italicized and for named serotypes, to emphasize that they are not separate species, the serotype name is not italicized and the first letter is capitalized (Brenner *et al.*, 2000).

The majority of *Salmonella* serotypes belong to *Salmonella enterica* subsp. I (*S. enterica* subsp. *enterica*) (Appendix 1). Within *S. enterica* subsp. I (*S. enterica* subsp. *enterica*), the most common O-antigen serogroup are A, B, C1, C2, D and E. Strains in these serogroup cause approximately 99% of *Salmonella* infections in humans and warm-blooded animals (Le Minor and Popoff, 1997). Serotypes in *S. enterica* subsp. II (*S. enterica* subsp. *salamae*), IIIa (*S. enterica* subsp. *arizonae*), IIIb (*S. enterica* subsp. *diarozonae*), IV (*S. enterica* subsp. *indica*), and V (*S. enterica* subsp. *bongori*) are usually isolated from cold - blooded animals and the environment but rarely from humans.

Strains of *Salmonella* are classified into serovars on the basis of extensive diversity of lipopolysaccharide (LPS) (O) antigens and flagellar protein (H) antigens in accordance with the Kauffmann/White scheme. Except for serotypes *S. Typhi*, *S. Paratyphi A*, and *S. Paratyphi C*, which are strictly human and whose only reservoir is man, all serotypes can be considered zoonotic or potentially zoonotic (Acha and Szyfres, 2001; Baird-Parker, 1990).

2.2. Physiology

Salmonella grows between 8 °C and 45 °C (optimally at 37 °C) and at a pH of 4 to 9. A temperature higher than 70 °C rapidly kills them. Pasteurization at 71.1 °C for 15 seconds is sufficient to destroy salmonellae in milk (Acha and Szyfres, 2001). Unlike the other enteric bacteria, except for *Yersinia*, salmonellae are frequently facultative intracellular parasites. The invasive strains are taken up by macrophages and spread is via the lymphatic system (Carter and Chengappa, 1986). These bacteria can resist dehydration for a very long time ($a_w \geq 0.93$), both in feces and in foods for human and animal consumption. In addition, they can survive for several months in brine with 20% salinity, particularly in products with a high protein or fat content, such as salted sausages; they also resist smoking. It has been indicated that they can survive for a long time in soil, water (WHO, 1988) and dried foodstuffs (Sleigh and Duguid, 1989).

Salmonellae grow on defined media without special growth factors. They can use citrate as a carbon source and are usually aerogenic, producing gas from glucose. They can selectively utilize tetrathionate or sodium selenite; thus very small number can be detected in highly contaminated specimens using these substrates as enrichment media. Carbohydrates are generally fermented with the production of acid and gas. *Salmonella Typhi*, *S. Gallinarum* and rare anaerogenic variants in other serotypes, e.g. *S. Typhimurium*, form only acid. Salmonellae are lactose non-fermenters, and this property is used for initial selection in the clinical microbiology laboratory. *Salmonella enterica* subsp. *arizone* exhibit important biochemical differences from typical salmonellae. Less than 1% of strains ferment lactose promptly (pink on MacConkey agar plates, like *Escherichia coli*) (Hohmann, 2001; ISO 6579, 1998) and others ferment it after several days' incubation of the test (Sleigh and Duguid, 1989). Salmonellae ferment glucose, resulting in a

typical acid butt and alkaline slant in triple sugar iron agar (TSI). They generally produce hydrogen sulphide (H₂S), which is detectable, using Fe⁺³ ions in selective agar as a black reaction product and serves initially to distinguish isolates from *Shigella*, which also gives an alkaline/acid TSI reaction (Keusch, 1991).

Salmonellae decarboxylate the amino acids lysine, ornithine and arginine, but not glutamic acid. Most salmonellae are indole negative, methyl-red positive, and acetyl methyl carbinol not produced (i.e. Voges-Proskauer negative). Citrate is utilized, except by *S. Typhi* and *S. Paratyphi A*. Urease is not produced. Hydrogen sulphide produced in ferrous chloride-gelatin medium, except by *S. Paratyphi A*, *S. Choleraesuis* (Jawetz *et al.*, 1984).

Salmonellae have several virulence factors that contribute to causing diarrhea, bacteremia, and septicemia. These factors include the lipopolysaccharide of the outer wall, pili, flagella, cytotoxin, and enterotoxin (Murray, 1986).

2.3. Epidemiology

Although primarily intestinal bacteria, *Salmonella* are widespread in the environment and commonly found in farm effluents, human sewage, and in any material subject to fecal contamination. Salmonellosis has been recognized in all countries but appears to be most prevalent in areas of intensive animal husbandry, especially poultry and swine production (Wray and Davies, 2003). Approximately 2500 different *Salmonella* serovars have been described, and the number increases annually as new serovars are recognized (Wray and Davies, 2003).

2.3.1. Susceptibility

All animals are at increased risk of developing disease if their normal flora is disrupted (stress, antibiotics). These circumstances render animals susceptible to exogenous exposure or activation of silent infections. Young animals are more susceptible to salmonellosis than older ones. Poor sanitation, overcrowding, unfavorable weather, stress of hospitalization and surgery, parturition, parasitism, transportation, and concurrent viral infections are all factors which predispose

animals to clinical salmonellosis (Clarke and Gyles, 1993). Many animals suffer inapparent infections during their life times. This is especially true of swine (Clarke and Gyles, 1993) and poultry fed rations that contain *Salmonella*. In the subclinical form, the animal may have a latent infection and harbor the pathogen in its lymph nodes, or it may be a carrier and eliminate the agent in its fecal material briefly, intermittently, or persistently (Acha and Szyfres, 2001).

2.3.2. Mode of transmission

The feco-oral route is the most important mode of transmission of *Salmonella* in animals. Infection in cattle may also occur via other routes, including the respiratory tract, by inhalation of aerosol (Gay, 2003). However, the cycle of infection may be more complex in some animal populations; in poultry, for example, the primary source of infection may be contaminated feed, and subsequent spread may occur via the feco-oral route or from egg to chick in the hatchery. A variable percentage of animals, once infected, remain carriers and shed the organism intermittently (Gillepsie and Timoney, 1981).

Animal feeds are frequently contaminated by a variety of serotypes, which usually enter the feed mixture in the protein supplement. Meat and bone meal, fish meal, and soybean meal have all been shown to be frequently and heavily contaminated (Wray and Sojka, 1977). The *Salmonella* enter these materials during or after processing. In the case of meat and bone meal, the percolator phase that removes fat after cooking is an important contaminative stage: the organisms are maintained and multiply in the material as it cools (Acha and Szyfres, 2001).

2.3.3. Carrier and sources of infection

Because salmonellae are facultative intracellular organisms that survive in the phagolysome of macrophages, they can evade the bactericidal effects of antibody and complement. Thus, persistence of infection in animals and in the environment is an important epidemiological feature of salmonellosis (Radostits *et al.*, 1994). Although *Salmonella* may survive for long periods in the environment (Gay, 2003; Wray and Sojka, 1977), it is the carrier state that provides the major source of infection for animals and humans. The carrier state is characterized by the

absence of evidence of disease in animals that are able to transmit infection to susceptible individuals. Certain stress factors have been shown to promote excretion of *Salmonella* by carriers and to lead to activation or reactivation of disease in carrier animals (Acha and Szyfres, 2001; Wray and Sojka, 1977).

Transportation of animals, overcrowding, and administration of corticosteroids, parturition, and concurrent viral and protozoan infections have all been shown to increase susceptibility of animals to disease (Clarke and Gyles, 1993). Intensification of husbandry in all species is recognized as a factor contributing significantly to an increase in the new infection rate. A typical example is the carrier rate of 54 % observed in intensive piggeries in New Guinea compared to the 9 % in village pigs (Radostits *et al.*, 1994).

The carrier domestic (including poultry) and wild animals, turtles and other pets shed salmonellae. Human patients, both sick and convalescent, and subclinical carriers may also shed organisms. Other sources are whole eggs, especially duck eggs, egg products, meat and meat products, poultry, and fertilizers and animal feeds prepared from bones, fish meals, and meat (Carter and Chengappa, 1986). Insects, particularly flies, may have some role as mechanical vectors in very contaminated environments. In developing countries, the source of infection is mainly the contaminated environment and water sources where animals crowd together (Acha and Szyfres, 2001). Salmonellosis is an important zoonosis and, although human-to-human transmission does occur, especially in pediatric wards, nurseries and nursing homes (WHO, 1988), animals and their products constitute the most important source of the organism for humans (D'Aoust, 1989).

2.4. Pathogenesis

Colonization of the distal small intestine and the colon is a necessary first step in the pathogenesis of enteric salmonellosis. Indigenous fusiform bacteria that lie in the mucous layer infesting the epithelium of the large intestine normally inhibit growth of *Salmonella* by producing volatile organic acids. The normal flora also blocks access to attachment sites needed by the pathogens. Factors, which disrupt the normal colonic flora, such as antibiotic therapy, diet, and water deprivation, greatly increase the host's susceptibility to enteric and septicemic

salmonellosis. Reduced peristalsis also enhances colonization by *Salmonella* because it allows temporary overgrowth to occur, especially in the small intestine. Peristalsis is stimulated by an active indigenous microflora, suppression of which increases the host's susceptibility to colonization (Venter *et al.*, 1994; Gillepsie and Timoney, 1981).

Following an adhesion-dependent attachment of salmonellae to luminal epithelial cells, the invasive pathogen is internalized within an epithelial cell by a receptor-mediated endocytotic process. Cytotoxin localized in the bacterial cell wall suggestively may facilitate *Salmonella* entry into the epithelial layer. Cytoplasmic translocation of the infected endosome to the basal epithelial membrane culminates in the release of salmonellae in the lamina propria. During this invasive process, *Salmonella* secretes a heat-labile enterotoxin that precipitates a net efflux of water and electrolytes into the intestinal lumen. *Salmonella* enterocolitis, which brings profuse loss of intestinal fluids, is the result of enterotoxin-mediated activation of adenylcyclase localized in the cytoplasmic membrane of host epithelial cells (D'Aoust, 1991a). The activation of adenylcyclase may be due to the effects of prostaglandins induced by the inflammatory response to the invading *Salmonella*. However, some strains of *S. Typhimurium* are known to produce enterotoxin-like substances. Inflammatory enteritis quickly develops and is characterized by extensive neutrophil invasion of villous cores with acute ileitis and colitis. Neutrophils are also shed in the stool, and their presence has diagnostic value (Venter *et al.*, 1994; Gillepsie and Timoney, 1981).

The pathogenesis of the septicemic phase of salmonellosis appears to be related to the effects of endotoxin released from bacterial cells (Wray and Davies, 2000; Gillepsie and Timoney, 1981). In *Salmonella*, endotoxic activity resides in the lipopolysaccharide of the cell wall. The lipopolysaccharide is composed of an O- specific chain, a core oligosaccharide common to all *Salmonella*, and a lipid A component. The latter is the part of the lipopolysaccharide molecule that contains endotoxin activity. The effect of endotoxin on the host includes fever, mucosal hemorrhages, leucopenia followed by leukocytosis, thrombocytopenia, and depletion of liver glycogen with prolonged hypoglycemia and shock. The shock effect may be severe and irreversible and may lead to death (Gillepsie and Timoney, 1981).

2.5. Clinical signs

The disease manifestations depend on the virulence of different *Salmonella* serovars; the number of *Salmonella* ingested and host immunity. Many *Salmonella* infections are opportunistic infection in compromised hosts. The majority of *Salmonella* infections in a herd over time are subclinical; the clinical infections are only the tip of the iceberg, even during outbreaks of clinical disease (Gay, 2003). A sudden change in herd resistance caused by concurrent disease, for example, fascioliasis, bovine virus diarrhea virus, nutritional stress, or severe weather can result in clinical disease (Wray and Davies, 2003).

Salmonellosis is manifested in animals in three major forms: enteritis, septicemia, and abortion. However, in an outbreak, or even in a single animal, any combination of the three may be observed (Wray and Sojka, 1977). Fever, inappetence, and depression are commonly observed in acutely ill animals. Enteritis caused by *Salmonella* results in the passage of foul-smelling, watery feces, which may contain fibrin, mucus, and sometimes blood (Wray and Sojka, 1977). When the enteric disease is severe, death may result from dehydration, electrolyte loss, and acid-base imbalance (Clarke and Gyles, 1993). Septicaemic form often leads to abortion. *Salmonella* Dublin has been associated with outbreaks of abortion in cattle, and several other serovars adapted to animal hosts have a particular association with abortion (Wray and Sojka, 1977). The signs and lesions are not pathognomonic and in many cases, especially in poultry and pigs, *Salmonella* infections may be inapparent (OIE, 2000).

2.6. Immunity

According to Wray and Davies (2000) the relative importance of humoral and cell-mediated immunity has been the subject of debate, but it is now generally accepted that both play a part in producing protection. Nevertheless, there is widespread agreement that cell-mediated immunity is more important than humoral antibodies in the resistance to salmonellosis. Cell-mediated immunity has its basis in enhanced microbicidal activity of the host's macrophages for the organism and is not serotype specific. The bactericidal actions of phagocytes supported by the lytic actions of T-killer lymphocytes and activated serum complement generally circumscribe the

non-typhoid infection to a local intestinal manifestation with no systemic involvement (D'Aoust, 1991a). Humoral immunity contributes to bacterial clearance, and is serotype-specific (Gillepsie and Timoney, 1981). Both live and inactivated vaccines have been used to prevent salmonellosis by immunization (Wray and Davies, 2000) but not the infection or carrier status (Acha and Szyfres, 2001). Although many publications attest to the efficacy of live vaccine in experimental trials, few are commercially available (Wray and Davies, 2000). Live vaccine stimulates a greater cell-mediated immune response than bacterins, which primarily promote a humoral response with little or no association with protection. Oral administration (whether bacterins or live vaccine) has the advantage of producing local immunity in the intestine and reducing elimination of salmonellae in feces (Acha and Szyfres, 2001).

2.7. Diagnosis

Diagnosis is based on the isolation of the organism either from tissues collected aseptically at necropsy or from feces, milk, blood, rectal swabs or environmental samples (Acha and Szyfres, 2001; OIE, 2000; Wray and Davies, 2000). When infection of the reproductive organs or conceptus occurs, it is necessary to culture fetal stomach contents, placenta and vaginal swabs and, in the case of poultry, egg contents. However, salmonellosis is particularly difficult to determine in clinically normal carrier animals. Because of the multitude of *Salmonella* serovars serotyping is of great importance. It represents an important prerequisite for the detection of the source of infection and the route of transmission (Mohr and Pollex, 1998).

Salmonellae may be isolated by a variety of techniques, which may include pre-enrichment in non-selective medium to resuscitate sub-lethally damaged salmonellae, enrichment media that contain inhibitory substances to suppress non-*Salmonella* organisms, and selective plating agars to differentiate salmonellae from other Enterobacteriaceae (OIE, 2000; ISO 6579, 1998).

Various biochemical and serological tests can be applied to the pure culture to provide a definitive confirmation of an isolated strain. Salmonellae possess antigens designated somatic (O), flagellar (H) and virulent (Vi), which may be identified by specific typing sera, and the serovar may be determined by reference to the antigenic formulae in the Kauffman-White scheme.

A number of serological tests, such as the serum agglutination test (OIÉ, 2000; Quinn *et al.*, 1994) and indirect enzyme-linked immunosorbent assays have been developed for diagnosis of *Salmonella* (Gabert *et al.*, 1999; OIÉ, 2000). Serological tests are useful for the identification of infected herds but are inadequate for the identification of persistently infected animals (OIÉ, 2000).

Over the last decade, several molecular methods have increasingly become the preferred tools for diagnosis of foodborne pathogens, including *Salmonella*. A molecular method used for diagnosis of *Salmonella* serovars includes polymerase chain reaction (PCR), DNA-DNA hybridization (Southern blot) and finger printing (genotyping) (Gebreyes, 2003). PCR has been one of the most promising new methods shown to be well suited for both rapid and sensitive detection of *Salmonella* contamination in various foods (Oliveira *et al.*, 2002; Ferretti *et al.*, 2001). Methods based on genotyping tend to have a high discriminatory power and offer rapid and sensitive subtyping, complementing conventional approaches that are based on phenotypes, for example, serotyping and phage typing (Gebreyes, 2003).

2.8. Treatment and Control

2.8.1. Treatment

Nursing care is the principal treatment for the enteric form of salmonellosis. Treatment of the systemic form includes nursing care and appropriate antimicrobial therapy as determined by retrospectively acquired susceptibility data. Treatment options may be compromised due to acquisition of resistance (R) plasmids encoding resistance to multiple antibiotics (Hirsh, 1999; D'Aoust 1989). In man, antibiotics are not indicated in salmonellae gastroenteritis, except in very young and those over 60 (Davis *et al.*, 1980) and to individuals with severe invasive infection (Lesser and Miller, 2001; Fey *et al.*, 2000; Lee *et al.*, 1993), since the disease is brief and limited to the gastrointestinal tract. In addition, the unnecessary use of antibiotics prolongs salmonellae excretion, promotes the incidence of the carrier state, and favors the acquisition of resistance by the infecting strain (Hohmann, 2001; D'Aoust, 1991a; Baird-Parker, 1990; D'Aoust, 1989; Davis *et al.*, 1980).

2.8.2. Control

The control of *Salmonella* in meat animals and derived products is a most challenging task because of the complexity and interdependence of various aspects of animal husbandry, slaughtering, and food processing (D'Aoust, 1989). Because of the complexity of *Salmonella* virulence factors, little progress has been made in converting the available knowledge into therapeutics. Good Agricultural Practices (GAP), Good Manufacturing Practices (GMP), Hazard Analysis Critical Control Point (HACCP), system appropriate food handling, and adequate water treatment remain the best preventive measures for most *Salmonella* infection, although the typhoid vaccines are effective against *S. Typhi* in humans, and vaccines for several other serovars have shown promise in food animals (IFT, 2003; Seifert, 1996).

In order to record marked reductions in the prevalence of *Salmonella* in food coordinated control efforts at various critical points from the farm to the “table” is essential. Additionally, comprehensive educational programs for the consumer and food handler, both in commercial establishments and in the home, about correct cooking and refrigeration practices for foods of animal origin, and about personal and environmental hygiene is of paramount importance (IFT, 2003; Acha and Szyfres, 2001; D'Aoust, 1989; WHO, 1988; Schnurrenberger and Hubbert, 1981). Veterinary meat and poultry inspection and supervision of milk pasteurization and egg production are important for consumer protection (Acha and Szyfres, 2001). The detection of personnel who are carriers of *Salmonella*, through regular stool culture is important to the food industry because it may prevent future contamination of products (Guthrie, 1992). Adequate nutrition and hygiene prior to and during transport and within the abattoir could be an important intervention to prevent spread of salmonellosis (Wray and Davies, 2003).

2.9. Public Health Significance

All strains of *Salmonella* are known to infect man and many other animal species (Guthrie, 1992).

2.9.1. Source of infection

Infection and epidemics are usually traceable to various food products derived from meat, eggs, milk, and poultry. Other means of infection derive from food and water contaminated with wild living birds and rodent feces, from infected food handlers, and from contaminated equipment and utensils. A more common hazard arises through cross-contamination from raw to cooked meat or other foods, and subsequent time – temperature abuse (ICMSF, 1986). Sporadic cases occur from direct contact with an infected animal or person (Carter and Chengappa, 1986). This is investigated for developed countries where consumers have no chance of contact with the animals from which they feed. The higher the number of contacts, the higher will be the risk of direct transmission. Epidemiological surveillance of animals, including birds, is of the utmost importance, since the source of the large majority of nontyphoid salmonellosis cases is food of animal origin. There are no sufficient data from developing countries in this regard (Acha and Szyfres, 2001; WHO, 1988). On the other hand workers in food processing or production plants once infected with *Salmonella* are also sources of infection. In such case, regular stool culture to assure that the workers are not shedders of this pathogen is essential. If this pathogen reaches the hands of the workers, then they are potentially dangerous to the products, because of the likelihood of transfer from hands to foods (Guthrie, 1992).

2.9.2. Occurrence in man

Salmonellosis occurs both in sporadic cases and outbreaks affecting a family or several hundreds or thousands of people in a population (Acha and Szyfres, 2001). The disease in human occurs in a wide variety of forms presenting a broad clinical spectrum (Guthrie, 1992). The true incidence is difficult to evaluate, since many countries do not have an epidemiological surveillance system in place, and even where a system does exist, mild and sporadic cases are not usually reported. In

countries with a reporting system, the number of outbreaks has increased considerably in recent years; this increase is in part real and in part due to better reporting (Acha and Szyfres, 2001).

In a large number of cases salmonellosis in man is recognized as a type of food poisoning, the severity of which can vary from mild diarrhea to acute gastroenteritis (Andrews and Walton, 1977). Gastrointestinal salmonellosis and its serious sequelae are linked to a wide variety of illnesses and therapies that affect the body's multiple defenses against enteric and intracellular pathogens. Gastric hypoacidity in infants, in pernicious anemia, or caused by antacids may predispose individuals to salmonellosis (Hohmann, 2001). The disease may also occur as a focal infection in any organ of the body, or as a systemic febrile infection (Guthrie, 1992). Salmonellosis is more common in human lower socio-economic groups (Schnurrenberger and Hubbert, 1981).

2.9.3. The disease in man

Salmonellosis is perhaps the most widespread zoonosis in the world. The nontyphoid salmonellae serovars differ in their degree of human pathogenicity with *S. Pullorum* and *S. Gallinarum* being among the least pathogenic, and *S. Choleraesuis*, *S. Dublin*, and *S. Enteritidis* are being the most pathogenic (Jay, 2000) and often invasive with sustained bacteremia (Lesser and Miller, 2001; Baird-Parker, 1990). Salmonellae of animal origin cause an intestinal infection in man characterized by a 6 to 72 hour incubation period after ingestion of the implicated food, and sudden onset of fever, myalgias, headache, and malaise. The main symptoms consist of diarrhea, vomiting, abdominal pain, and nausea (Acha and Szyfres, 2001). Gastroenteritis caused by nontyphoidal *Salmonella* is usually self-limited (Lesser and Miller, 2001; D'Aoust, 1991a). Diarrhea resolves within 3 to 7 days and fever within 72 hours. Stool cultures remain positive for 4 to 5 weeks after infection and, in rare cases of chronic carriage (<1%), remain positive for more than 1 year (Lesser and Miller, 2001). Conversely, the carrier state is persistent in infection due to *S. Typhi* or paratyphoid salmonellae. Up to 5% of patients with nontyphoidal *Salmonella* gastroenteritis have positive blood cultures, and 5 to 10% of these bacteremic persons develop localized infections (Lesser and Miller, 2001). Although salmonellosis may occur in persons of all ages, incidence is much higher among children and the elderly (D'Aoust, 1991a). Dehydration can be serious (Acha and Szyfres, 2001).

In the developing world, HIV infection is a prominent risk factor for nontyphoidal salmonellosis and bacteremia. In recent series of HIV-infected African adults with documented blood stream infections, nontyphoidal *Salmonella* spp. were isolated from up to 35% of adults (Mycobacteriae were most commonly isolated) (Hohmann, 2001). Salmonellosis usually heals without complications and the only treatment recommended is rehydration and electrolyte replacement (Acha and Szyfres, 2001; D'Aoust, 1997; Guthrie, 1992). A small proportion of patients, particularly those weakened by other diseases (AIDS, neoplasia's, diabetes, etc.), neonates and the elderly can suffer from bacteremia. Antibiotic therapy should be used in such patients (Lesser and Miller, 2001; Guthrie, 1992). Although rare, there may also be different localizations such as infections of joints, lungs, pleura, endocardium (Acha and Szyfres, 2001; Lesser and Miller, 2001), abdominal organs, central nervous system, bone, urinary and genital tracts (Lesser and Miller, 2001). The average mortality rate is 4.1%, varying from 5.8% during the first year of life, to 2% between the first and 50th year, and 15% in persons over 50. Among the different serotypes of *Salmonella*, *S. Choleraesuis* has been reported to produce the highest mortality (21%) (Jay, 2000).

2.10. Economic implications

Salmonellosis, a common human intestinal disorder primarily caused by *Salmonella*-contaminated meat and poultry, is estimated to cost nations billions of dollars annually thereby draining funds that could have been used for development (Radostits *et al.*, 1994; WHO, 1988).

The economic losses associated with *Salmonella* infections have attracted increasing attention in developed countries in recent years, in particular in the United States, Canada and Europe, although the impact may well be greater in developing countries for which little data is available (Socket, 1991). Financial costs are not only associated with investigation, treatment and prevention of human illness but may affect the whole chain of food production. Thus, the costs of salmonellosis, as with other foodborne illness, fall into both the public and private sectors and may be surprising, both in terms of the level of costs incurred and the variety of areas affected. In the public sector, resources may be diverted from preventative activities into the treatment of patients and investigation of the source of infection. In the private sector considerable financial burdens may be imposed on industry in general and on the food industry in particular and last but

not least, on the affected individual and his or her family (Sockett, 1991). The number of deaths from foodborne disease like salmonellosis is likely to be underestimated (Helms *et al.*, 2003) as most estimate of mortality are short term and do not take into account coexisting illnesses. Infections with *Salmonella* were associated with increased long-term mortality (Helms *et al.*, 2003). Costs of individual outbreaks can be very high and may range from thousands to millions of dollars depending on the type of outbreak implicated (Sockett, 1991). Chronic disease sequels, which include reactive arthritis and malabsorption syndrome, require costs for treatments over short or extended period of time (Radostits *et al.*, 1994; Sockett, 1991; WHO, 1988). In the USA alone, nontyphoidal salmonellosis is estimated to be responsible for the deaths of more than 500 people each year, with costs of \$1.1billion to \$1.5 billion annually (Mead *et al.*, 1999).

Salmonella infection has substantial financial and social impacts. Medical costs and lost production are examples of tangible costs which are easily measured in monetary terms, while costs such as loss of leisure and pain and discomfort are intangible costs difficult to measure (Sockett, 1991). Costs to society include costs of illness which fall directly on the ill persons and their immediate family; costs to the national economy which relate to sickness absence from work; and cost to producers, manufacturer or retailer when food products are implicated in food poisoning outbreak (Sockett, 1991).

Salmonellosis is a significant cause of economic loss in farm animals because of the costs of clinical disease that include deaths in a small proportion of cases, decreased milk and meat production, reduced value of contaminated products, diagnosis and treatment of clinical cases, diagnostic laboratory costs, the costs of cleaning and disinfections, and the costs of control and prevention (Radostits *et al.*, 1994).

2.11. Antimicrobial Resistance

During the past decade, bacteria that cause human disease have developed resistance to many of the antibiotics commonly used for treatment (Witte, 1998).The incidence of zoonotic foodborne *Salmonella* infection has increased in most industrialized countries (Mølback *et al.*, 1999). In recent years, testing of *Salmonella* isolates has shown that an increasing proportion of isolates are resistant to several antimicrobial agents both in developing and developed countries. The strains

of *S. Typhimurium* known as definitive phage type 104 (DT 104) have become a worldwide health problem causing illness in humans and animals. It is usually resistant to five drugs: ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracyclines (White *et al.*, 2001; Hohmann, 2001; Lesser and Miller, 2001; Cloeckaert *et al.*, 2000; Mølbak *et al.*, 1999; Tollefson *et al.*, 1998; Glynn *et al.*, 1998). A report from the United Kingdom suggests that infections caused by this five-drug-resistant *S. Typhimurium* might be associated with greater morbidity and mortality than other *Salmonella* infections (Lesser and Miller, 2001; Wall *et al.*, 1994). Other serovars, which have been reported to develop single, or multiple resistances to antibiotics are the Dublin, Gallinarum, Choleraesuis, Newport, Saintpaul, Enteritidis, Infantis, Heidelberg, Derby, and Sandiego serovars (Guthrie, 1992). Antimicrobial resistance can be spread throughout populations by epidemic spread of a particular isolate or through exchange of genetic material. The emergence of multidrug resistance (MDR) *Salmonella* at increasing frequency limits therapeutic options both in humans and animals (Gebreyes *et al.*, 2000).

The reservoir for most nontyphoidal *Salmonella* in developed countries like USA is food producing animals, and therefore the emerging resistance in *Salmonella* is largely a consequence of the use of antimicrobial agents in animals (Acha and Szyfres, 2001; Hohmann, 2001; Olsen *et al.*, 2001; Gorbach, 2001) as well as the indiscriminate prescription-drug treatment of people and animals (Acha and Szyfres, 2001). In the developing world, nosocomial and community-acquired multidrug-resistant salmonellosis have been recurrent problems. The origins of resistance in the developing world are unknown (Olsen *et al.*, 2001). Although the wisdom and merits of subtherapeutic (prophylactic) and growth-promoting antibiotic regimens in animal husbandry remain a highly controversial issue, recent evidence suggest that such treatments facilitate the emergence and persistence of drug-resistant *Salmonella* strains and other foodborne bacterial pathogens in farm animals and derived meat products (Gorbach, 2001; D'Aoust, 1989). Moreover, the magnitude of the problem is amplified by the current inability of slaughtering plants to effect significant pathogen reductions on meat carcasses and to eliminate potential sources of product cross-contamination within the plant environment (D'Aoust, 1989; WHO, 1988). Resistance in *Salmonella* limits the therapeutic options available to veterinarians and physicians in the treatment of certain cases of salmonellosis (Witte, 1998).

Drug resistance in *Salmonella* increases the frequency and severity of infection with this pathogen, limits treatment options, and raise health care costs. These effects may be related to

enhanced shedding and augmented virulence of resistant strains, increased rates of transmission of this strain, and the ineffectiveness of initial regimens of antimicrobial therapy against such strains (Gorbach, 2001). Inappropriate antibiotic treatment of a septicemic patient infected with a resistant *Salmonella* strain could lead to a fatal outcome if the therapeutic drug administered upon hospitalization subsequently proved to be identical to the resistance phenotype. Such an occurrence would likely stem from the antibiotic-dependent inactivation of resident microflora in the intestinal tract and from the rapid colonization, growth, and dissemination into deeper tissues of antibiotic-resistant salmonellae to a point beyond therapeutic management (D'Aoust, 1994; D'Aoust *et al.*, 1992). In the countries of the developing world, which are responsible for about 25% of world meat production, policies regulating veterinary use of antibiotics are poorly developed or absent (Witte, 1998). In developing countries, the principal cause of the emergence of multi-resistant *Salmonella* strains may be self-medication, made possible by the public's easy access to antibiotics without prescription (Acha and Szyfres, 2001). Recent increases in the rates of human isolation of chloramphenicol, ampicillin, and trimethoprim-sulfamethoxazole-resistant *Salmonella* have necessitated a major shift in the antibiotic treatment of human systemic salmonellosis and the sanitation of chronic carriers (D'Aoust, 1997).

3. MATERIALS AND METHODS

3.1. Study design

3.1.1. Study site

A cross-sectional survey of *Salmonella* in food items and personnel working in Addis Ababa markets was undertaken for six months between September 2003 and February 2004.

Addis Ababa is the capital city and administration center for the Federal Democratic Republic of Ethiopia. Currently there are 10 “*Kifle Ketemas*” in Addis Ababa city administration delineated on the basis of geographical set up, population density, asset and service providers’ distribution and convenience for administration (AACA, 2004). Addis Ababa is situated at latitude of 9°3’ North and 38°43’ East (ILCA, 1994). It lies in the central highlands of Ethiopia at an altitude of 2500 m.a.s.l. It has an average rainfall of 1800 mm per annum. The annual average maximum and minimum temperature is 26 °C and 11 °C, respectively; with an overall average of 18.7 °C. Highest temperatures are reached in May. The main rainy season extends from June to September. Addis Ababa has a relative humidity varying 70% to 80% during the rainy season and 40% to 50% during the dry season. The human population is estimated at about 3 million inhabitants (NMSA, 1999).

3.1.2. Addis Ababa abattoir and origin of study samples

Addis Ababa slaughterhouse serves people residing in and the surrounding of Addis Ababa. At Addis Ababa abattoir cattle, sheep, goats and swine are slaughtered. Animals for slaughter are derived from different regions of the country. Daily slaughtered animals are 700 cattle, 250 sheep, and 75 goats. About 50 swine’s are slaughtered per week. On average 153,000 cattle, 39,000 sheep, 3,200 goats and 750 swine are slaughtered annually. The sources of swines are Addis Ababa, Debre Zeit and Ziway swine farms. In the slaughterhouse three separate sections for slaughter of cattle, shoats and pigs are available.

The study was conducted on meat samples derived from apparently healthy slaughtered cattle, sheep, pig, chicken, and fish originating from the different corners of the country. Mutton and beef originate predominantly from indigenous sheep and zebu cattle (*Bos indicus*), respectively. Most of these cattle are steers at various stages of fattening, however, there are also some females which are unfit for breeding or have finished their breeding age. Animals are brought to the slaughterhouse either on hoof or truck. Veterinary professionals assigned by the government undertake regular meat inspection activities. It is expected that butchers and supermarkets in Addis Ababa get meat for sale from this slaughterhouse. The city municipality arranges transportation facilities along workers. The chicken meat originate from exotic commercial chickens raised under intensive management in and around Addis Ababa including Debre Zeit and few local chicken managed extensively, while that of the fish from rift valley lakes mainly from Ziway. The origin of cottage cheese was from local and exotic animals around Addis Ababa and regional states. The ice creams were made by the retail shops themselves or brought from elsewhere. Supermarket employees who volunteered to provide stool samples were also part of the study.

3.1.3. Study type

A cross-sectional survey of randomly selected supermarkets, out of the 42 supermarkets in Addis Ababa, was undertaken. The prevalence, distribution and antimicrobial susceptibility of *Salmonella* from purchased food items (minced beef, mutton, pork, chicken, fish, cottage cheese, and ice creams) and stool samples from apparently healthy personnel were determined following aseptic sampling techniques. First, the list of all the currently operational supermarkets registered in Addis Ababa was collected from the city municipality (sampling frame) and then using a simple random sampling technique the study supermarkets were identified. From these study supermarkets a simple random sample of food items were collected between September and February 2004. Stool samples were collected from voluntary and accessible supermarkets included in the study.

3.1.4. Sample size

The study involved a total of 1200 samples consisting of 194-pork meat, 142 minced beef, 212 mutton and 208 dressed chicken carcasses from supermarkets. Additionally 128 *Tilapia* fish meats, 190 cottage cheese, and 126 ice cream samples were collected from randomly selected fish shops, open markets and pastry shops in Addis Ababa, respectively. A sampling unit consisting of 100 g of food items were taken at random within a lot, 25 g portions (analytical units) were subsequently removed from each sample unit for analysis (ICMSF, 1986). Stool samples from 68 personnel working in the supermarkets were collected.

3.1.5. Study methodology

3.1.5.1. Sample collection and transportation

The meat samples were purchased randomly as sold for consumers (usually in a refrigerated display cases at 4 °C) from 32 randomly selected supermarkets, while cottage cheese, *Tilapia* fish, ice cream samples were purchased from 3 open markets, 6 fish shops and 17 pastry shops in Addis Ababa, respectively, between September 2003 and February 2004 (Appendix 2.1, 2.2 and 3). Out of the 93 supermarket workers sixty-eight personnel working in 22 supermarkets gave their stool sample for analysis during the study period. For collection of stool samples, sterile labeled containers with spoons were distributed for those who volunteered so that they could place their stool carefully and submit it immediately. Stool sample collection was done in collaboration with medical professionals. Twenty-five individuals working in 10 different supermarkets did not participate in the sampling mainly because they were not willing and some were not available for various reasons (e.g., leave). Out of thirty-two supermarkets enrolled for the study, two terminated selling after one visit while one became not cooperative to sell the small amount required for the study.

Samples were properly identified by date of collection, sources (supermarket, open market, pastry and fish shops) and sample type (Appendix 2.1 and 2.2). They were immediately transported in icebox to the Microbiology Laboratory of the Faculty of Veterinary Medicine, Addis Ababa University, Debre Zeit, which is 47 km from Addis Ababa on the main asphalt road

leading to Eastern and Southern parts of Ethiopia. Upon arrival, the samples were stored overnight in a refrigerator at 4 °C or frozen until examined the next day.

3.1.5.2. Bacteriological Examination

3.1.5.2.1. Culture methods

The technique recommended by the International Organization for Standardization ISO 6579 (1998) was employed in order to isolate and identify *Salmonella* organisms. Overnight frozen samples were allowed to thaw for 3 – 5 hours at room temperature before analysis. The bacteriological media used for the study were prepared following the instructions of the manufacturers (Appendix 3). For chicken carcass, sub samples from 5 different parts (breast, wing, thigh, neck and back skin and muscles) were cut, pooled together, mixed thoroughly and the appropriate analytical units (25 g) withdrawn. For other food items also 25 g was taken. This was aseptically cut into smaller fine pieces with sterile scalpel blades. Each sample was put in a sterile stomacher bag and 225 ml of buffered peptone water (BPW, Sifin, Germany) was added, and homogenized using a laboratory blender (Stomacher 400, Seward, England) for 2 minutes. Stool samples from personnel were pre-enriched in BPW in a ratio of 1 g of sample to 9 ml of BPW. The pre-enriched samples were incubated for 16 to 20 hours at 37 °C. Following this, 1 ml and 0.1 ml aliquot of the pre-enrichment broths was transferred aseptically into 10 ml of selenite cystine (SC) and 10 ml of Rappaport-Vassilliadis (RV) broth, mixed and then incubated for 18 to 24 hours at 37 °C and 42 °C, respectively. Following incubation, a loopful of each culture was streaked onto one plate of brilliant green-phenol red-lactose-sucrose agar (BPLS, SIFIN, Germany) and another plate of xylose lysine desoxycholate (XLD, SIFIN, Germany) medium and incubated at 37 °C for 24 to 48 hours. The plates (BPLS and XLD) were examined for the presence of *Salmonella* colonies. Most salmonellae give an alkaline reaction in BPLS medium and have red colonies. On XLD medium the majorities of *Salmonella* serotypes produces hydrogen sulphide and have red colonies with a black (H₂S) center (Quinn *et al.*, 1994). If growth was slight or if typical colonies of *Salmonella* were not present the plates were re-incubated for a further 18 to 24 hours and re-examined for the presence of typical *Salmonella* colonies. A single positive colony showing red color with a black center on XLD and red color on BPLS agars was further examined using recommended serological tests after growing overnight on nutrient agar at 37 °C. Rambach (Merck, Germany) agar was used following biochemical testing as a confirmatory test based on the red colony color typical for *Salmonella*.

3.1.5.2.2. Biochemical tests

All suspected non-lactose fermenting *Salmonella* colonies were picked from the nutrient agar and inoculated into the following biochemical tubes for identification: triple sugar iron (TSI) agar, lysine decarboxylate broth, Simmon's citrate agar, urea broth, tryptone water, MR-VP broth and incubated for 24 or 48 hours at 37 ° C. Colonies producing an alkaline slant with acid (yellow color) butt on TSI with hydrogen sulphide production, positive for lysine (purple color), negative for urea hydrolysis (red color), negative for tryptophan utilization (indole test) (yellow-brown ring), negative for Voges-Proskauer, and positive for citrate utilization were considered to be *Salmonella*-positive (Appendix 4) (ISO 6579, 1998; Quinn *et al.*, 1994).

3.1.5.2.3. Serology

Positive colonies of putative *Salmonella* organisms were further tested for agglutination by rapid slide agglutination test using *Salmonella* polyvalent O antiserum "I" and "II" (Sifin, Berlin, Germany) in accordance with the manufacturer's instructions and ISO 6579 (1998) recommendations. Briefly, some bacterial mass from suspected colonies grown on nutrient agar was mixed with a drop of polyvalent antiserum I on a carefully cleaned glass slide so as to get a homogenous and turbid suspension. Positive results were recorded if agglutination occurred within 1 to 20 times shaking against dark background or reactions observed using magnifying glass. In order to exclude any spontaneous agglutination (auto agglutination) a negative control using physiological saline solution and bacterial colony to be tested was done. *Salmonella* polyvalent antiserum II was used when suspected bacteria fail to agglutinate with *Salmonella* polyvalent O antiserum I.

3.1.5.2.4. Serotyping and phage typing

Those isolates tentatively identified as *Salmonella* were sub-cultured on brain-heart infusion agar and sent to Health Canada, Office International des Epizooties (OIE) Reference Laboratory for Salmonellosis in Guelph, Ontario, Canada for complete serotyping, and phage typing. A sample was considered positive if *Salmonella* was confirmed by serotyping. The somatic (O) antigens of the *Salmonella* isolates were determined by slide agglutination test as described by Ewing (1986).

The flagellar (H) antigens were identified using a micro-technique (Shipp and Rowe, 1980) that employs microtitre plates. The antigenic formula of Le Minor and Popoff (1997) was used to name the serovars. The standard phage typing technique described by Anderson and Williams (1956) was employed throughout. The phage typing scheme and phages for *S. Typhimurium* developed by Callow (1959) and further extended by Anderson (1964) and Anderson *et al.* (1977) were obtained from the Central Public Health Laboratory, Colindale, London and United Kingdom.

3.1.5.2.5. Antimicrobial susceptibility testing

The antimicrobial susceptibility test of all isolates of *Salmonella* was assayed in the Food Microbiology Laboratory, Laboratory Service Division, Animal Health Laboratory, University of Guelph, Guelph, Ontario; Canada. The National Committee for Clinical Laboratory Standards (NCCLS) (1999) guidelines was followed throughout the agar dilution testing procedure and interpretation of results as susceptible and resistant. Briefly, the isolates were grown to 0.5 – 1.0 McFarland density in Muller Hilton (MH) broth (Difco, Detroit, USA) and replica plated using a Cathra Replicator (Brown and Washington, 1978) on to MH agar plates (Difco, Detroit, USA). The list of panel of antimicrobials utilized, their symbols and concentrations to classify an isolate as susceptible or resistant were shown on Table 1. To determine resistance to florfenicol, Aquaflor[®] containing 50% florfenicol was dissolved in dimethylformamide and added to stock solution containing 20 mg/ml Aquaflor to MH agar to obtain plates with 16 and 32 µg/ml of florfenicol. An isolate was defined as resistant if it was resistant to one or more of the antimicrobial drugs tested whereas multiple resistance was defined as resistance to two or more antimicrobial drugs. Standard and reference strains, which include *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 29213 and *Enterococcus faecalis* ATCC 29212 were used following the recommendations of NCCLS (1999).

Table 1. Antimicrobials and concentrations used to test susceptibility of *Salmonella* isolates

Antimicrobial	Abréviations	Break points and Concentrations ^b	
		Susceptible at ≤µg/ml	Resistant at ≥µg/ml
Amikacin	AMK	16	ND ^c
Ampicillin	AMP	ND	32
Amoxicillin/clavulanic acid	AMC	ND	64/16 ^d
Apramycin	APR ^{e,f}	ND	32 ^g
Carbadox	CRB ^{e,f}	ND	30 ^h
Cephalothin	CEF	ND	32
Ceftriaxone	CRO	8	ND
Ceftiofur	CTF ^e	ND	8
Cefoxitin	FOX	ND	32
Chloramphenicol	CHL	ND	32
Ciprofloxacin	CIP	0.125 ⁱ	ND
Florfenicol	FLO ^{e,f}	ND	16 ^j
Gentamycin	GEN	ND	16
Kanamycin	KAN	ND	64
Nalidixic acid	NAL	ND	32
Neomycin	NEO ^{e,f}	ND	16 ^g
Nitrofurantoin	NIT	ND	64 ^k
Spectinomycin	SPT ^f	ND	64 ^g
Streptomycin	STR ^f	ND	32 ^g
Sulfisoxazole	SUL ^e	ND	512
Sulfamethoxazole/trimethoprim	SXT	ND	76/4
Tetracycline	TET	ND	16
Tobramycin	TOB	ND	8
Trimethoprim	TMP	ND	16

^b The breakpoint concentrations to determine susceptible and resistance were those specified by the NCCLS standards M31-A and M100-S12.

^c ND - not done.

- ^d The strains were considered resistant when growing on agar plates with amoxicillin/clavulanic acid at 64/16 µg/ml.
- ^e The abbreviations APR, CRB, CTF, FLO, NEO and SUL were self-chosen.
- ^f There are no interpretive standards specified by the NCCLS standards M31-A or M100-S12 for apramycin, carbadox, florfenicol, neomycin, spectinomycin and streptomycin.
- ^g Strains were considered to be resistant to apramycin, neomycin, spectinomycin and streptomycin at 32, 16, 64, and 32 µg/ml, respectively.
- ^h The strains were considered to be resistant to carbadox, a veterinary growth promoter for pigs, at 30 µg/ml
- ⁱ A 0.125 µg/ml of ciprofloxacin concentration determines reduced sensitivity to ciprofloxacin.
- ^j Strains were considered to be resistant to florfenicol at the level of 16 µg/ml.
- ^k Strains were considered to be resistant to nitrofurantoin at 64 µg/ml; human urinary tract isolates are considered to be resistant to nitrofurantoin at 128 µg/ml (NCCLS, M100-S12).

3.1.5.3. Data management and analysis

Microsoft Excel was employed for data entry, computation of descriptive statistics and drawing graphs. Descriptive statistics such as percentage, proportion, range, median, and mean were applied to compute some of the data. Prevalence was defined as the proportion of food items / personnel/ positive for *Salmonella* by bacteriological examination divided by the total number of food items / personnel examined. It was generated using the procedure of frequency (FREQ) and expressed in percent. The Pearson's chi-square test was used to determine the significance of difference or variation of prevalence and also to see whether *Salmonella* prevalence in Addis Ababa supermarkets was associated with the size of supermarkets. Fisher's exact test was used to see the significance of antimicrobial resistance between food items. Correlation analysis was used to see associations of *Salmonella* serotypes between food items and personnel handling them. A difference will be statistically significant if the P-value is less than 0.05. All statistical analysis was performed using Intercooled Stata 6.0 soft ware package.

4. RESULTS

4.1. Description of markets and shops

All the meat samples sold in the randomly sampled supermarkets included in the current study were sold either deep frozen (mostly for chicken and pork) or refrigerated (mutton and minced beef). All supermarkets possessed mincing machine to be used mainly for beef.

Cottage cheese originating from the vicinity of “*Legedadi*”, “*Sululta*” and “*Butajira*” areas were sold at “*Shola*”, “*Addisu Gebeya*” and “*Markato*” open markets, respectively. All the vendors were females, except one. The vendors displayed their cottage cheese in plastic buckets, polyethylene bags, clay pots, etc., covered with cloth or plastics. Based on visual assessment and experience of the investigator, the hygienic status of the containers and the vendors themselves is questionable and no cooling system was used at all. Majority of the vendors sold their cottage cheese using a jug or ladle while few used balance.

Ice creams of various types were sold in the shops, which used freezers. The hygienic condition at time of selling seemed good. Ice cream made in Addis Ababa was predominantly composed of vanilla, pineapple, banana, orange, straw berry, lemon, chocolate, milk and egg.

Tilapia fish were transported mainly from Lake Ziway to Addis Ababa, “*Atkilt Tera*”, loaded on medium sized trucks without cooling system. All fish selling shops considered in the study used deep freezers to store fish for sale. However, the methods of evisceration and hygienic measures practiced in preparing fillets seemed favorable for cross-contamination from the gut, knife, cutting board and hands of personnel

4.2. Prevalence and distribution

A total of 1268 samples originating from 32 supermarkets, 3 open markets, 17 pastry shops and 6 fish shops were cultured for *Salmonella*. The pathogen was detected from 21 supermarkets (Table 2), one open market and 2 fish shops (Table 3). The frequency of isolation of *Salmonella* varied between supermarkets and it ranged from 0 % to 40 %, with a median of 8% (Table 2).

Dressed chicken carcass samples were obtained from all supermarkets while minced beef, mutton, pork, and stool samples were obtained from 29, 19, 10 and 22 supermarkets, respectively. The results, as can be seen from Table 2, indicated that 50% (16/32), 31% (9/29), 57.9% (11/19) and 70% (7/10) of supermarkets had one or more *Salmonella* positive samples from dressed chicken carcass, minced beef, mutton and pork samples, respectively. Out of 22 supermarkets three supermarkets (13.6%) gave one or two *Salmonella* positive result from apparently healthy supermarket butchery workers. The specific prevalence of *Salmonella* from dressed chicken carcass, pork, mutton and minced beef collected from supermarkets were found to be 13.9%, 11.3% 10.8% and 8.5%, respectively (Table 4). Out of 68 stool samples collected from personnel of 22 supermarkets 7.4% (5/68) were *Salmonella* positive.

From a total of 190 cottage cheese samples collected from 3 open markets located in 3 “*Kifle Ketemas*” in Addis Ababa only 1 open market (33.3%) gave 4 positive samples (2.1 %). A prevalence of 2.3 % of *Salmonella* from *Tilapia* fish meat samples was calculated out of 128 fish samples obtained from 6 fish selling shops found in 2 “*Kifle Ketemas*”. On the other hand, none of the 126 ice cream samples collected from 17 pastry shops located in 6 “*Kifle Ketema*” gave positive results for *Salmonella* (Table 3). Though statistical analysis revealed absence of significant variation in prevalence of *Salmonella* between chicken carcass, pork, mutton and minced beef samples (Pearson’s $X^2 = 2.623$, $df = 3$, $p\text{-value} = 0.454$), chicken and pork samples resulted in relatively higher prevalence rates (13.9 % and 11.3 %, respectively). However, there was a statistically significant difference in the proportion of *Salmonella* isolation between meats (chicken carcass, pork, mutton and minced beef) and the rest of the samples (cottage cheese, fish and ice cream) (Pearson’s $X^2 = 37.569$, $df = 1$, $p\text{-value} = 0.000$).

The prevalence of *Salmonella* varied among the sampling sites / supermarkets, open markets, fish and pastry shops/ and sample type from 0% to 40% (Table 2 and 3). Samples examined from supermarkets 24, 32, 4, 19 and 1 with the prevalence of 40%, 22.7%, 19.8%, 18.2% and 16.5%, respectively, were the most contaminated (Table 2) compared with the other supermarkets accounting for 47.3 % of the isolates from supermarkets.

Table 2. Prevalence and distribution of *Salmonella* from Addis Ababa supermarkets

Supermarket No.	No. of positives/ No. Tested (%)				
	Chicken	Pork	Mutton	Mince beef	Total
1	0/7(0)	6/40 (15)	7/32 (21.9)	1/6 (16.7)	14/85 (16.5)
2	1/8 (12.5)	1/10 (10)	2/10 (20)	1/6 (16.7)	5/34 (14.7)
3	3/6 (50)	-	0/9 (0)	0/5 (0)	3/20 (15)
4	2/7 (28.6)	9/54 (16.7)	3/13 (23.1)	2/7 (28.6)	16/81 (19.8)
5	0/6 (0)	-	-	0/5 (0)	0/11 (0)
6	3/7 (42.9)	1/15 (6.7)	0/16 (0)	3/8 (37.5)	7/46 (15.2)
7	0/6 (0)	1/2 (50)	1/5 (20)	0/4 (0)	2/17 (11.8)
8	1/7 (14.3)	0/12 (0)	1/9 (11.1)	0/5 (0)	2/33 (6.1)
9	0/7 (0)	2/26 (7.7)	1/14 (7.1)	1/5 (20)	4/52 (7.7)
10	0/4 (0)	-	-	0/2 (0)	0/6 (0)
11	0/7 (0)	-	-	0/5 (0)	0/12 (0)
12	2/9 (22.2)	-	1/18 (5.6)	0/4 (0)	3/31 (9.7)
13	0/6 (0)	-	-	0/4 (0)	0/10 (0)
14	2/8 (25)	0/4 (0)	1/6 (16.7)	0/5 (0)	3/23 (13)
15	0/7 (0)	2/25 (8)	1/14 (7.1)	0/6 (0)	3/52 (5.8)
16	0/9 (0)	-	0/20 (0)	0/7 (0)	0/36 (0)
17	0/6 (0)	-	-	1/5 (20)	1/11 (9.1)
18	1/5 (20)	-	0/3 (0)	0/4 (0)	1/12 (8.3)
19	2/7 (28.6)	-	-	0/4 (0)	2/11 (18.2)
20	0/1 (0)	-	-	0/1 (0)	0/2 (0)
21	1/7 (14.3)	-	0/5 (0)	0/5 (0)	1/17 (5.9)
22	0/7 (0)	-	-	-	0/7 (0)
23	2/8 (25)	-	-	0/7 (0)	2/15 (13.3)
24	4/10 (40)	-	-	-	4/10 (40)
25	1/7 (14.3)	-	3/20 (15)	1/6 (16.7)	5/33 (15.2)
26	0/2 (0)	-	-	-	0/2 (0)
27	0/7 (0)	-	-	0/4 (0)	0/11 (0)
28	0/7 (0)	0/6 (0)	0/2 (0)	0/5 (0)	0/20 (0)
29	1/6 (16.7)	-	0/10 (0)	0/5 (0)	1/21 (4.8)
30	0/1 (0)	-	0/1 (0)	0/1 (0)	0/3 (0)
31	1/8 (0)	-	-	1/6 (16.7)	2/14 (14.3)
32	2/8 (25)	-	2/5 (40)	1/5 (20)	5/18 (22.7)
Total	29/208 (13.9)	22/194 (11.3)	23/212 (10.8)	12/142 (8.5)	86/756 (11.4)

Table 3. Prevalence of *Salmonella* from open markets, fish and pastry shops in Addis Ababa

Kifle Ketema	Type of sample	No. Examined	No. Positive (%)
Gulele	Cottage cheese	12	0 (0%)
Addis Ketema	Cottage cheese	76	0 (0%)
Yeka	Cottage cheese	102	4 (3.92%)
Total		190	4 (2.11%)
Arada	Fish	100	3 (3 %)
Kirkos	Fish	28	0 (0%)
Total		128	3 (2.3%)
Bole	Ice cream	35	0 (0%)
Arada	Ice cream	45	0 (0 %)
Yeka	Ice cream	18	0 (0%)
Kirkos	Ice cream	18	0 (0%)
Gulele	Ice cream	3	0 (0%)
Nifassilk Lafto	Ice cream	7	0 (0%)
Total		126	0 (0%)

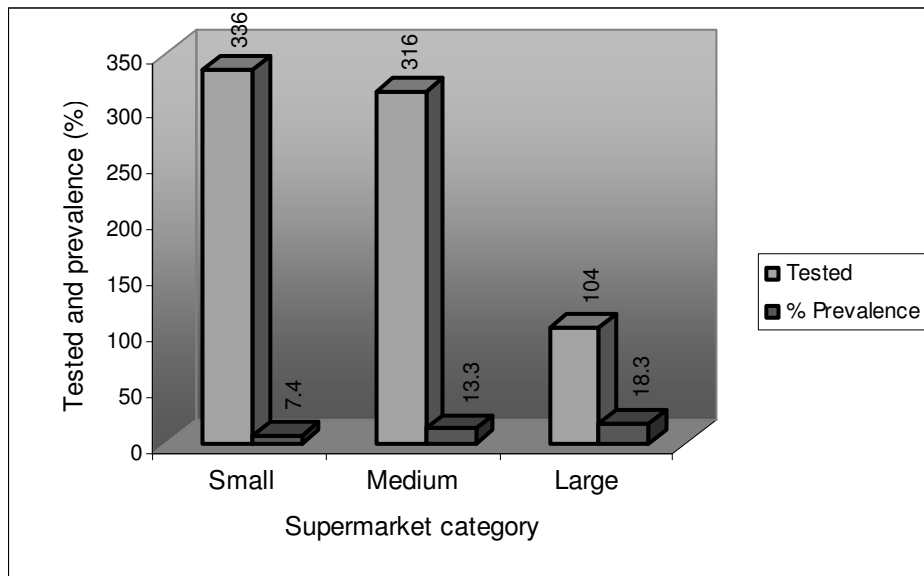
Table 4. Summary of prevalence of *Salmonella* and number of different serotypes detected

Sample type	Number of samples			95 % CI	No. of different serotypes detected
	Exam.	Pos.	%		
Chicken	208	29	13.9	9.54 – 19.40	Newport (4), Braenderup (12), Hadar (6), Typhimurium (3), Kentucky (2), Bovismorbificans (1), Anatum (1)
Pork	194	22	11.3	7.24 – 16.66	Newport (12), Dublin (2), Haifa (5), Infantis (2), Kottbus (1)
Minced beef	142	12	8.5	4.44 – 14.29	Newport (3), Typhimurium (1), Dublin (2), Kentucky (1), Anatum (2), Saintpaul (1), Infantis (1), 1: 9,12:- (1)
Mutton	212	23	10.8	7.00 – 15.83	Newport (12), Typhimurium (3), Dublin (2), Hadar (2), Infantis (1), Bovismorbificans (2), Zanzibar (1)
Cottage cheese	190	4	2.1	0.58 – 5.30	Newport (3), Haifa (1)
Fish	128	3	2.3	0.49 – 6.70	Newport (2), Zanzibar (1)
Ice cream	126	0	0	0 – 2.89*	-
Stool	68	5	7.4	2.43 – 16.33	Newport (5)
Total	1268	98	7.7	6.30 – 9.32	

* One –sided 97.5 % CI, $X^2 = 36.41$, P-Value = 0.000

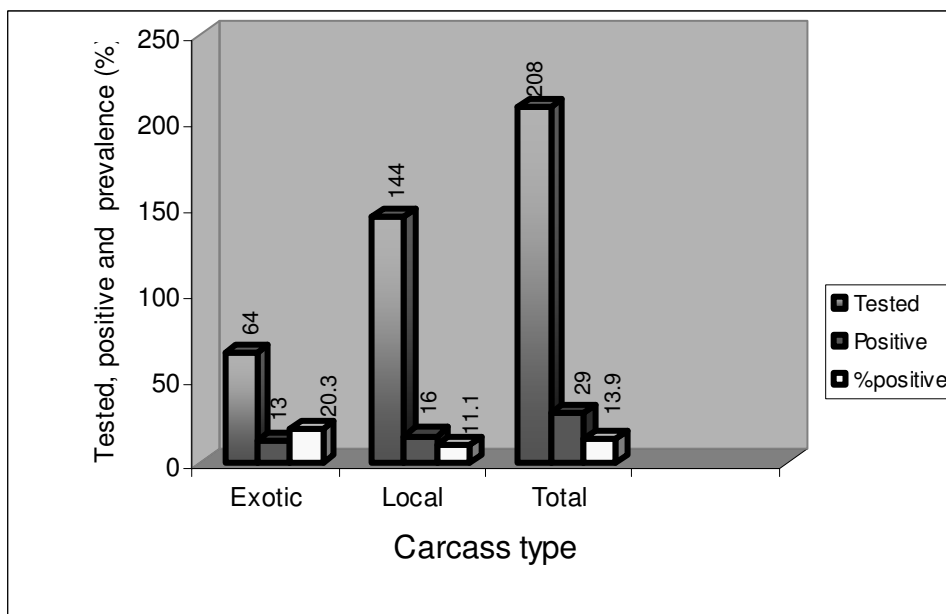
Attempt was made to determine the prevalence of *Salmonella* based on the size of the supermarket classified into large, medium and small on the basis of number of personnel working in the butchery (Appendix 5). The hypothesis behind was that the prevalence of *Salmonella* would be higher from large supermarkets than medium and small, as more personnel and meat contribute for cross-contamination. With respect to supermarket size approximately 18.3%, 13.3% and 7.4% of the samples were positive for *Salmonella* from large, medium and small supermarkets, respectively (Figure 1). There was a statistically significant (Pearson's $X^2 = 11.213$, $df = 2$, p -value = 0.004) difference in the prevalence of *Salmonella* based on supermarket size.

Figure 1. Prevalence of *Salmonella* based on supermarket category



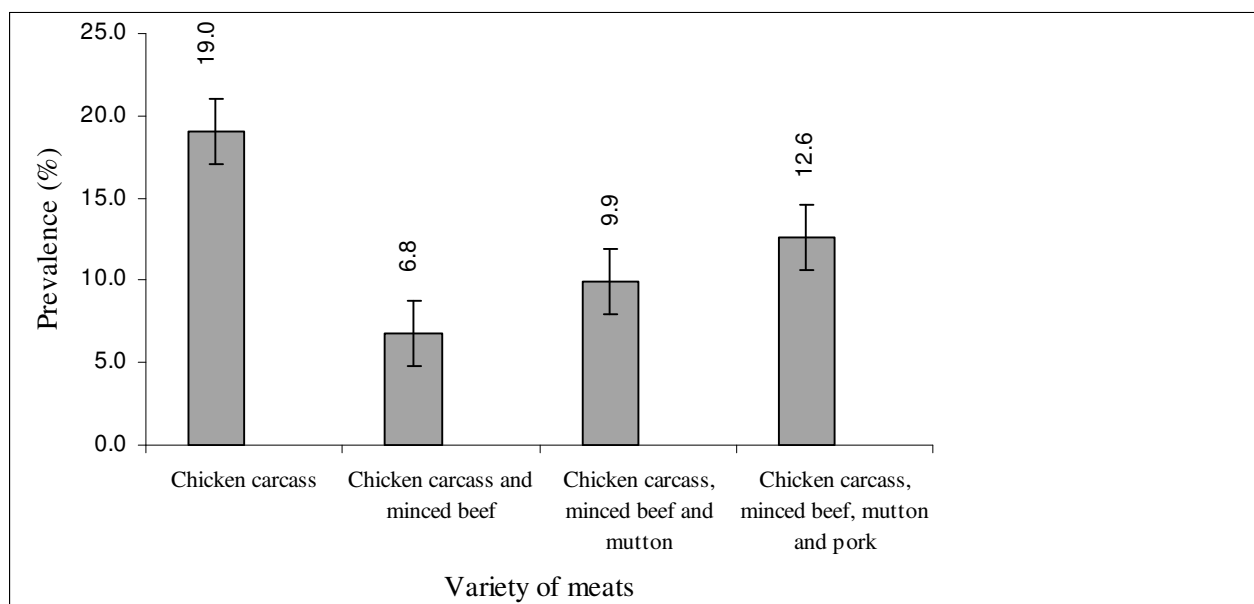
Although equal number of samples was not taken from exotic and local chicken, the result showed a relatively higher *Salmonella* prevalence in exotic than local chicken carcasses (20.3% vs. 11.1%, Figure 2). However, this difference is not statistically significant (Pearson's $X^2 = 3.127$, $df = 1$, $p\text{-value} = 0.077$).

Figure 2. Prevalence of *Salmonella* in exotic and local chicken carcass



Except for chicken carcass (where high prevalence in supermarket No. 24 raised the prevalence), there was a tendency for *Salmonella* prevalence to increase as the variety of meat types retailed increased (Figure 3).

Figure 3. Distribution of *Salmonella* based on meat types sold by supermarkets



4.3. Serotyping and phage typing

From the 98 *Salmonella* isolates presently isolated, 14 different serotypes were recognized. The most prevalent *Salmonella* serotype isolated was *Salmonella* Newport, which comprised 41.8% of the positive samples, followed by *Salmonella* Braenderup, *Salmonella* Hadar, *Salmonella* Typhimurium, *Salmonella* Dublin and *Salmonella* Haifa which accounted for 12.2%, 8.2%, 7.1%, 6.1% and 6.1% of the isolates respectively, Table 5. *Salmonella* serotypes less commonly isolated include, *S. Infantis*, *S. Kentucky*, *S. Bovismorbificans*, *S. Anatum*, *S. Zanzibar*, *S. Kottubus*, *S. Saintpaul* and *Salmonella* 1: 9, 12:- (Table 5). *Salmonella* Braenderup was isolated only from chicken carcass, whereas *S. Kottbus* was isolated only from pork and *S. Saintpaul* and *S. 1:9, 12:-* were both isolated only from minced beef samples.

Eight different serotypes were isolated from minced beef, while seven, five, and two different serotypes were detected from chicken carcass and mutton, pork, cottage cheese and fish samples,

respectively (Table 4). *Salmonella* Braenderup was predominant serotype isolated from chicken carcass comprising 41.4% of the serotypes. *Salmonella* Hadar, *S. Newport*, *S. Typhimurium* were serotypes most commonly isolated from chicken carcass after *S. Braenderup*, comprising 20.7%, 13.8% and 10.3% of the isolates, respectively.

Table 5. Distribution of *Salmonella* serotypes from food items and personnel in Addis Ababa

Serotype	Antigens	Phage type	Chicken	Pork	Mutton	Minced Beef	Fish	Cottage cheese	Ice cream	Stool	Total
<i>Salmonella</i> Newport	6,8:eh:2		4	12	12	3	2	3		5	41
<i>S. Braenderup</i>	6,7:eh:z15		12								12
<i>S. Hadar</i>	6,8:z10:x		6		2						8
<i>S. Typhimurium</i>	4,5:i:2	2	1		3	1					5
<i>S. Typhimurium</i>	4,5:i:2	193	1								1
<i>S. Typhimurium</i>	4,5:i:2	atypical	1								1
<i>S. Dublin</i>	9,12:gp:-			2	2	2					6
<i>S. Haifa</i>	4:z10:2			5				1			6
<i>S. Infantis</i>	6,7:r:5			2	1	1					4
<i>S. Kentucky</i>	8,20:iz6		2			1					3
<i>S. Bovismorbificans</i>	6,8:r:5		1		2						3
<i>S. Anatum</i>	10:eh:6		1			2					3
<i>S. Zanzibar</i>	10:k:5				1		1				2
<i>S. Kottbus</i>	6,8:eh:5			1							1
<i>S. Saintpaul</i>	4:eh:2					1					1
<i>S.1:9,12:-:-</i>	9,12:-:-					1					1
Total			29	22	23	12	3	4	0	5	98

S. Newport (54.5%) was the dominant serotype from pork samples followed by *S. Haifa* (22.7%), *S. Dublin* (9.1%), *S. Infantis* (9.1%) and *S. Kottbus* (4.5%). Among *Salmonella* isolates from mutton, *S. Newport* (52.2%) was dominant followed by *S. Typhimurium* (13%), while *S. Hadar*, *S. Dublin* and *S. Bovismorbificans* each accounted for 8.7% of the mutton isolates.

Overall six *Salmonella* Typhimurium (6.1% of positive samples) were isolated. Among the *S.* Typhimurium isolates, phage type (DT) 2 (4 of 6) was most commonly encountered. This phage type was isolated from three mutton and one minced beef samples. *S.* Typhimurium phage type 193 (DT 193) and atypical, each one from chicken carcass were detected (Table 5).

Out of 21 supermarkets, which gave positive *Salmonella* isolates, 13 (61.9%) supermarkets gave multiple serotypes while only 1 serotype was detected from the other 8 supermarkets.

Table 6. Number of positive sources and samples by *Salmonella* serotypes/serogroup/

Serotype (serogroup)	Positive samples (n=98)		Supermarkets (n=21)		Open market (n=1)		Fish shop (n=2)	
	No.	%	No.	%	No.	%	No.	%
	<i>S.</i> Typhimurium (B)	7	7.1	6	28.6			
<i>S.</i> Haifa (B)	6	6.1	1	4.8	1	100		
<i>S.</i> Saintpaul (B)	1	1	1	4.8				
<i>S.</i> Braenderup (C1)	12	12.2	8	38.1				
<i>S.</i> Infantis (C1)	4	4.1	2	9.5				
<i>S.</i> Newport (C2)	41	41.8	13	61.9	1	100	1	50
<i>S.</i> Hadar (C2)	8	8.2	6	28.6				
<i>S.</i> Bovismorbificans(C2)	3	3.1	3	14.3				
<i>S.</i> Kentucky (C3)	3	3.1	3	14.3				
<i>S.</i> Kottbus (C2)	1	1	1	4.8				
<i>S.</i> Dublin (D1)	6	6.1	4	19				
<i>S.</i> Anatum (E1)	3	3.1	3	14.3				
<i>S.</i> Zanzibar (E1)	2	2	1	4.8			1	50
<i>S.</i> :1:9,12:-*	1	1	1	4.8				

* Not typable

Most of the positive samples belonged to serogroup C2 (54.1%), followed by serogroup C1 (16.3%), B (14.3%), D1 (6.1%), E1 (5.1%) and C3 (3.1%) (Table 6).

4.4. Antimicrobial resistance

Of the 98 *Salmonella* serotypes subjected to antimicrobial susceptibility test, using a panel of 24 different antimicrobials (Table 1), 32 isolates (32.7%) were resistant to one or more of the antimicrobials used. A total of 66 (67.3%) *Salmonella* isolates belonging to *S. Newport*, *S. Typhimurium*, *S. Infantis*, *S. Bovismorbificans*, *S. Anatum*, *S. Zanzibar*, *S. Kottbus*, *S. Saintpaul* and *S. I: 9, 12:-* were found to be susceptible to all antimicrobials tested. However, 32 *Salmonella* isolates (32.7%) belonging to *S. Braenderup*, *S. Hadar*, *S. Dublin*, *S. Haifa* and *S. Kentucky* were resistant to one or more of the 24 antimicrobials tested (Table 7 and 8). All *Salmonella* isolates belonging to *S. Dublin* (isolated from pork, mutton and minced beef) were resistant to carbadox and *S. Haifa* (isolated from pork and cottage cheese) was resistant to ampicillin, streptomycin and tetracycline. About 83% of *S. Braenderup* isolated from chicken carcass and 87.5% of *S. Hadar* isolated from chicken carcass and mutton were also resistant to one or more of the antimicrobials tested. In relation to the total *Salmonella* isolates tested, 24.5% were found resistant to streptomycin, while 19.4%, 15.3%, 13.3% and 13.3% were resistant to ampicillin, tetracycline, spectinomycin and sulfisoxazole, respectively.

With regards to source of the 32 resistant *Salmonella* isolates, chicken carcass accounted for 56.3% (18/32) while pork, mutton, minced beef and cottage cheese accounted for 21.9% (7/32), 9.4% (3/32), 9.4 % (3/32) and 3.1 % (1/32), respectively. All *Salmonella* isolates from personnel and fish were susceptible to all antimicrobials tested (Table 7).

None of the *Salmonella* isolates showed resistance for the following antimicrobials: amikacin, apramycin, ceftriaxone, ceftiofur, cefoxitin, chloramphenicol, florfenicol, kanamycin, neomycin, nitrofurantoin and tobramycin.

Table 7. Distribution of antimicrobial resistant *Salmonella* from food items and personnel in Addis Ababa

Sample type	No. of samples		<i>Salmonella</i> (No.)	Serotypes	No. of resistant isolates
	Examined	Positive (%)			
Chicken carcass	208	29 (13.9)	<i>S. Braenderup</i> (12)		10
			<i>S. Hadar</i> (6)		6
			<i>S. Newport</i> (4)		
			<i>S. Typhimurium</i> (3)		
			<i>S. Kentucky</i> (2)		2
			<i>S. Bovismorbificans</i> (1)		
Pork	194	22 (11.3)	<i>S. Newport</i> (12)		
			<i>S. Haifa</i> (5)		5
			<i>S. Dublin</i> (2)		2
			<i>S. Infantis</i> (2)		
			<i>S. Kottbus</i> (1)		
Mutton	212	23 (10.8)	<i>S. Newport</i> (12)		
			<i>S. Typhimurium</i> (3)		
			<i>S. Hadar</i> (2)		1
			<i>S. Dublin</i> (2)		2
			<i>S. Bovismorbificans</i> (2)		
			<i>S. Infantis</i> (1)		
Minced beef	142	12 (8.5)	<i>S. Zanzibar</i> (1)		
			<i>S. Newport</i> (3)		
			<i>S. Dublin</i> (2)		2
			<i>S. Anatum</i> (2)		
			<i>S. Typhimurium</i> (1)		
			<i>S. Infantis</i> (1)		
			<i>S. Kentucky</i> (1)		1
<i>S. Saintpaul</i> (1)					
Cottage cheese	190	4 (2.1)	<i>S. 1:9,12:-</i> (1)		
			<i>S. Newport</i> (3)		
			<i>S. Haifa</i> (1)		1
Fish	128	3 (2.3)	<i>S. Newport</i> (2)		
			<i>S. Zanzibar</i> (1)		
Stool	68	5 (7.4)	<i>S. Newport</i> (5)		

Out of the 32 resistant *Salmonella* isolates, 23 (23.5%) were multidrug resistant (MDR) (Table 8). The proportion of MDR *Salmonella* isolates varied between sample types being highest in chicken carcass (65.2%, 15/23), cottage cheese (25%, 1/4) and pork 21.7%, 5/23). It is lowest in minced beef (8.3%, 1/12) and mutton (4.3%, 1/23) samples. Among MDR isolates resistance to streptomycin, spectinomycin, sulfisoxazole, ampicillin and tetracycline was most often observed (Table 10). Serotypes isolated from chicken carcass (*S. Braenderup* and *S. Kentucky*) showed resistance pattern for up to ten antimicrobials, while those isolates from pork (*S. Haifa*) showed resistance pattern for up to four antimicrobials. None of the mutton and cottage cheese isolates showed resistance for more than three antimicrobials and only one serotype from minced beef showed resistance for 8 antimicrobials. The most frequent combination of resistance was seen in *S. Braenderup* for the following antimicrobials: ampicillin, spectinomycin, streptomycin, sulfisoxazole, sulfamethoxazole / trimethoprim and trimethoprim. The three *S. Kentucky* serotypes isolated from exotic chicken carcass (one), local chicken carcass (one) and minced beef (one) samples were found to have MDR pattern for 10, 9 and 8 antimicrobials, respectively. Although 12 different antimicrobial resistance patterns were seen in this study, the two most common resistance patterns were Amp Spt Str Sul Sxt Tmp (9 isolates from chicken) and Amp Amc Str Tet (4 isolates from pork). Resistance to trimethoprim and sulfamethoxazole / trimethoprim was seen only in *S. Braenderup* and *S. Kentucky* isolated from chicken carcass while to carbadox was seen only among *S. Dublin* isolates from pork, mutton and minced beef (Table 8 and 9).

Table 8. Antimicrobial resistance of *Salmonella* isolates by serotype

Serotype	No. of strains			Resistance Pattern (number of strains)
	Tested	R (%)	MR (%)	
<i>S. Newport</i>	41	-	-	- Amp Spt Str Sul Sxt Tmp (9)
<i>S. Braenderup</i>	12	10 (83.3)	10 (83.3)	Amp Amc Spt Str Sul Sxt Tmp (1) Str Tet (3) Str (2)
<i>S. Hadar</i>	8	7 (87.5)	3 (37.5)	Tet (2)
<i>S. Typhimurium</i>	7	-	-	- Crb Tet (1)
<i>S. Dublin</i>	6	6 (100)	1 (16.7)	Crb (5) Amp Amc Str Tet (4)
<i>S. Haifa</i>	6	6 (100)	6 (100)	Amp Str Tet (2)
<i>S. Infantis</i>	4	-	-	- Amp Amc Cef Cip Gen Nal Spt StrSulTet (1) Amp Amc Cef Cip Nal Spt Str Sul Tet (1)
<i>S. Kentucky</i>	3	3 (100)	3 (100)	Amp Amc Cip Nal Spt Str Sul Tet (1)
<i>S. Bovismorbi- ficans</i>	3			
<i>S. Anatum</i>	3			
<i>S. Zanzibar</i>	2			
<i>S. Kottbus</i>	1			
<i>S. Saintpaul</i>	1			
<i>S. :1:9,12:-</i>	1			
Total	98	32 (32.7)	23 (23.5)	

R = Resistant, MR = Multiple Resistance

Looking at individual antimicrobial drug, resistance to streptomycin was most frequently observed, followed by ampicillin, tetracycline, spectinomycin, and sulfisoxazole (Table 10).

Isolates resistant to these antimicrobials were detected predominantly from chicken carcass and pork.

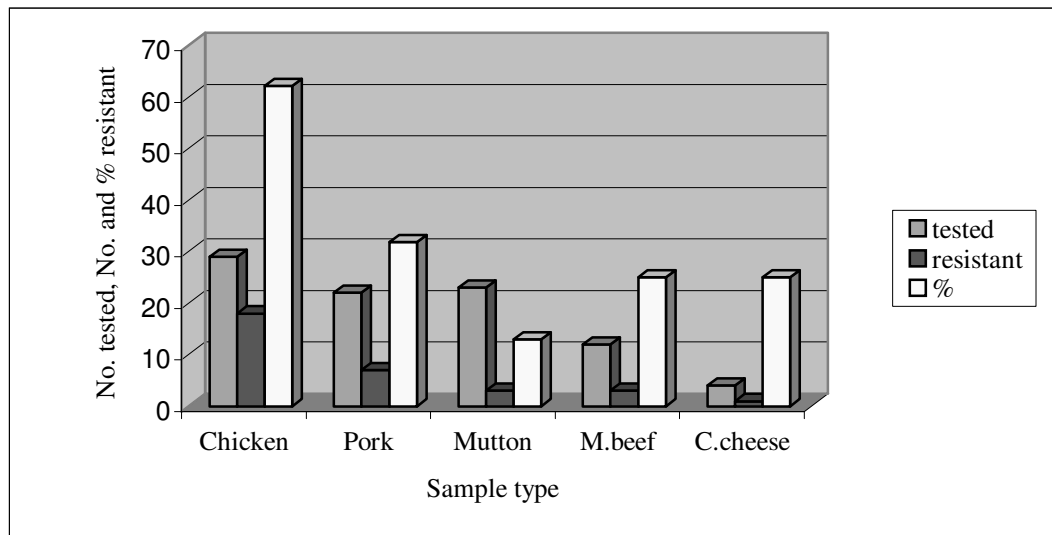
Table 9. Multiple antimicrobial resistances of *Salmonella* serotypes isolated from food items of Addis Ababa markets

Number of antimicrobial resistance	Antimicrobial resistance pattern (No.)	No. of isolates (%)
Zero	none	66 (67.3)
	Str (2)	
	Tet (2)	
One	Crb (5)	9 (9.2)
	Str Tet (3)	
Two	Crb Tet (1)	4 (4.1)
Three	Amp Str Tet (2)	2 (2)
Four	Amp Amc Str Tet (4)	4 (4.1)
Six	Amp Spt Str Sul Sxt Tmp (9)	9 (9.2)
Seven	Amp Amc Spt Str Sul Sxt Tmp (1)	1 (1)
Eight	Amp Amc Cip Nal Spt Str Sul Tet (1)	1 (1)
Nine	Amp Amc Cef Cip Nal Spt Str Sul Tet (1)	1 (1)
Ten	Amp Amc Cef Cip Gen Nal Spt Str Sul Tet (1)	1 (1)

Table 10. *Salmonella* isolates resistance by antimicrobial type

Antimicrobial drug	Total No. (%) of isolates resistant					
	Total resistant isolates (n=32)	Chicken carcass (n=18)	Pork (n=7)	Mutton (n=3)	Minced beef (n=3)	Cottage cheese (n=1)
AMP	19 (59.4)	12 (66.7)	5 (71.4)	0 (0.0)	1 (33.3)	1 (100)
SPT	13 (40.6)	12 (66.7)	0 (0.0)	0 (0.0)	1 (33.3)	0 (0.0)
STR	24 (75)	17 (94.4)	5 (71.4)	0 (0.0)	1 (33.3)	1 (100)
SUL	13 (40.6)	12 (66.7)	0 (0.0)	0 (0.0)	1 (33.3)	0 (0.0)
SXT	10 (31.3)	10 (55.6)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
TMP	10 (31.3)	10 (55.6)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
TET	15 (46.9)	6 (33.3)	5 (71.4)	2 (66.7)	1 (33.3)	1 (100)
CEF	2 (6.3)	2 (11.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
AMC	8 (25)	3 (16.7)	4 (57.1)	0 (0.0)	1 (33.3)	0 (0.0)
CIP	3 (9.4)	2 (11.1)	0 (0.0)	0 (0.0)	1 (33.3)	0 (0.0)
GEN	1 (3.1)	1 (5.6)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
NAL	3 (9.4)	2 (11.1)	0 (0.0)	0 (0.0)	1 (33.3)	0 (0.0)
CRB	6 (18.8)	0 (0.0)	2 (28.6)	2 (66.7)	2 (66.7)	0 (0.0)

Figure 4. Proportion of samples with antimicrobial resistant salmonellae



5. DISCUSSION

5.1. Prevalence and distribution

Over the last decade the number of supermarkets in Addis Ababa city has been growing considerably due to the favorable economic policy. Currently there are about 42 supermarkets selling meats in parallel with other items. These supermarkets are important settings, which serve the city dwellers to buy different livestock and poultry products. These products are acquired directly from private farms, agro-industries and abattoirs. It would be logical to consider and include these settings as critical sites /areas/ in designing surveillance studies on foodborne *Salmonella*.

As shown in the present study, *Salmonella* was detected in almost all food items sampled, except ice cream, indicating that many kinds of food items could act as vehicle of *Salmonella* spp. Many authors outlined the consumption of raw meat and meat products (Oliveira *et al.*, 2002; Haeghebaert *et al.*, 2001; Fey *et al.*, 2000; D'Aoust, 1997; Tauxe, 1991; WHO, 1988; Silliker, 1982), raw egg and egg products (Jay, 2000; Dodhia *et al.*, 1998; Caffer and Eiquer, 1994; Tauxe, 1991) and raw milk and milk products (Jay, 2000; WHO, 1988; Silliker, 1982; Fontaine *et al.*, 1980) as principal causes of the increase of *Salmonella* gastroenteritis worldwide.

Costalungn and Tondo (2002) in their study of salmonellosis in Brazil, during the periods 1997 – 1999 demonstrated that among food vehicles, meat and meat products accounted for 16.5% of outbreaks of *Salmonella* spp. while milk and dairy products and ice cream accounted for 2.9% and 0.7%, respectively.

Although we were not able to take equal number of samples from all supermarkets category, *Salmonella* prevalence was relatively higher in larger and medium supermarkets than small supermarkets, suggesting size of supermarkets as a possible risk factor for *Salmonella* occurrence. The difference observed in recovery of *Salmonella* between large (18.3%), medium (13.3%) and small supermarkets (7.4%) could be attributed to the differences in management practices. The variety of slaughtered animal carcasses retailed and supermarket butchery employees are higher in large supermarkets. These workers perhaps pay less attention to hygienic

aspects of the meats and equipment; thereby leaving more opportunity for cross-contamination. The higher the number of supermarket butchery employees the higher will be the chance of contaminating the meats. Additionally, one of the large supermarkets sells all meat samples except chicken carcass after mincing thereby increasing chance of cross-contamination. Tibaijuka (2001) also reported that the risk of *Salmonella* contamination to be higher in larger supermarkets.

There was a substantial difference in the prevalence of *Salmonella* among the different samples analyzed ($p = 0.000$). From the outcome of this research undertaking it was evident that chicken, followed by pork yielded the highest proportion of salmonellae isolates. This is consistent with various reports made in the country and elsewhere in the world (Ejeta *et al.*, 2004; Tibaijuka *et al.*, 2003; Tauxe, 1991; D'Aoust, 1989).

Although the history of the animals before slaughter is unknown by the investigator, it is likely that stresses due to transport, improper feeding and poor hygiene, etc. might happen to these animals considering the prevailing socioeconomic conditions, knowledge and awareness of the people, particularly those from rural areas. Different authors (Deen *et al.*, 2001; Wray and Davies, 2000; Vella and Curshieri, 1995; Oosterom, 1991; Baird-Parker, 1990; Adesiyun and Oni, 1989; Moo *et al.*, 1980) have attributed various stress factors to be in favor of increased *Salmonella* prevalence. Under normal circumstances the meat of animals should not contain pathogens like *Salmonella*. However, it is logical to think that the manual slaughtering of animals coupled with inadequate hygienic practices in the abattoir environment, transportation and supermarket level significantly increases the possibility of carcass contamination and spreading of *Salmonella*. Partly the results of the present findings could be attributed to contamination of the red meat at abattoir, at any stage during butchering, from the lymph nodes (Opuda-Asibo *et al.*, 1990; Lammerding *et al.*, 1988; Moo *et al.*, 1980; Samuel *et al.*, 1980) and feces of slaughtered animals, which are asymptomatic carriers (Wray and Davies, 2000; Fedorka-Cray *et al.*, 1998; Gay *et al.*, 1994; Opuda-Asibo *et al.*, 1990; Bean and Griffin, 1990; Norval, 1981; Peel and Simmons, 1978). It could also be due to contamination from abattoir equipment, floor and hands of personnel, as has been reported by various authors (Baird-Parker, 1990; Smeltzer *et al.*, 1980b; Smeltzer *et al.*, 1980a; Smeltzer *et al.*, 1979; Watson, 1975). According to Arroyo and Arroyo (1995) it was mentioned that the primary cause for the presence of higher number of *Salmonella* mainly in offals, but also in carcasses, was due to cross-contamination taking place at

slaughterhouse, during transport, or at the markets themselves, rather than other sources, e.g. disease. Even with the best manufacturing practices, cross-contamination occurs during slaughtering, dressing and further processing (Silliker, 1982).

5.1.1. Chicken

Different workers from different parts of the world gave varying frequency of *Salmonella* isolation from market ready dressed chicken carcass; some of which agree while others disagree with the findings of the present study.

The present finding of 13.9% (95% CI = 9.49 to 19.32) prevalence of *Salmonella* in chicken samples was lower than the results of earlier studies made by Molla *et al.* (1999a) who reported 28.6%, 22.6%, and 15.4% in chicken gizzard, liver, and heart, respectively. It is also lower than the findings of Molla and Mesfin (2003) who detected *Salmonella* in 21.1% of samples originating from chicken carcass and giblets in central Ethiopia and that of Tibaijuka *et al.* (2003) who indicated 17.5% prevalence (54/301) in chicken meat and edible offals. However, the present *Salmonella* prevalence from chicken carcass agrees with the study made in Ireland by Whyte *et al.* (2000) who detected *Salmonella* in 32 (16%) of the 198 skin samples using the traditional culture methods. On the other hand our result was markedly different from 66% prevalence reported by Jerngklinchan *et al.* (1994) from Thailand, 29.7% by Plummer *et al.* (1995) from whole bird in UK, 38.3% reported by Rusal *et al.* (1996) from poultry carcass arising from wet markets and processing plants in Malaysia. Arumugaswamy *et al.* (1995) from Malaysia also reported a much higher *Salmonella* isolation rate from chicken portions (39%), liver (35%) and gizzard (44%). The level of contamination of dressed chicken meat in our study (13.9%) was also found to be slightly higher than the 11.5% prevalence report by Živković *et al.* (1997) on market ready dressed chicken meat, in Zagreb, Croatia and 4.2% by Zhao *et al.* (2001) from Greater Washington D.C. area. On the other hand the present finding was lower than the results of Hang'ombe (1999) who published 20.5% frequency of isolation for *Salmonella* (the second highest frequency of isolation after *E. coli*) from dressed chicken carcass in Lusaka, Zambia. Variation in the frequency of isolation of *Salmonella* between the present and earlier studies in Ethiopia might stem from either actual difference in prevalence of *Salmonella* in carrier chicken in the flock of origin or the fact that, unlike our studies, giblets were included in previous

studies, which contributed substantially for the difference in prevalence. Unlike the previous studies made on chicken in Ethiopia, it is of interest to note that 144 (69.2%) of dressed chicken carcass sampled in this research work originated from indigenous backyard local chicken with different management from commercial farms. According to D'Aoust (1989) high prevalence of *Salmonella* in chicken carcass is attributable to problems associated with poultry husbandry, processing, and cross-contamination of carcasses in slaughtering plant through common scalding, de-feathering, and chilling processes. The same author also showed that cross-contamination from the hands of workers, equipment and utensils can spread the bacterium to uncontaminated carcass and parts. The relatively high prevalence of *Salmonella* in dressed chicken carcass might have emerged, in part, from their feeding habits i.e., their daily ration comprises of animal proteins, as source of essential amino acids and minerals, that might have been contaminated with *Salmonella* (D'Aoust, 1989; Pegram, 1981).

Majority of dressed chicken carcasses were being stored for varying duration at deep freezer temperature at the time of purchase. Although difficult to exactly tell the duration, prolonged storage at deep freezer temperature was likely to damage the pathogen under study thereby affecting the rate of detection (Plummer *et al.*, 1995).

Dressed chicken carcasses are usually delivered to supermarkets after being properly wrapped in transparent polyethylene bags, which reduces the possibility of cross-contamination within each other and to other type of meats sold in the supermarket (Plummer *et al.*, 1995). Contamination of poultry carcass by *Salmonella* is a worldwide problem, particularly with *Salmonella* Typhimurium and *Salmonella* Enteritidis (D'Aoust, 1989). Many authors have shown that chicken carcass harbor diverse *Salmonella* serotypes (Molla and Mesfin, 2003; Tibajuka *et al.*, 2003; Molla *et al.*, 1999a; Uyttendaele *et al.*, 1998; Rusal *et al.*, 1996; Plummer *et al.*, 1995; Jerngklinchan *et al.*, 1994).

5.1.2. Pork

The present study on pork revealed 11.3% (22/194) (95% CI = 7.24 – 16.66) *Salmonella* contamination rate. To the best of our knowledge so far, except the preliminary work done by Ejeta *et al.* (2004) who indicated 16.4% contamination rate, no other investigation of prevalence on *Salmonella* in pig and pork has been conducted in Ethiopia, to compare with and to elucidate their role as source of *Salmonella*. Our finding was therefore, slightly lower than the previous results of Ejeta *et al.* (2004), which was carried out on pork samples from similar supermarkets in Addis Ababa. The slight difference in *Salmonella* contamination rate of pork meat could probably stem out from the variation in sample size where only 55 pork samples were analyzed in their study.

A prevalence of 11.3% for *Salmonella* on pork in this study was comparable with the result of White *et al.* (2001) who detected 16 % *Salmonella* contamination rate in ground pork samples obtained from 3 retail supermarkets from Washington D.C. area between June and September 1998. However, it was much higher than the 3.3 % reported by Zhao *et al.* (2001) from the Greater Washington, D.C. area. The relatively high prevalence of *Salmonella* in pork meat in the present study was a reflection of the poor sanitary condition under which pigs are raised in the farms, transported to slaughterhouse and kept in the lairage. It could probably be also associated with feeding habit of pigs (scavengers), which increases the chance of infection through feeding on excreta of infected animals and infected carcass. It could also be an indication of inadequate hygienic practices exercised while transportation of carcass and retail sale in supermarkets of Ethiopia, which probably add up to the cross-contamination of carcass from other meats, equipment and surrounding environment. We should not also exclude the role of carrier pigs contributing partly to the present prevalence findings. With regards to the contribution of carrier pigs as source of *Salmonella* during and after transporting to slaughtering plant, Isaacson *et al.* (1998) reported that such pigs are of great threat to contamination of the meat after slaughter.

In Ethiopia data disclosing the role of swine as a source of *Salmonella* serotypes causing disease in humans is lacking. However, reports from elsewhere in the world indicated that pork products were implicated in 2.9% of all *Salmonella* outbreaks during the year 1983 to 1987, compared to 18.2% of those originating from poultry and eggs, and 11.2% from beef (Tauxe, 1991).

Consumption of pork had been incriminated with large outbreaks of foodborne illness caused by *Salmonella* Infantis in Denmark (Isaacson *et al.*, 1999).

According to Sinell *et al.* (1984), 63.7% of porcine organs from central wholesale meat market in Berlin contained *Salmonella*, which is significantly higher than the present findings (11.3%). Our results were unmatched with the findings of another study from northern region of Thailand (Chiang Mai) by Patchanee (2002) which revealed 69.5% overall prevalence at farm level and 82.5% overall prevalence of pre-slaughter *Salmonella* infection at the slaughter level. The explanation of this difference may be related to the large herd size, feeding and intensive swine husbandry practiced in developed nations, and suitable geographical locations, which favored *Salmonella* propagation. The deviation could also be due to the fact that in our study pork meat cut samples were analyzed which were less likely to contain *Salmonella* in comparison with edible offals.

Morrow *et al.* (2002) indicated that any practice that increases the proportion of pigs excreting *Salmonella* organism is likely to increase the proportion of pork products that are contaminated. The spillage material from the intestinal tract, mainly as a result of intestinal perforation during evisceration, is the major source of carcass contamination (Deen *et al.*, 2001).

5.1.3. Mutton

Of the 212 mutton samples examined for *Salmonella*, 23 were positive (10.8% prevalence, 95% CI = 6.94 – 15.69). It was lower than the 14.1% (12/ 85) prevalence rate reported by Ejeta *et al.* (2004) from Addis Ababa and 13.1% reported by Kotova *et al.* (1988) and much lower than the 50% and 33.3% reported by Arroyo and Arroyo (1995) from lamb liver and heart from markets in Madrid, Spain, respectively. However, the present findings agree with the findings of Sierra *et al.* (1995) who indicated 10% frequency of *Salmonella* isolation from sheep carcasses.

The extensive range production system of sheep in Ethiopia should contribute to low prevalence of *Salmonella* in Ethiopia. However, the duration the sheep stay collectively in the hands of traders prior to selling and at abattoir before killing might further increase the spread of infection. The slaughtering process adds to carcass contamination (Wray and Davies, 2003). Weldemariam

(2003) in his abattoir survey at Debre Zeit, Ethiopia, indicated 6.3% carrier rate and 6.5% and 8.4% contamination rate of abdominal and diaphragmatic muscles of sheep, respectively; 80% of serotypes recovered from these muscles being also detected from liver, spleen and feces, evidencing the importance of carrier sheep in cross-contaminating carcasses. The presence of *Salmonella* in edible red meat of sheep (mutton), like that of other animal meats, might indicate cross-contamination of carcasses from feces, lymph nodes and internal organs of carrier sheep at slaughterhouse, transport and in supermarkets. Kumar *et al.* (1973) indicated that 3.1% of 812 slaughtered sheep in India were *Salmonella* carriers as demonstrated through isolation of *Salmonella* made from feces, mesenteric lymph nodes, liver and spleen. Nabbut and Al-Nakhli (1982) reported 14.3% (40/280) *Salmonella* prevalence from lymph node, feces and spleen of sheep slaughtered in Riyadh public abattoir, Saudi Arabia. Norval (1981) also showed that contamination of red meat by salmonellae to occur at the abattoir (from lymph nodes, animal excreters, contaminated abattoir equipment and floors) and during butchering.

5.1.4. Minced beef

The prevalence of *Salmonella* in minced beef was found to be 8.5% (12/142) (95% CI = 4.44 – 14.3). Factors, which account for increased prevalence, could be the grounding of meat, which reduces the particle size thereby providing greater surface areas for growth of *Salmonella* organisms. In addition cutting knives, saws, hoes, meat grinders, cutting boards and storage utensils, which are not properly cleaned and disinfected, serve as means of cross-contamination for *Salmonella* from contaminated meat to clean meats (Jay, 2000). This is especially true when fat embedded infected lymph nodes, which contain higher number of *Salmonella*, are cut and same knife is used on other meats.

The prevalence of *Salmonella* in minced beef samples found in this study agrees with the findings of previous studies made in Ethiopia by Nyeleti *et al.* (2000) and Ashenafi (1994) who revealed prevalence of *Salmonella* in minced beef and raw beef from butcher's shop to be 7.9% and 9%, respectively. It also agrees with the findings of studies made in USA (USDA, 1996) on minced beef, which revealed 7.5% prevalence. However, our finding was lower than the 14.4% (23/160) prevalence reported by Ejeta *et al.* (2004) and much lower than the 40% (20/50) prevalence reported by Molla *et al.* (1999a). Our result was also much lower than the report made

by Tegegne and Ashenafi (1998) on raw “kitfo” samples that indicated 42% *Salmonella* isolation rate. On the other hand *Salmonella* prevalence from minced beef in the present finding was higher than the 1.9% and 6% reported by Zhao *et al.* (2001) and White *et al.* (2001), respectively.

In a study to determine prevalence of *Salmonella* from abdominal and diaphragmatic muscles Alemayehu *et al.* (2003) reported 2.8% and 3.1%, respectively. Another study by Nyeleti *et al.* (2000) indicated the contamination rate due to *Salmonella* from abdominal and diaphragmatic muscles to be 9.8% and 11.9%, respectively. The same author revealed low prevalence of *Salmonella* from feces (2.1%) and lymph node (4.2%) indicating the significance of cross-contamination in the abattoir and low carrier rate in the herd of origin. The difference in prevalence between our study and that of Molla *et al.* (1999a) and Tegegne and Ashenafi (1998) could probably emanate from small samples, limited markets investigated in both studies. It could also be attributed to the way “kitfo” (a traditional Ethiopian spiced minced beef) is made in food establishments, which further add or amplify *Salmonella* contamination from kitchen personnel and equipment due to the unhygienic method of preparation. Methodology of isolation employed such as variation in media used for isolation, sampling procedures (swabs versus 25 g of meats) and one versus multiple picks of suspect colonies for confirmation could also contribute for difference in prevalence between various studies indicated above.

5.1.5. Cottage cheese

Cottage cheese or “ayib” is an Ethiopian traditional dairy product made from sour milk after the removal of the fat by churning and cooking the curd to a temperature of 40 – 70 °C. “Ayib comprises 79% water, 14.7% protein, 1.8% fat, 0.9% ash and 3.1% soluble milk constituents (O’Mahony, 1988). In this study *Salmonella* prevalence in cottage cheese was low. Only 2.1% (4 of 190) of samples gave positive results. The low level of detection could probably be due to the low prevalence of this pathogen in milk used to prepare “ayib”. It could also be due to the fact that the methodology of cooking (time-temperature factor) used in preparing “ayib” from the curd might have destroyed the pathogen of interest originally present. Those *Salmonella* isolates detected (4 out of 190) might arise from post-preparation contamination of the products from one or more of the following: unclean environment, equipment, poor hygiene of handlers, starting from site of preparation up until the site of retail selling. This indicates the need to monitor and

improve simple hygienic measures practiced in the production and marketing of this product; such as proper washing of utensils and hands of personnel who handle the product. There is a possibility that the heat treatment was not adequate to kill the *Salmonella* present or *Salmonella* tolerates the heat treatment and hence appear in the product tested. The low pH of 'ayib', which varies from 3.3 to 4.6 (Ashenafi, 1990), was an important limiting factor, which was not favorable for survival, growth and multiplication of this pathogen thereby contributing significantly for the low recovery rate of *Salmonella*. In a study by Ashenafi (1993) to determine the fate of *Salmonella* Enteritidis and *Salmonella* Typhimurium during the fermentation of "Ergo", traditional Ethiopian sour milk, growth of lactic acid bacteria resulted in complete inhibition of *Salmonella* Enteritidis and *Salmonella* Typhimurium between 48 and 60 hours of fermentation of milk in non-smoked and smoked containers.

Isolation of *Salmonella* from cottage cheese (2.1%) is important because this raw eaten food contains 1.8% fat which may trap the *Salmonella* present, even if few in number, and assists to survive the acidic condition of stomach to subsequently attach to and invade the enterocytes lining the intestine (D'Aoust, 1994).

On the other hand, although the method of preparation and product pH were different from that of Ethiopian cottage cheese, several episodes of human illness following the consumption of *Salmonella* containing cheese have been reported in recent years (D'Aoust, 1994). Cheddar cheese contaminated with *Salmonella* Heidelberg was incriminated in outbreak in USA with 2700 cases of illness (Fontaine *et al.*, 1980).

5.1.6. Fish

During the study period 128 meat samples from *Tilapia* fish were examined for the presence of *Salmonella* and three of the samples were found contaminated with *Salmonella*. In Ethiopia studies made on *Salmonella* isolation from fishery products were non-existent. Similarly foodborne illness associated with the consumption of *Salmonella* contaminated fishery products had not been clearly documented so far. In Ethiopia, *Tilapia* fish are mostly consumed following cooking, which renders it microbiologically safe for consumption, except among few groups of people who consume it raw or lightly cooked. Under such circumstances foodborne infection due

to *Salmonella* is likely to occur. The present study indicates a relatively low prevalence of *Salmonella* in *Tilapia* fish meat sold in retail markets of Addis Ababa. The prevalence reported in this study was much lower than that reported by Mohamed Hatha and Lakshmana Perumalsamy (1997) from South India, who indicated that 14.3% of fishes were contaminated with *Salmonella*. However, in their study they examined body surfaces, gills and alimentary canals of fish, (using swab techniques) where *Salmonella* isolation rate was comparatively higher. Additionally, climate, type of fish and season might contribute to the difference in isolation rates. In this study, during the sampling process attempt was made to remove external covering of fish and only the meat part was considered as analytical unit. This might reduce isolation of *Salmonella*, the chance of detecting those *Salmonella* present on the surface of the body being minimized. Heinitz *et al.* (2000) in their study of incidence of *Salmonella* in fish and seafood's reported 12.2% incidence in raw import fish, which was higher than the present finding. Feeding of raw meat scraps and offal, animal feeds, night soil and antibiotics at sub-therapeutic dose for the farmed fish and shellfish is favorable for *Salmonella* contamination (D'Aoust, 1997). However, the fish in our study originate from natural fresh water lake (Ziway) where artificial feeding of the aforementioned type was absent, thereby contributing for low prevalence.

The detection of *Salmonella* from fish was important not only from public health point of view but also because of its substantial role as source of infection for domestic animals through feeding fish meal. According to Seifert (1996) 34% of the fish meal imported to Germany in 1984 was contaminated with *Salmonella*.

Contamination of the water body where fishes live from the surrounding run off (flooding) and aquatic birds coupled with poor hygienic practice at all stages of fish handling contribute significantly for *Salmonella* to prevail in fishes.

5.1.7. Ice cream

Eggs and food containing raw or undercooked eggs are usually confirmed or suspected vehicles responsible both for outbreaks and sporadic cases (Fantasia and Filetici, 1994). Absence of salmonellae in ice cream samples in this study might be due to the low incidence of *Salmonella* in the ingredients used to prepare ice cream or due to the inadequate sample size. It could also be

due to the good standard of hygiene exercised in the preparation of ice cream and use of the right temperature for the storage of this product, which is not favorable for the growth and multiplication of *Salmonella*. Absence of *Salmonella* in ice cream might represent low risk or absence of risk for the consumers. But it by no means implies or rules out the absence of *Salmonella* Enteritidis in flocks of chicken from which the eggs used to prepare ice cream came from. In Ethiopia, so far meager study had been done to estimate prevalence of *Salmonella* from egg and milk to relate the results with the current findings. On the other hand, egg survey undertaken by Public Health Laboratory Service of UK has shown that 0.7% of eggs from high street retail outlets were contaminated with *Salmonella* Enteritidis (de Louvois, 1993). Humphrey (1994) mentioned isolation of few number of *Salmonella* Enteritidis from contents of clean intact shell eggs as a result of infection of reproductive tissue (oviduct) of laying hens. This is vertical transmission (Caffer and Eiger, 1994), the subsequent growth being governed by temperature and length of storage. Hassen *et al.* (2000) determined 1.5% prevalence of *Salmonella* from milk filter samples in New York dairy herds. Another study made on bulk tank milk from eastern South Dakota and Western Minnesota reported 6.1% *Salmonella* prevalence (Yayarao and Henning, 2001). Generally, there is a need to undertake more comprehensive studies by analyzing larger samples to further clarify the role of ice cream as a vehicle to cause foodborne *Salmonella* illnesses in Ethiopia. On the other hand, contrary to this, different authors have incriminated ice cream as a food vehicle for outbreak of acute *Salmonella* gastroenteritis. Bean and Griffin (1990) indicated ice cream to be the fourth leading food vehicle known for salmonellosis outbreaks in United States during 1973 – 1987 being responsible for 28 outbreaks (3.5%). Again, ice cream produced from milk that was transported in tanker trucks that had previously hauled liquid egg was responsible for one of the largest outbreak of salmonellosis (due to *Salmonella* Enteritidis) that occurred in USA involving more than 224,000 persons (Jay, 2000). Dodhia *et al.* (1998) in their epidemiological investigation indicated that home made ice cream for a children's birthday party was source of infection for outbreak of food poisoning caused by *Salmonella* Enteritidis PT 6, isolated from stool of sick individuals. As opposed to the present finding, Al-Hindawi and Rished (1979) reported high contamination rate of cake samples (61.1%, i.e., 22/36) and attributed to food handlers or to constituents like cream and egg.

5.1.8. Human subjects

A prevalence of 7.4% (95% CI = 2.43 – 16.33) (5/68) of *Salmonella* from stool samples of supermarket workers is significant and is of concern to the food service providing establishments because of the perceived risk of cross-contamination of foods by infected food handlers and potentiation of foodborne disease outbreaks (D'Aoust, 1991). The finding was in consonance with earlier studies made by Nyeleti *et al.* (2000) who reported 6% (18/300) prevalence of *Salmonella* in Addis Ababa abattoir workers. The 7.4% prevalence in the present study indicate that significant proportion of the study group are shedders or carriers of *Salmonella* with increased likelihood of transfer of the infection to others through contamination of food.

Salmonella belonging to serogroup A, B, C, D and E have been isolated in Ethiopia (Mache, 2002; Mache *et al.*, 1997; Ashenafi and Gedebou, 1985; Gedebou and Tassew, 1981) predominantly from stool and blood samples of humans. The present result of *Salmonella* isolation from personnel in Addis Ababa supermarkets has close resemblance to that of Mache *et al.* (1997) from Addis Ababa and Jiang *et al.* (2002) from India (Goa) and Jamaica (Montego Bay) who reported *Salmonella* prevalence of 7.9%, 10.2% and 7.8%, respectively. Our result was significantly lower than the report of Mache (2002) from Jimma and Saha *et al.* (2001) from Calcutta, India, who indicated prevalence of 15.4% and 15.3%, respectively. On the other hand our result was higher than that of Ashenafi and Gedebou (1985) from Addis Ababa, who reported a prevalence of 4.5%. However, unlike our case, samples examined by all the aforementioned studies originated from outpatients or pathological specimen of clinically sick individuals with diarrhea (adult and children) and the methods employed to culture *Salmonella* were slightly different. Failure to detect *Salmonella* from one time examination of stool sample from 63 supermarket employees may not be significant because even if infected, these individuals may shed the pathogen when expressing diarrhea, otherwise they shed intermittently (Guthrie, 1992).

In the course of this study it has been observed that some local and expatriate people purchase meat for their pet animals. Feeding salmonellae contaminated meat to these animals may lead to the establishment of the infection and subsequent transfer to human being, particularly to children who frequently come in contact with contaminated ground and infected pet animals. WHO (1988) indicated that uncooked, raw, fresh or frozen food of animal origin used for pet food constituted a danger not only to the pet animal but also to the domestic kitchen environment

where cross-contamination might occur. Some of this food must always be suspected of being contaminated with salmonellae, and cooking prior to the feeding of pet animals is strongly recommended.

5.2. Serotypes and phage types

Serotypes well known to exist in prevalence and distribution studies made in Ethiopia from chicken carcass (*S. Braenderup*, *S. Typhimurium*, *S. Anatum*, *S. Hadar* and *S. Bovismorbificans*), Pork (*S. Infantis*), mutton (*S. Bovismorbificans* and *S. Infantis*) and minced beef (*S. Typhimurium*, *S. Dublin* and *S. Anatum*) (Ejeta *et al.*, 2004; Molla and Mesfin, 2003; Tibaijuka *et al.*, 2003; Nyeleti *et al.*, 2000; Molla *et al.*, 1999a) also were encountered in the current investigation. However, the more prevalent serotype (*S. Braenderup*) reported by Ejeta *et al.* (2004) from mutton and pork was not isolated on our study. Unlike the observation of Ejeta *et al.* (2004), *S. Infantis* which occurred in 43.5% of minced beef, was detected only in 8.3% of our minced beef isolates, and no *S. Vejle* was encountered in our samples. *Salmonella* Enteritidis that was reported earlier by Molomo (1998) and Molla *et al.* (1999a) were not isolated in any of our samples.

Some of the presently isolated serotypes have also been reported earlier from abroad. Al-Hindawi and Rished (1979) noted, among others, *S. Newport* and *S. Typhimurium* to be the most predominant serotypes in foods (cakes, meats, raw milk). Guthrie (1992) in his comparison of *Salmonella* strains isolated from humans and animal sources in USA during 1987 indicated that *S. Newport* was among the top 10 most frequently reported serotype. Jerngklinchan *et al.* (1994) reported *S. Hadar*, *S. Anatum* and *S. Kentucky* from supermarket chicken carcasses. Rusel *et al.* (1996) also isolated *S. Haifa*, *S. Newport*, *S. Hadar* and *S. Bovismorbificans* from broiler carcasses. Plummer *et al.* (1995) and Uyttendaale *et al.* (1998) isolated more frequently some of the present serotypes from chicken carcasses.

Isolation of *S. Newport*, from 12 supermarkets (57.1% of positive supermarkets), one fish shop and one open market (from three cottage cheese sellers) shows that the serotype is widespread in different settings and food types with the consequent high risk for the consumer. This is supported by the fact that all isolates from supermarket personnel, too, were *S. Newport*, showing that there was a substantial correlation between human and food isolates due to this non-host-specific serotype. The possible explanations for the introduction of *S. Newport* to Ethiopia might be through importation of animals (poultry and dairy cattle), food items, or through travelers from countries where this serotype is endemic. The isolation of *S. Newport* at such high rate

(41.8% of isolates) and 3.2% of all samples, which has never been reported from Ethiopia before, is of concern as this serotype like *S. Typhimurium*, *S. Infantis* and *S. Saintpaul* was frequently associated with salmonellosis (Uyttendaale *et al*, 1998). The recovery of *S. Newport* at higher frequency from pork (6.2% of samples) and mutton (5.7% of samples) in this study indicated the serotype is most prevalent and ubiquitous. It may also reflect the importance of cross-contamination probably along the chains from abattoir to vending sites. Conversely, rare serotypes like *S. Kottbus*, *S. Saintpaul* and *S.:* 1: 9, 12: - were detected each from three different supermarkets on one occasion only. The isolation of invasive serotypes like *S. Typhimurium*, which is of global health importance commonly associated with outbreak of human food poisoning, and *S. Dublin* is remarkable in view of their veterinary and human health hazard implication. Tauxe (1991) indicated *S. Typhimurium* as the serotype most frequently associated with human cases of disease in the USA. *Salmonella Typhimurium* and *S. Dublin* are common serotypes isolated from cattle in European countries. In veterinary sector, *S. Typhimurium* and *S. Dublin* induce serious economic loss due to their high morbidity and mortality, particularly in young animals (Rabsch *et al.*, 2003). *S. Dublin* can cause septicemia and often metastatic abscesses (Acha and Szyfres, 2001). All *Salmonella Haifa* isolates from pork samples were recovered from the same supermarket indicating possibility of cross-contamination possibly from the mincing machine (pork, mutton and minced beef samples purchased from this supermarket were all sold following mincing, packing and labeling).

Although the percentage and variety of serotypes recovered was different from ours, Neyleti *et al.* (2000) also isolated 4 different *Salmonella* serotypes from minced beef namely *S. Anatum*, *S. Dublin*, *S. Saintpaul* and *S. Rough form*. Similar to the present finding Ejeta *et al.* (2004) also reported eight different serotypes from minced beef of which *S. Infantis*, *S. Dublin*, *S. Anatum* and *S. Saintpaul* were also encountered in our case. *Salmonella Dublin* predominantly affects cattle. In our study this serotype occurred only in 2 minced beef samples (16.7% of positive cases). Neyleti *et al.* (2000) also reported 4 cases of *S. Dublin* out of 26 *Salmonella* isolates from minced beef (15.4%). The finding of *S. Dublin* in pork (2/22) and mutton (2/23) was probably an indication of contamination at the level of supermarkets.

Salmonella isolated from diarrrheal cases in Ethiopia have been identified at serogroup level with serogroup C, B and D being dominant (Mache 2002; Mache *et al.* 1997; Ashenafi and Gedebeu, 1985). This is in close agreement with the present finding where serogroup C ranked first (73.5%,

72/98) and serogroup B ranked second (14.3%, 14/98) out of the total isolates. These results also showed that serogroup isolated from food items are also common isolates from human beings. Except serogroup, no sufficient information on distribution of serotypes of *Salmonella* was available from the public health sectors in Ethiopia in order to compare serotypes isolated from food and people. However, Nyeleti *et al.* (2000) found 3 serotypes: namely: *S. Anatum*, *S. Dublin* and *S. Meleagridis* common between animals and abattoir workers; which accounted for 61.1% of serotypes isolated. On the other hand none of these human isolates were detected in our study and all the human isolates belonged to *S. Newport*. Although number of supermarket workers affiliated to butchery included in this study was not very large (n=93) to undertake more precise statistical analysis, it seems that there was an apparent association ($r = 0.35$) between *Salmonella* isolates from food samples and stools of personnel handling the foods. This is further evidenced by the fact that *Salmonella Newport*, the only serotype isolated from 7.4% of stool samples of supermarket butchery workers, was also the only serotype isolated at a frequency of 5.8% (3/52) from meat samples originating from one of the supermarket where two shedders were found. Therefore, isolation of similar *Salmonella* serotypes from food and stool samples investigated presently support the idea that food of animal origin constitutes an important source of *Salmonella* for people.

Detection of multiple serotypes from 13 supermarkets (61.9%, 13/21) may indicate or reflect cross-contamination of different types of meats along the chain from abattoir or natural infection at the farm level. The detection of 14 different *Salmonella* serotypes from food samples studied in Addis Ababa markets suggest that *Salmonella* serotypes are widely distributed and the studied markets are important sources of *Salmonella*. This is to be expected as the samples pass through different critical points before reaching into the hands of consumers, which might augment further contamination.

Salmonella Haifa, *S. Newport*, *S. Dublin* and *S. Kottbus* from pork, *S. Hadar*, *S. Dublin*, *S. Typhimurium*, *S. Zanzibar* and *S. Newport* from mutton, *S. Newport* and *S. Zanzibar* from *Tilapia* fish, *S. Newport* and *S. Haifa* from cottage cheese and *S. Newport* from stool samples were reported for the first time from Ethiopian retail food items and personnel.

5.3. Antimicrobial resistance profiles

Antimicrobial resistance recognizes no geographical boundaries and increasing rate of resistance of *Salmonella* isolates have been reported from developing and developed countries. Some of the antimicrobial drugs for which *Salmonella* serotypes / serogroups were resistant in our study have been reported earlier from Ethiopia (Molla *et al.*, 2003; Alemayehu *et al.*, 2003; Tibaijuka *et al.*, 2003; Mache, 2002; Molla *et al.*, 1999b; Mache *et al.*, 1997; Ashenafi and Gedebou, 1985; Gedebou and Tassew, 1981), other African countries (Leegaard *et al.*, 1996; Adesiyun and Oni, 1989; Hadfield *et al.*, 1985; Hummel, 1979) and elsewhere (White *et al.*, 2001; Gebreyes *et al.*, 2000; Tellefson *et al.*, 1998; Lee *et al.*, 1993; D'Aoust *et al.*, 1992d; Wray *et al.*, 1991).

The finding of 32.7% antimicrobial resistant *Salmonella* isolates from food samples examined was remarkable. It represents public health hazards due to the fact that food poisoning outbreaks would be difficult to treat and this pool of multi-drug resistant *Salmonella* in food supply represents a reservoir for transferable resistant genes (Diaz De Aguayo *et al.*, 1992).

Among the important findings of the antimicrobial resistance testing was that 62.1% (18/29) of chicken carcass, 31.8% (7/22) of pork, 25% (1/4) of cottage cheese, 13% (3/23) of mutton and none of the fish and human isolates were antimicrobial resistant (Table 7). The level of resistance was significantly higher for chicken carcass and pork isolates ($p = 0.003$) (Figure 4). Tibaijuka *et al.* (2003) also reported 60% antimicrobial drug resistance from chicken meat, which was similar to our findings. D'Aoust *et al.* (1992d) also indicated a high antimicrobial resistance among poultry isolates as compared to *Salmonella* isolated from other sources. Out of the 32 resistant isolates 24 (75%), 19 (59.4%) and 15 (46.9%) were resistant for streptomycin, ampicillin and tetracycline, respectively (Table 10). The significantly high frequency of resistant salmonellae for these antimicrobials was probably an indication of their frequent usage both in livestock and public health sectors. The high prevalence of *Salmonella* isolates resistant to these relatively cheaper and commonly available antimicrobials is disturbing because of the limited access and high cost of newer cephalosporins and quinolone drugs (D'Aoust, 1989) for poor citizens of developing countries like Ethiopia. Furthermore, systemic spread of such resistant isolates in human host could lead to serious complications or to a fatal outcome (D'Aoust, 1991a).

Our antimicrobial drug resistance result indicated that resistance to some extended spectrum cephalosporins (ceftriaxone, ceftiofur), aminoglycosides and newer quinolones was absent, perhaps due to their limited usage in veterinary and public health sectors of Ethiopia. On the other hand the occurrence of resistance to the quinolone (nalidic acid) and fluoroquinolone (ciprofloxacin) in 9.4% of resistant isolates from chicken carcass and minced beef or 3.1% of the total *Salmonella* isolates (*S. Kentucky*) was striking because development of resistance undermines the value of this first line drug (ciprofloxacin) for human systemic salmonellosis. Reasons for the emergence of resistance against these drugs were unknown and deserve investigation. However, introduction of resistant *Salmonella* with importation of food items and travelers (D'Aoust, 1994) and usage in human medicine in Ethiopia were suspected. MDR was higher in *Salmonella* isolates from chicken carcass and pork. Thus, 65.2% of MDR isolates were *S. Braenderup*, *S. Hadar* and *S. Kentucky* from chicken carcass and 21.7% of MDR isolates from pork were *S. Haifa*. These all show that antimicrobial resistant *Salmonella* serotypes are widespread and more common particularly from chicken carcass, cottage cheese and pork samples as compared to mutton and minced beef. The isolation of susceptible *S. Newport* among supermarket butchery workers and food items examined indicate that the source of contamination could be either from reservoir animals or personnel. The reasons for the recovery of antimicrobial resistant *Salmonella* serotypes was most likely due to the indiscriminate use of antimicrobials (Guthrie, 1992; WHO, 1988), self-medication due to easy access to antibiotics without prescription (Acha and Szyfres, 2001) in public health sector and the administration of sub-therapeutic dose of antimicrobials to livestock for prophylactic or nutritional purpose. Such agricultural practices introduce selective pressures that potentiate the emergence and distribution of resistant salmonellae in meats and other products (D'Aoust, 1989). Therefore, attempts should be made to reduce the magnitude of the problem at various levels through prudent use of antimicrobials. The tendency of salmonellae for intra- and inter-generic exchange of cytoplasmic DNA (R plasmid) that encodes for single or multiple antimicrobial resistances is another contributing factor (D'Aoust *et al.*, 1992d; D'Aoust, 1991a; D'Aoust, 1989; WHO, 1988). Nonetheless, there is a need to relate the type and amount of antimicrobial drugs used in intensive farms with data from systematic survey of resistant *Salmonella* infection to monitor changing resistance and to determine if change in the frequency and pattern of resistance are related to specific pattern of antimicrobial usage (Lee *et al.*, 1993).

None of the *S. Typhimurium* isolates were found resistant to any of the antimicrobial drugs used. In contrast, Alemayehu *et al.* (2003) detected MDR strain of *S. Typhimurium* phage type 2 and Molla *et al.* (1999b) reported 60% of *S. Typhimurium* isolates from chicken and minced beef to be MDR. Leegaard *et al.* (1996) also reported MDR *S. Typhimurium*. The absence of antimicrobial resistant *Salmonella* isolates from minced beef in Neyleti *et al.* (2000) and 25% (3/12) resistant isolates in the present investigation was contrasting and suggests that antimicrobial resistant salmonellae from minced beef are emerging through time.

The study demonstrated that supermarket meat samples particularly dressed chicken carcass and pork, were important sources of antimicrobial resistant *Salmonella* serotypes for consumers and stressed the need to regulate the ethical usage of antimicrobials and regular monitoring of antimicrobial resistance.

5.4. Synopsis

Generally, the variation in overall prevalence of *Salmonella* between this study and those made elsewhere may be explained, in part, by variation in laboratory practice (methods), difference in management (particularly use of antimicrobials and flock or herd size), type of sample (meat, offals), variation in sampling methods and geographical locations (climate).

As shown in the present study, different food items contained *Salmonella* at varying frequencies, and hence with varying degrees of risk for consumers. Therefore, the presence of *Salmonella* in the samples suggests that unless proper heat treatment is done and cautious culinary and personal hygiene practiced (to prevent cross-contamination to other raw eaten foods), the risk of acquiring *Salmonella* by the consumers is undoubtedly high. It was found that chicken meat is the most risky food for *Salmonella* followed by pork and mutton. This is further supported by the findings of different authors.

In developing countries like Ethiopia, where surveillance system for foodborne diseases is non-existent, it is difficult to estimate the problems associated with *Salmonella* food poisoning. Even in countries where surveillance services are very efficient, the precise incidence of salmonellae food poisoning is not known, as outbreaks are often not reported to public health authorities (Jay, 2000)

The present study does not necessarily indicate the prevalence of *Salmonella* in the animal population. It does, however, reflect the role of supermarkets, open markets and fish selling shops in the transmission of *Salmonella* to humans. The present work in general contributes considerably towards understanding the prevalent *Salmonella* serotypes and common food vehicles, which might be involved in foodborne *Salmonella* infection in Addis Ababa. It also provides information on antimicrobial resistant *Salmonella* serotypes and role of supermarket workers as possible sources of infection.

The prevalence rate of *Salmonella* reported in this study particularly from meats, chicken and cottage cheese, in conjunction with available current literature on *Salmonella* clearly attest that salmonellae are widespread in food items and may pose a health hazard to consumers due to consumption of raw or under cooked products, direct or indirect contact through such contaminated materials, and cross-contamination of food items in the kitchen (ICMSF, 1986). This is especially important in developing countries like Ethiopia where the habit of consuming raw animal products like meat in the form of “Kurt”, raw or medium cooked “Kitfo” and cottage cheese (served as made with various chilly sauces) is very common. The risk of acquiring *Salmonella* is further augmented in view of the low socio-economic status of the people and the increasing number of sensitive segment of population, mainly due to HIV/AIDS. Patients with AIDS are at increased risk of acquiring nontyphoidal *Salmonella* bacteremia and they should avoid consuming raw or under cooked foods (Thamlikitkul *et al.*, 1996; Baird-Parker, 1990). The findings also suggest the need for developing educational program to address issues related to the consumption of raw animal products. Consumer education about proper handling of food as a means of combating food poisoning should be initiated and inculcated in to the minds of adults and children at various levels of government, educational institutions and industry (ICMSF, 1986; Silliker, 1982). Generally the prevention of contamination of meat and dairy products by *Salmonella* as well as other foodborne pathogens should not be the responsibility of one body, rather every individual involved from “farm” to “table” should contribute in the attempt to minimize the risk of contamination in line with the Good Agricultural Practice (GAP), Good Manufacturing Practices (GMP), and Hazard Analysis and Critical Control Point (HACCP) programs, which are the most effective strategies to assure the safety of food products for human consumption.

There is also an urgent need of assessing the most important critical points in the production, processing, transport and distribution of meat and chicken carcasses in order to alleviate the contamination rate and subsequently ensuing economic loss. The overall results of the present study showed that rate of *Salmonella* contamination particularly from supermarkets are undesirably high.

6. CONCLUSIONS

Microbiological analysis of food items from retail outlet for foodborne organisms like *Salmonella* is of paramount importance in ensuring the supply of safe food for the consumers. The information collected in this cross-sectional survey coupled with other similar studies at different critical points preceding retail outlet markets can be used as a basis to undertake qualitative microbiological risk assessment and provide indispensable information in designing monitoring and surveillance programs.

In view of the fact that most samples investigated presently are consumed raw or under cooked by significant proportion of the population and existence of immense export potential, further monitoring of their microbiological quality deserves special attention in order to limit further spread and ensure public health standards. The following specific and important points could be concluded from this cross-sectional survey:

- *Salmonella* contamination was widespread in food items (except ice cream) (7.8%) and supermarket personnel (7.4%) in Addis Ababa and the magnitude of the problem is especially high in meat samples as compared to others and represents a real public health hazard.
- A total of 14 different serotypes were identified, *Salmonella* Newport, *Salmonella* Braenderup, *Salmonella* Hadar, *Salmonella* Typhimurium and *Salmonella* Dublin being the most dominant. Serotype Newport and Kentucky were reported from Ethiopia for the first time.
- Although all *Salmonella* Newport isolates from personnel and food items were susceptible to all antimicrobial drugs tested, some supermarket workers who are shedders of *Salmonella* in their stool could be source of infection as evidenced by isolation of same serotype (*Salmonella* Newport) from personnel and meat samples.
- There is a marked difference in isolation rate of *Salmonella* between supermarkets, open markets and fish shops.

- Ice cream is not a significant source of *Salmonella* for consumers.

- Significant proportions of *Salmonella* isolates were resistant for antimicrobials (32.7%), of which 23.5% were MDR. This could make treatment of humans' clinical salmonellosis and other bacterial diseases difficult should food poisoning by similar resistant *Salmonella* serotype ensue. Among MDR serotypes, *Salmonella* Kentucky was resistant to up to ten antimicrobials.

7. RECOMMENDATIONS

- ♣ Adoption of HACCP principle at various critical points from farm to table, as a tool to determine the precise sources of contamination, is of paramount importance in designing appropriate strategies to substantially reduce the prevalence and associated risk for consumer and trade partners. Along this, continuous monitoring of antimicrobial resistance pattern and phage type of isolates (finger printing of isolates) assists in tracing origin of strains causing human salmonellosis thereby limiting prevalence.
- ♣ Encouraging prudent and judicious use of antimicrobial drugs in veterinary and public health sectors.
- ♣ Establishment and enforcement of legislation which prohibit handling food unless free from *Salmonella* or excretion (shedding) is terminated.
- ♣ Improve method and hygiene of meat transport from slaughterhouse to retail markets such as use of refrigerated transport vehicle.
- ♣ There is a need to gather sufficient information on the occurrence and distribution of *Salmonella* serotypes starting from farm level in order to control food contamination.
- ♣ The relative importance of size of supermarket and type of chicken carcass (local versus commercial) as risk factor along with management practices on *Salmonella* prevalence requires further investigation.
- ♣ There is a need to educate consumers, food handlers and all others who have access to food about the importance of hygiene, cooling system, etc.

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Appendix 3. Composition and method of preparation of media and anti-sera used for laboratory work

1. Buffered Peptone Water (BPW) – SIFIN, Berlin, Germany

Typical formula (g/l):

- Peptone from casein.....10.0
- Sodium chloride5.0
- Di-sodium hydrogen phosphate.....3.5
- Potassium dihydrogen phosphate.....1.5

Direction: Dissolve 20 g in 1 litre aqua dist. And sterilize by autoclaving at 121 °C for 15 minutes.

2. Selenit-Cystine of 500 g (SIFIN, Berlin, Germany)

Typical formula (g/l):

- Peptone from casein.....5.0
- L(-)cystine.....0.01
- Lactose.....4.0
- Phosphate buffer10.0
- Sodium hydrogen selenite4.0

Direction: Suspend 23 g in 1 litre of de-mineralized water at room temperature. If necessary, warm shortly (max. 60 °C) filter-sterilize if storage is planned; dispense into suitable containers. Don't autoclave. pH :7.0 ±0.2 at 25 °C

3. Magnesium chloride Malachite Green Broth acc. To Rappaport – Vassiliadis (RV-Medium) of 500 g. (SIFIN, Berlin, Germany).

Typical formula (g/l):

- Peptone from casein and soy.....5.0
- Magnesium chloride (anhydrous).....18.73
- Sodium chloride.....8.0
- Potassium dihydrogen phosphate.....1.6
- Malachite green.....0.04

pH 5.2 ±0.2

Direction: Dissolve 33.4 g in 1 litre aqua. Dest., mix well and dispense 10 ml in tubes.

Sterilize by autoclaving 20 minutes at 115 °C.

4. Triple Sugar Iron Agar (Difco, Detroit, USA)

Approximate formula per liter:

- Beef extract.....3.0 g
- Yeast extract.....3.0 g
- Pancreatic digest of casein.....15.0g
- Proteose peptone N0. 3.....5.0g
- Dextrose.....1.0g
- Lactose.....10.0g
- Sucrose.....10.0g
- Ferrous sulfate.....0.2g
- Sodium chloride.....5.0g
- Sodium thiosulfate.....0.3g
- Agar.....12.0g
- Phenol red.....0.024g

Directions: Suspend 65 g of powder in 1 litre of purified water. Mix thoroughly. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder. Dispense into tubes and autoclave at 121 °C for 15 minutes. Cool in a slanted position so that deep butts are formed.

4. Lysine Decarboxylase Broth of 500 g (Difco, Detroit, USA).

Formula per litre:

- Bacto peptone.....5 g
- Bacto yeast extract.....3 g
- Bacto dextrose.....1 g
- L-lysine.....5 g
- Bacto Brom Cresol Purple... ..0.02 g

Directions: Suspend 14 grams in 1 litre distilled water or deionized water and boil to dissolve completely. Sterilize at 121 – 124 °C for 15 minutes. Final pH: 6.8 ± 0.2 at 25 °C.

5. Simmons Citrate agar of 500 g (Difco, Detroit, USA).

Formula per litter:

- Magnesium sulfate0.2 g
- Ammonium Dihydrogen phosphate.....1 g
- Dipotassium phosphate.....1 g
- Sodium citrate.....2 g
- Sodium chloride.....5 g
- Bacto Agar.....15 g
- Bacto BromThymol Blue.....0.08 g

Direction: Suspend 24.2 g in 1 litre distilled or deionized water and boil to dissolve completely. Sterilize at 121 – 124 °C for 15 minutes. Final pH: 6.8 ± 0.2 at 25 °C.

6. Urea Broth (Merck, Germany)

Typical formula (g/l):

- Yeast extract0.1
- Potassium dihydrogen phosphate.....9.1
- Disodium hydrogen phosphate.....9.5
- Urea.....20.0
- Phenol red0.01

Preparation: Dissolve 38.5 g/l, if necessary heat up to a temperature of 60 °C. Sterilize by filtration or dispense aliquots of approximately 3 ml into test tubes and sterilize for 5 minutes in a current of steam under mild conditions. Don't autoclave!. pH = 6.8.±0.1. The broth is clear and yellow-orange. If filter sterilization or heat sterilization is not possible, the medium must be inoculated as soon as it has been prepared.

7. Brilliant Green Phenol Red Agar acc. to Edel and Kampelmache (BPLS Agar, mod.) of 500 g. (Sifin, Berlin, Germany).

Typical formula (g/l):

- Peptone..... 10.0
- Meat extract.....5.0
- Yeast extract.....3.0
- Lactose.....10.0
- Saccharose.....10.0
- Disodium hydrogen phosphate.....1.0
- Sodium dihydrogen phosphate.....0.6
- Brilliant green.....0.005
- Phenol red.....0.09
- Agar.....10.0

pH 7.0 ± 0.2

Directions: Suspend 49.7 g in 1 litre of distilled water; carefully bring to the boil with frequent agitation to dissolve completely. Don't autoclave! Mix well and pour into petridishes.

8. XLD – Agar acc. ISO 6579 (Xylose-lysine-desoxycholate agar) of 500 g (Sifin, Berlin, Germany).

Typical formula (g/l):

- Yeast extract.....	3.0
- L-Lysine hydrochloride.....	5.0
- Xylose.....	3.75
- Lactose.....	7.5
- Sucrose.....	7.5
- Sodium deoxycholate.....	1.0
- Sodium chloride.....	5.0
- Sodium thiosulphate.....	6.8
- Iron (III) ammonium citrate.....	0.8
- Phenol red.....	0.08
- Agar.....	16.5

pH: 7.4 ± 0.2

Directions: Suspend 57 g in 1 liter of distilled water; carefully bring to the boil with frequent agitation to dissolve completely. Don't autoclave! Mix well and pour into Petri dishes.

Storage: Dry, tightly closed, at 10 – 25 °C.

9. Rappaport – Vassiliadis (RV) enrichment broth of 500 g (Oxoid, England)

Typical formula (g/l):

- Soya peptone5.0 g
- Sodium chloride.....8.0 g
- Potassium dihydrogen phosphate1.6 g
- Magnesium chloride40.0 g
- Malachite green.....0.04 g

Directions: Weigh 30 g (the equivalent weight of dehydrated medium per liter) and add to 1 liter of distilled water. Heat gently until completely dissolved. Dispense 10 ml volumes into screw-capped bottles or tubes and sterilize by autoclaving at 115 °C for 15 minutes. This medium is very hygroscopic and must be protected from moisture.

10. Nutrient agar of 500 g (Oxoid, England).

Typical formula (g/l):

- Lab-Lemco powder.....1.0
- Yeast extract2.0
- Peptone.....5.0
- Sodium chloride.....5.0
- Agar.....15.0

pH: 7.4 ± 0.2

Directions: Suspend 28 g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilize by autoclaving at 121 °C for 15 minutes.

11. Rambach Agar (Merck, Germany)

Typical composition (g/l):

Peptone 8.0; Sodium chloride 5.0; Sodium deoxycholate 1.0; Chromogenic mix 1.5; Propylene glycol 10.5; Agar-agar 15.0.

Preparation:

1. Add 1 vial of liquid- mix to 250 or 1000 ml distilled water and mix by swirling until completely dissolved. (The water quantity is dependent on the respective pack – sizes)
2. Add 1 vial of nutrient powder and mix by swirling until completely suspended.
3. Heat in a boiling water bath or in a current of steam, while carefully shaking from time to time. The medium is totally dissolved, if no visual particles stick to the glass-wall. The medium should not be heat treated further. Standard time for complete dissolution (shaking in 5 minutes sequence):

250 ml: 20 – 25 minutes

1000 ml: 35 – 40 minutes

Don not autoclave, do not over heat.

4. Cool the medium as fast as possible in a water-bath (45 – 50 °C). During this procedure 9 (max 30 minutes) gently shake the medium from time to time. Pour into plates.
5. In order to prevent any precipitate or clotting of the chromogenic- mix in the plates, it is advisable to place Petri-dishes during pouring procedure-on a cool (max. 25 °C) surface.
6. The ready plates are opalescent and light pink. Before inoculation, the plates should be dry. pH: 7.3 ± 0.2 at 25 °C.
7. Shelf life and storage conditions of fresh prepared plates:

Room temperature: 12 hrs.

In the fridge (not below 6 °C) unsealed: 3 weeks.

In the fridge (not below 6 °C) sealed in plastic pouch or with tape: 3 months.

12. Brain Heart Infusion Agar (Merck, Germany)

Typical composition (g/l):

Nutrient substrate (extracts of brain and heart, and peptone) 27.5; D (+) Glucose 2.0; Sodium chloride 5.0; di-sodium hydrogen phosphate 2.5; Agar-Agar 15.0.

Preparation:

Suspend 52 g in 1 liter of de-mineralized water by heating in a boiling water bath or in a current of steam; autoclave (15 minutes at 121 °C). pH: 7.4 ± 0.2 at 25 °C.

13. Polyspecific test serum “anti-salmonella II” (Sifin, Germany). This consists of sera from immunized rabbits and /or sheep. It contains O agglutinins against the groups F to 67. It is an unabsorbed serum. The serum is lyophilized and ready for use after dissolution in 1 or 5 ml of distilled water. Preservative: sodium azide 1 mg/ml.

Applications: The test serum is used to detect *Salmonella* strains from test material of both human and animal origin in slide agglutination. First, the bacteria of suspected colonies are tested with the polyspecific test reagent “Anti-*Salmonella* I”. This test reagent covers more than 90 % of all *Salmonella* isolates. The “Anti-*Salmonella* II” unabsorbed polyspecific test serum should only be applied, if the test with the test reagent “Anti-*Salmonella* I” did not yield a positive result and the *Salmonella* genus has been confirmed by biochemical tests.

Appendix 4. Interpretation of the biochemical tests (ISO 6579, 1998; D' Aoust and Purvis, 1998)

Test ¹⁾ and reaction	Observation		Typical <i>Salmonella</i> reaction	% of <i>Salmonella</i> inoculations showing the reactions ²⁾
	Positive	Negative		
TSI – glucose utilization	+, butt yellow		Positive	100
TSI–gas formation	+, gas pocket in the medium	-, no gas	Positive	91.9 ³⁾
TSI- lactose utilization	+, slant turns yellow	-, color of slant unchanged	Negative	99.2 ⁴⁾
TSI – sucrose utilization	+, slant turns yellow	-, color of slant unchanged	Negative	99.5 (a)
TSI – H ₂ S Production	+, black butt and/or slant	-, no blackening	Positive	91.6 (b)
Urea splitting, urease production	+, slant turns pink / red	-, color of slant unchanged	Negative	99
Lysine decarboxylation, lysine decarboxylase production	+, butt remain purple	-, butt turns yellow	Positive	94.6 ⁵⁾
Beta-galactosidase reaction	+, yellow color	-, colorless after 24 hours	Negative	98.5 ⁴⁾
Voges-Proskauer (MR-VP broth), acetoin derived from glucose	+, red	-, colorless	Negative	100
Indole test (tryptophan utilization	+, reagent layer deep red (tryptose water)	-, reagent layer yellow	Negative	98.9
Simmons citrate, citrate utilization	+, blue	-, green	Positive	

- 1) Ewing, W.H. and Ball, M.M. “*The biochemical reactions of members of the genus Salmonella.*” National Communicable Disease Center, Atlanta, Georgia, USA (1996).
- 2). These percentages indicate only that not all strains of *Salmonella* show the reactions marked + or -. These percentages may vary from country and food product to food product.
- 3). *Salmonella typhi* is anaerogenic.
- 4). The *Salmonella* Subgenus III (Arizona) gives positive or negative lactose reactions but is always beta – galactosidase positive. The *Salmonella* subgenus II gives a negative lactose reaction, but gives a positive beta-galactosidase reaction. For the study of strains, it may be useful to carry out complementary biochemical tests.
- 5). *S. paratyphi* A is negative.
 - (a)= Some strains can utilize one or both substrates
 - (b)= Slow H₂S producers may be encountered. The H₂S reaction may be inhibited in lactose and / or sucrose –utilizing strains.

Appendix 5. Location of sampling sites, type and number of samples used for the study

Code	Source	Name	Type of sample	NSBW	TNS	Kifle Ketema
1	Supermarket	Shisolomon -1	C, MB, M, P & S	6	90	Kirkos
2	Supermarket	Loyal	C, MB, M, P & S	4	38	Arada
3	Supermarket	Berta	C, MB, M & S	3	23	Kirkos
4	Supermarket	Bambis	C, MB, M, P & S	8	89	Kirkos
5	Supermarket	Betelihem	C & MB	2	11	Yeka
6	Supermarket	Fantu - 1	C, MB, M, P & S	4	51	Bole
7	Supermarket	Felix	C, MB, M, P & S	4	21	Kirkos
8	Supermarket	Fantu -2	C, MB, M, P & S	3	34	Bole
9	Supermarket	Novis - 1	C, MB, M, P & S	5	55	Bole
10	Supermarket	Eyo-ta-1	C & MB	1	6	Kirkos
11	Supermarket	Ethio	C & MB	1	12	Kirkos
12	Supermarket	Hadiya	C, MB, M & S	2	32	Kirkos
13	Supermarket	Supersave	C, MB & S	1	11	Nifas silk lafto
14	Supermarket	Fantu - 3	C, MB, M, P & S	8	31	Nifas silk lafto
15	Supermarket	Novis -2	C, MB, M, P & S	5	56	Nifas silk lafto
16	Supermarket	Solsis	C, MB & M	3	36	Lideta
17	Supermarket	Belonias	C, MB & S	2	13	Arada
18	Supermarket	Fantu-4	C, MB, M & S	3	15	Kirkos
19	Supermarket	Gebreal	C, MB & S	2	13	Nifas silk lafto
20	Supermarket	Eyo-ta-2	C &MB	1	2	Kirkos
21	Supermarket	The twins	C, MB &M	1	17	Bole
22	Supermarket	Shopper's mart	C &S	2	9	Nifas silk lafto
23	Supermarket	Victory	C, MB & S	4	19	Nifas silk lafto
24	Supermarket	Alamaz	C	1	10	Kirkos
25	Supermarket	Central	C, MB, M & S	2	34	Bole
26	Supermarket	Meskerem	C	1	2	Bole
27	Supermarket	Negash	C, MB & S	1	12	Lideta
28	Supermarket	Novis-3 (Hilton)	C, MB, M, P	2	19	Kirkos
29	Supermarket	Shisolomon-2	C, MB, M & S	3	22	Kirkos
30	Supermarket	Abrico	C, MB, M	3	3	Bole

31	Supermarket	Blue	C, MB & S	2	16	Arada
32	Supermarket	Populare	C, MB, M & S	4	22	Lideta
I	Pastry shop	Pelican	Ice cream		15	Bole
II	Pastry shop	Saay	Ice cream		12	Bole
III	Pastry shop	Chentro	Ice cream		9	Arada
IV	Pastry shop	Sofnia	Ice cream		9	Arada
V	Pastry shop	Maleda	Ice cream		12	Arada
VI	Pastry shop	Romina	Ice cream		12	Arada
VII	Pastry shop	Top view	Ice cream		1	Arada
VIII	Pastry shop	Fasika	Ice cream		3	Gulele
IX	Pastry shop	Betelihem	Ice cream		12	Yeka
X	Pastry shop	Lidia	Ice cream		6	Yeka
XI	Pastry shop	Classic	Ice cream		6	Bole
XII	Pastry shop	Purple café	Ice cream		12	Kirkos
XIII	Pastry shop	London café	Ice cream		1	Bole
XIII	Pastry shop	Bekele Molla	Ice cream		1	Bole
XV	Pastry shop	Dandi	Ice cream		6	Kirkos
XVI	Pastry shop	Soul kid	Ice cream		2	Arada
15	Supermarket	Novis-2	Ice cream		7	Nifas silk lafto
A	Fish shop	Melese Debella	Tilapia fish		33	Arada
B	Fish shop	TenkirW/Mariam	Tilapia fish		38	Arada
C	Fish shop	Asamirt.G.D	Tilapia fish		28	Kirkos
D	Fish shop	MintidegG/michael	Tilapia fish		8	Arada
I	Fish shop		Tilapia fish		8	Arada
J	Fish shop		Tilapia fish		13	Arada
F	Open market	Addisu Gebeya	Cottage cheese		12	Gulele
G	Open market	Shola	Cottage cheese		102	Yeka
H	Open market	Merkado	Cottage cheese		76	Addis Ketema

TNS= Total Number Sampled

NSBW=Number of Supermarket Butchery Workers

C = Chicken carcass, P = Pork, M = Mutton, MB = Minced beef,

10. CURRICULUM VITAE

PERSONAL DATA

Name: Endrias Zewdu Gebremedhin
Sex: Male
Address: Ambo College of Agriculture. P.O.Box 19 Ambo - Ethiopia
Tel. 251-1-36 24 63 (office), 36 26 10 (home)
e-mail: endriaszewdu@yahoo.com
Place of birth: Messella, Ethiopia
Date of birth: 4 August, 1965
Citizenship: Ethiopian
Marital status: Married
Children: 2 daughters and a son.
Home address: Ambo College of Agriculture. P.O.Box, 19, Ambo, Ethiopia
Tel. 251-1-36 26 10 (home)
Language: English, Amharic and Oromiffa

EDUCATIONAL BACKGROUND

Name and place	From to	Description (Degree, Diploma, Certificate)
Messella Kindergarten School, Messella	1970 - 1971	-
Messella Primary and Junior Secondary School, Messella	1972 - 1979	Certificate
Harar Medhanealem Comprehensive Secondary School, Harar	1980 - 1982	-
Dire Dawa Comprehensive Secondary School, Dire Dawa	1983	Certificate
Addis Ababa University, Faculty of Veterinary	1984 - 1989	DVM (Doctor of

Medicine		Veterinary Medicine with Distinction)
Introduction to Applied Statistics for African Animal Scientists, ILCA, Addis Ababa	June 18-29, 1990	Certificate
Teaching and Learning, Debre Zeit, Ethiopia	August 5-27, 1991	Certificate
Larenstein International Agr. College, The Netherlands	August 1992 to June 1993	Diploma (Tropical Animal Production

OTHER

- Certificate on managing and administering higher Education in Ethiopia, from August 19 to 23, 2002, organized by Ministry of Education in collaboration with The British Council Ethiopia.
- Computer skill on word processing, spreadsheet and database
- September 2002 - on wards, graduate student of the Addis Ababa University, Faculty of Veterinary Medicine, Debre Zeit.

EMPLOYMENT RECORD

POSITION

Date of employment: - September 1989
 Post: - Lecturer and veterinarian
 Employer: - Higher Education Main Department.
 Ministry of Education

TASKS:

- ❖ Offering lecture in the field of Animal health and disease control from 1989 up till now and apiculture starting 1999. Additionally I have taught Introduction to Animal Breeding & Animal Feed & Nutrition courses at time of manpower shortage in the department.
- ❖ Veterinarian in charge of Ambo College of Agriculture's dairy, beef, poultry, sheep and bee farms
- ❖ Initiator and developer of ox fattening project at Ambo College of Agriculture & responsible person for health & routine management aspects since 1995.
- ❖ Head of Department of Animal Sciences from Feb. 1998 to Jan. 2000.
- ❖ Head of the Registrar Office from Feb. 2000 to September 2002.

RESEARCH / PROJECT WORK EXPERIENCE

- ❖ Sero-prevalence study of bovine brucellosis in selected sites of Sidamo region, DVM thesis, 1989.
- ❖ Pilot study on bovine tuberculosis in cattle and its implication in man in selected sites of Ethiopia (collaborative research)
- ❖ Developed project, together with department members about practical training in bee keeping & small-scale poultry production for disabled individuals (FAO Sponsored)
- ❖ Developed project on beekeeping for Muger Cement Enterprise in August 2000

- ❖ Provide community service in the area of livestock health, production and management for the surrounding small scale farmers and private investors.

EXTRA CURRICULAR ACTIVITIES

- Member of Research, Extension & publication Committee from 1995 – 2001.
- Member of Curriculum Review & Development Committee form 1997 – 2001.
- Chairman of Credit & Saving Association from 1994 – 1996.
- Chairman of Discipline Committee 1997 – 1999.
- Member of Farm Management Technical Committee: 1998 – 2000.
- Served the college in several Ad-hoc Committees.

MEMBERSHIPS TO SCIENTIFIC SOCIETIES

- ♣ Ethiopian Veterinary Association (EVA)
- ♣ Ethiopian Society of Animal Production (ESAP)

HOBBIES

- Reading, music, indoor games and tour