PREVALENCE AND ANTIBIOTIC SUSCEPTIBILITY PROFILE OF *SALMONELLA* ISOLATES AMONG DIARRHOEAL PATIENTS VISITING DESSIE REFERRAL HOSPITAL, NORTH EAST ETHIOPIA

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Prevalence and Antibiotic Susceptibility Profile of *Salmonella* Isolates among Diarrhoeal Patients Visiting Dessie Referral Hospital, North East Ethiopia

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As Thesis Research advisor, we hereby certify that we have read and evaluated this thesis prepared, under our guidance, by Samuel Chane, entitled **''Prevalence and Antibiotic Susceptibility Profile of *Salmonella* Isolates among Diarrhoeal Patients Visiting Dessie Referral Hospital, North East Ethiopia''**. We recommend that it can be submitted as fulfilling of the thesis requirement.

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DEDICATION

This thesis is dedicated to my beloved parents who have devoted their lives to support my educational career.

STATEMENT OF THE AUTHOR

By my signature below, I declare and affirm that this thesis is my own work. I have followed all ethical and technical principles of scholarship in the preparation, data collection, data analysis and compilation of this thesis. Any scholarly matter that is included in the thesis has been given recognition through citation.

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LIST OF ACRONYM AND ABBREVATIONS

CDC	Center for Disease Control
DRH	Dessie Referral Hospital
DRL	Dessie Regional Laboratory
EFSA	European Food Safety Authority
LIA	Lysine Iron Agar
MDR	Multi Drug Resistance
NCCLS	National Committee for Clinical Laboratory Standards
NTS	Non Typhoidal *Salmonella*
OPD	Outpatient Department
SPSS	Statistical Package for Social Sciences
TMP–SXT	Trimethoprim–Sulfamethoxazole
TSI	Triple Sugar Iron
WHO	World Health Organization
XLD	Xylose-Lysine Desoxycholate

BIOGRAPHICAL SKETCH OF THE AUTHOR

The author was born on January 21, 1986 in North Wollo Zone, Woldia town. He attended his elementary education at Etegie Taitu Bitul Primary School. He also attended his high school and preparatory education at Woldia Secondary and Preparatory School, respectively. He joined Addis Ababa University in 2005 and graduated with Bachelor of Science Degree in Biology in July 2007 and he directly joined Postgraduate Program of Biology Department, Haramaya University in September 2008 to pursue his post-graduate study in Applied Biology by the sponsorship of Ministry of education.

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ABSTRACT

Salmonellosis, a disease caused by Salmonella, remains an important public health problem worldwide, particularly in the developing countries. A cross sectional descriptive survey study was conducted to determine the prevalence and antibiotic susceptibility profile of Salmonella species among diarrhoeal patients who were visiting Dessie Referral Hospital (DRH), Dessie, North East Ethiopia from November 2016 - January 2017. A total of 384 stool samples were collected using sterile stool cups. Out of these 20 (5.21%) were found to be positive for Salmonella species. The distribution of positive samples among the three age groups indicated that Salmonella species were predominantly prevalent in the age group 0.25 (three months) to 4 year's old patients. Abdominal pain, vomiting, watery consistency of stool and 1 to 5 days of diarrhea were the clinical features that were significantly associated with salmonellosis. Consumption of raw vegetables and fruits, consumption of street vended foods, cohabitation of animals, use of water from unprotected source, absence of latrine and consumption of raw products of animals such as eggs and raw milk were the risk factors that were significantly associated with prevalence of Salmonella species. Antibiotic sensitivity test was performed for the isolated Salmonella species against 5 currently recommended antibiotics. The antimicrobial sensitivity study carried out using the Kirby-Bauer disk diffusion method showed that 100% and 80% of the Salmonella isolates were sensitive to Ciprofloxacin and Nalidic acid respectively. Of the 20 Salmonella isolates, 100%, 85% and 80% were resistant to Ampicillin, Tetracycline and Trimethoprim-sulfamethoxazole, respectively. This study indicated that Salmonella species were prevalent among diarrheal patients who were visiting DRH and should, therefore, be considered routinely in the diagnosis of patients with diarrhea cases. The physicians should also prescribe the appropriate drugs either after sensitivity testing or referring to updated information on local antibiotic sensitivity patterns.

***Key words**: - Antibiotic resistance, Dessie, Diarrhoea, Risk factors, Salmonellosis.*

1. INTRODUCTION

Human diarrheal diseases have been recognized since the beginning of civilization and remain one of the most prevalent public health problems of today. Diarrhoea is a condition of having three or more loose or liquid bowel movement per day (WHO, 2010). The disease is known to be caused by several factors including infectious and non-infectious agents such as metabolic disorders, food allergy and other organic compounds. Diarrhoea, which is usually occurring due to bacterial, viral, or parasitic infection, is a common problem that usually lasts 1 or 2 days and resolves on its own without special treatment (Chen *et al*., 2010). Infectious diarrhea is characterized by an alteration in the normal bowel movement, an increase in the volume of water content or frequency of stools, nausea, vomiting, cramps and abdominal discomfort. Diarrhoeal infection spreads through the ingestion of contaminated food or drinking-water, or person-to-person contact as a result of poor hygiene. There are three clinical types of diarrhoea: (i) acute watery diarrhoea which lasts several hours or days and includes cholera; (ii) acute bloody diarrhoea, also called dysentery; and (iii) persistent diarrhoea that lasts 14 days or longer (Kosek, *et al*., 2003). Diarrhoea alone kills more children than AIDS, malaria, and measles combined (WHO/UNICEF, 2009).

In developed countries acute infectious diarrhoea results in more than 1.5 million visits to doctors and 200,000 hospitalizations annually (Kotloff *et al*., 2013). Diarrhea in young children living in poverty can be so severe that it results in death by dehydration. In Ethiopia, as in other sub-Saharan African countries, morbidity from diarrheal diseases is a serious health problem (Kosek *et al*., 2003). Moreover, during a period of draught and famine, acute childhood diarrhea becomes the leading cause of death across all age groups in Ethiopia (Yemane *et al*., 2006).

The estimated number of yearly childhood deaths in Ethiopia attributable to diarrhea at 95,000 based on an overall under-five mortality rate of 170 deaths /1000 and the assumption that 20% of those were caused by diarrhea (Yemane *et al*., 2006).

Diarrhea can be caused by many etiological agents, but mainly by *Enterobacteriaceae* such as *Escherichia coli*, *Salmonella* species, *Shigella* species, *Campylobacter jejuni*, *Vibrio cholerae* as well as parasites such as *Entamoeba histolytica* and *Giardia intestinalis*, and some rotaviruses (Guerrant *et al*., 2002). Among the different pathogens responsible for diarrheal diseases, *Salmonella* plays an important role.

Salmonellosis, the disease caused by *Salmonella,* is one of the most frequently occurring food-borne diseases worldwide (Puthucheary *et al*., 2004). As a result it continues to be a major health burden worldwide. Even in developed countries, for instance in USA, there was an estimate of 1.4 million non-typhoid *Salmonella* infections, resulting in 168,000 visits to physicians, 15,000 hospitalizations and 580 deaths annually (WHO, 2005). As reported by Mikhail *et al*. (1990), there was a prevalence of 2.9% *Salmonella* infection in human diarrheal cases in Djibouti. In Ethiopia, Getachew *et al*. (2014) reported 10.5% *Salmonella* prevalence in Butajira. Getnet and Haimanot (2014) reported 6.2% *Salmonella* prevalence in Jimma Health Center while Alex Ayalu *et al*. (2011) reported a prevalence of 11.5% *Salmonella* infection in Harar among patients who were admitted to hospital. Getnet *et al*. (2011) reported 5.3% *Salmonella* infection in children of Addis Ababa and Jimma at Tikur Anbessa Hospital and Jimma University Hospital, respectively.

Salmonellosis is characterized by acute onset of fever, abdominal pain, diarrhea, nausea and sometimes vomiting. In a small percentage of cases, septicemia and invasive infections of organs and tissues can occur, leading to diseases such as osteomyelitis, pneumonia, and meningitis (CDC, 2001). These diseases can generally become serious problems in an area in the presence of other diseases and factors that weaken the immune system as well as with the development of antibiotic resistance in *Salmonella* species.

The genus *Salmonella* is divided into two species: *Salmonella enterica* (comprising six subspecies) and *Salmonella bongori*. Over 99% of human *Salmonella* spp. infections are caused by *S. enterica* subsp. *enterica* (Bell and Kyriakides, 2002; Crum-Cianflone, 2008).

Salmonella Enteriditis and *Salmonella* Typhimurium are ubiquitous serovars which affect humans and other animals causing gastroenteritis that is less severe than enteric fever (Velge *et al*., 2005). These two serovars are the predominant serotypes associated with human disease in most countries (EFSA, 2005).

They have been reported as having the potential to cause epidemics and become the dominant serovars in many countries in the foreseeable future (WHO, 2005). The spread of *S*. Typhimurium in sub-Saharan Africa is a public concern too. As a result of the wide spread of HIV in Sub-Saharan Africa, it is believed that there is a high prevalence of non-typhoidal *Salmonella*, mainly *S*. Typhimurium and *S*. Enteriditis serotypes that cause bacteremia in these areas. Some *S*. Typhimurium strains are particularly important because of their multidrug resistance genes and worldwide dissemination (Ruiz *et al*., 2008).

Salmonella Typhi and *Salmonella* Paratyphi are specifically associated with infections in humans, leading to severe disease called enteric fever. *S*. Typhi and *S*. Paratyphi produce clinical syndromes referred to as typhoid and paratyphoid fever, respectively. Enteric fever is rare in developed countries, with the majority of cases associated with overseas travel (Darby and Sheorey, 2008). The global incidence of typhoid is estimated to be about 21 million with 700,000 deaths each year primarily in South East Asia, Africa and Latin America attributed to rapid population growth, inadequate and improper waste disposal, and lack of safe water supply. That is why, in the late 1970s, typhoid fever was the major health problem in Addis Ababa (Getnet *et al*., 2008).

Salmonella spp. are transmitted by the faecal-oral route by either consumption of contaminated food or water, person-to-person contact, or from direct contact with infected animals (Jay *et al*. 2003).

In Dessie town, particularly the rural area of Dessie, there are a number of risk factors which lead to gastrointestinal infections. For example, poor sanitary conditions, cattle living together with people, drinking raw milk and eating raw meat, and consumption of street vended foods are common practices. These factors are directly or indirectly related to consumption of water and foods. According to Puthucheary *et al*. (2004), contaminated water and foods are responsible for initiating salmonellosis.

Among Dessie town health institutions, particularly at Dessie Referral Hospital, the episode of diarrhoeal diseases record is very high (Personal communications with the Hospital Director). There was, however, information gap on major etiological agents of diarrhoeal diseases and the profile of their antibiotic resistance in the study area. So far, no report exists regarding the prevalence and antibiotic resistance patterns of *Salmonella* species in the selected study area (Personal communications with the Hospital Director).Thus, this study was proposed to investigate the prevalence and antibiotic resistance patterns of *Salmonella* among diarrhoeal patients visiting DRH, Dessie town, North East Ethiopia.
The specific objectives were:

1. To determine the prevalence of *Salmonella* spp. among diarrheal patients visiting DRH.

2. To determine the antibiotic susceptibility of *Salmonella* isolates to common antimicrobial agents.

3. To investigate the association between risk factors, clinical features and *Salmonella* infection.

2. LITERATURE REVIEW

2.1. General Characteristics of *Salmonella* species

Members of the genus *Salmonella* are ubiquitous pathogens found in humans and livestock, wild animals, reptiles, birds, insects (Getenet, 2008) and can multiply under various environmental conditions outside the living hosts (Pui *et al*., 2011). *Salmonella* make up a large genus of gram-negative bacilli within the family *Enterobacteriaceae* and it constitutes 2,579 *Salmonella* serotypes that are highly adapted for growth in both humans and animals and that cause a wide spectrum of disease (Grimant and Weill, 2007). The growth of *Salmonella* Typhi and *Salmonella* Paratyphi is restricted to human hosts, in whom these organisms cause enteric (typhoid) fever. The remainder of the *Salmonella* serotypes, referred to as non-typhoidal *Salmonella*, can colonize the gastrointestinal tracts of a broad range of animals, including mammals, reptiles, birds, and insects. More than 200 of these serotypes are pathogenic to humans, in whom they often cause gastroenteritis and can also be associated with localized infections and/or bacteremia (Fuaci and Jameson, 2005).

Salmonellae are gram-negative, non-spore forming, facultative anaerobic bacilli, and 2 to 3 by 0.4 to 0.6 μm in size (Getenet, 2008). They do not require sodium chloride for growth, but can grow in the presence of 0.4 to 4% Nacl. Most *Salmonella* serotypes grow at a temperature range of 5 to 47°C with optimum temperature of 35 to 37°C but some can grow at temperatures as low as 2 to 4°C or as high as 54°C. They are sensitive to heat and often killed at a temperature of 70°C or above. *Salmonella* grows in a pH range of 4 to 9 with the optimum being between 6.5 and 7.5. They require high water activity (aw) between 0.99 and 0.94 (pure water, aw=1.0); yet they can survive at a water activity of less than 0.2 such as in dried foods. Complete inhibition of growth occurs at temperatures less than 7°C, pH less than 3.8 or water activity less than 0.94 (Pui *et al*., 2011). Like other members of the family *Enterobacteriaceae,* they produce acid on glucose fermentation; reduce nitrates to nitrite, and don't produce cytochrome oxidase. In addition, all salmonellae except *S.* gallinarum-pullorum are motile by means of peritrichous flagella, and all but *S*. Typhi produce gas (H_2S) on sugar fermentation (Fuaci and Jameson, 2005; Getenet, 2008). *Salmonella* are non- capsulated except *S*. Typhi, *S*. Paratyphi C and some strains of *S*. Dublin (Getenet, 2008).

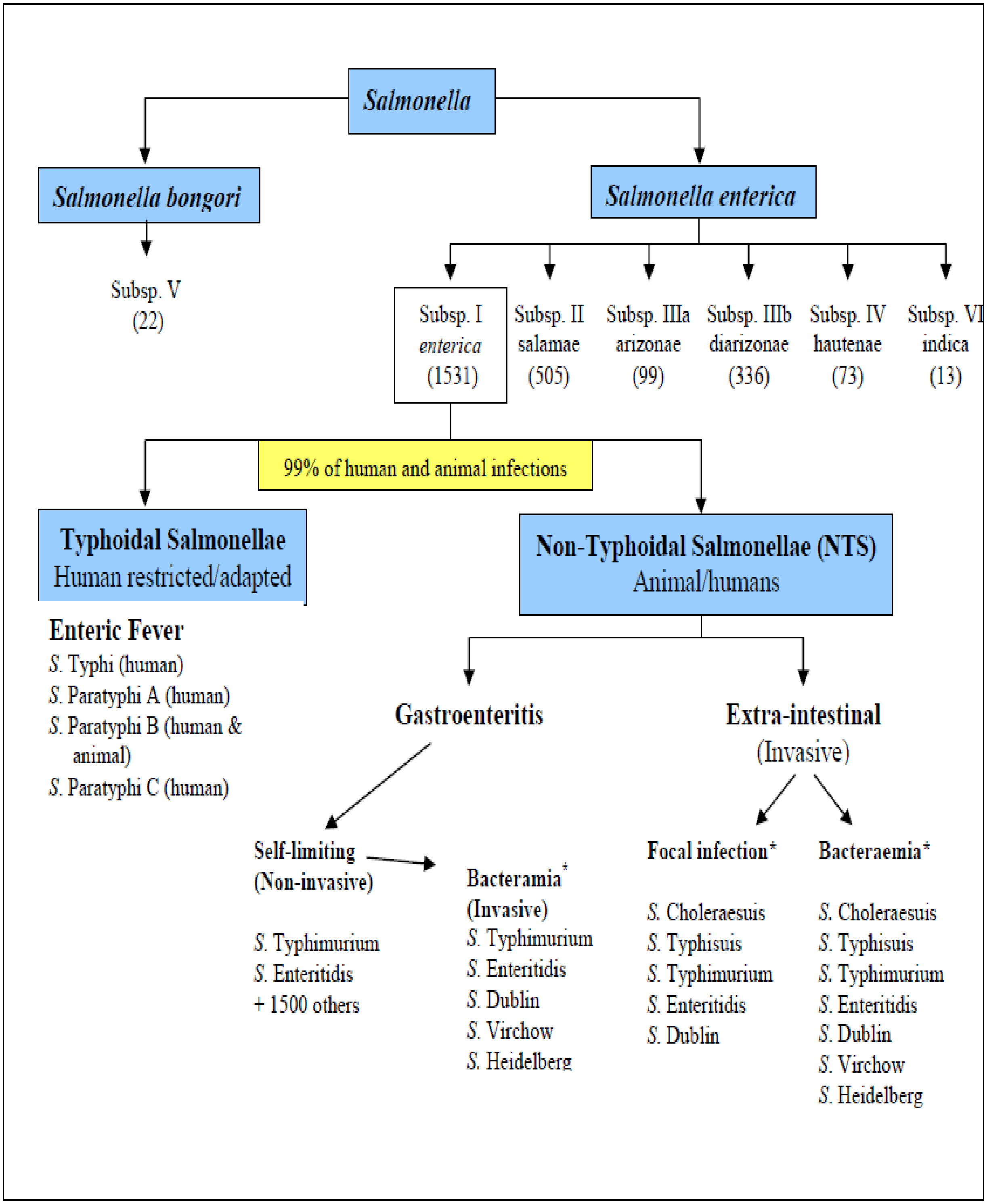
2.2. Taxonomy and Nomenclature of *Salmonella* species

A non-human *Salmonella* spp., *Salmonella* Choleraesuis, was isolated from a swine's intestine by Theobald Smith (1859-1934) under the direction of Daniel E. Salmon (1850-1914) in 1885 (Galanis *et al*., 2006). *Salmonella* nomenclature is complex, and scientists use different systems to refer to and communicate about this genus. The current usage often combines several nomenclatural systems that inconsistently divide the genus into species, sub species, subgenera, groups, subgroups, and serotypes (serovars) which causes confusion (Brenner *et al.*, 2000).

The nomenclature of the genus *Salmonella* has evolved from the initial one serotype-one species concept proposed by Kauffmann on the basis of the serologic identification of O (somatic) and H (flagellar) antigens. A capsular polysaccharide, the VI antigen is present on *Salmonella* Typhi and few other serovars of *Salmonella*, including *Salmonella* Dublin (Heyndrickx *et al.*, 2005). The defining development in *Salmonella* taxonomy occurred in 1973 when Corsa demonstrated by DNA-DNA hybridization that all serotypes and subgenera I, II and IV of *Salmonella* and all serotypes of "Arizona" were related at the species level, thus belonging to a single species. The single exception subsequently described is *Salmonella bongori* previously known as sub species V which by DNA-DNA hybridization is a distinct species (Tindall *et al.*, 2005).

The current nomenclature used by CDC is based on the recommendations from the WHO collaborating centre and it adequately addresses the concern and requirements of clinical and public health microbiologists (Popoff *et al.*, 2001). The nomenclature is summarized in the figure 1. Currently, there are 2,579 *Salmonella* serotypes (Grimant and Weill, 2007). Most of these serotypes, including *Salmonella*, serotype Typhi, belong to sub species I (1,531 recognized serotypes) and are found in O groups A, B, C, C2, D and E. The two most commonly isolated serotypes in the United States are *Salmonella* serotypes Typhimurium and Enteritidis (Bopp *et al*., 2003).

Serotypes belonging to sub species II (505 serotypes), IIIa (99 serotypes), IIIb (336 serotypes), IV (73 serotypes), VI (13 serotypes) and *S. bongori* (22 serotypes) are found primarily in O groups (Grimant and Weill, 2007).



Note. Numbers in brackets indicate the total number of serotypes included in each subspecies. * Common serotypes are listed but other serotypes may cause bacteraemia or focal Infection; subsp = subspecies.

Figure 1. Classification of the genus *Salmonella.* Source: Langridge *et al.* (2008).

2.3. Salmonellosis and Its Pathogenesis

With respect to human disease, *Salmonella* serotypes can be divided into three groups that cause distinctive clinical syndromes, typhoid fever, bacteremia and enteritis (Santos *et al*., 2001). The non-typhoid *Salmonella* serotypes can cause protean manifestations in humans, including acute gastroenteritis, bacteremia and extra intestinal localized infections involving many organs (Chiu *et al*., 2004). Within *Salmonella enterica* subspecies I (*S. enterica* subspecies *enterica*), the most common O antigen serogroups are A, B, C1, C2, D, and E. Strains within these serogroups cause approximately 99% of *Salmonella* infections in humans and warm blooded animals. Serotypes in other subspecies are usually isolated from cold-blooded animals and the environment, but rarely from humans (Velge *et al*., 2005).

Salmonellae avoid host defense in the stomach and reach the intestines, and interact with the non-phagocytic cells such as the epithelial cells of the intestinal mucosa (Henselm, 2004). They adhere to the intestinal epithelial cells by adhesive structures (fimbriae) that promote binding and invade epithelial cells to provoke gastroenteritis. *Salmonella* produces various virulence factors including exotoxin (cytotoxin, enterotoxin) and endotoxin that mediate the development of diarrhea (Murray, 1986). Bacterial enterotoxins initiate diarrhea by binding to receptors that stimulate the second messenger systems of cyclic Adenosine Monophosphate(cAMP) and cyclic Guanosine Monophosphate (cGMP), which secondarily activate enterocyte fluid hyper secretion (Jones and Blikslager, 2002).

The organisms have virulence factors such as virulence-plasmids, toxins, fimbriae and flagella that help in establishing an infection (Alphons *et al*., 2005). Most *Salmonella* virulence factors are clustered on chromosome referred to as *Salmonella* pathogenicity islands (SPIs) which divided into five regions (Shea, 2004).

SPI-1 which encodes a secretion apparatus, transcriptional regulators and effectors proteins that facilitate uptake of *Salmonella* by enterocyte, SPI-2 responsible for intra macrophage survival, SPI-3 required for growth in Mg^{+2} limiting conditions, SPI-4 which may play role in invasion and SPI-5 required for enteric salmonellosis (Songer and Post , 2005).

Pathogenicity is mediated by certain factors such as strain virulence, infectious dose, route of infection and host susceptibility. Microfold (M) cell is the target cells of *Salmonella* pathogenicity (Ohl and Miller, 2001). Some of the mechanisms of pathogenesis are bacterial mediated endocytosis, neutrophil recruitment and migration, epithelial cell cytokine secretion, fluid and electrolyte secretion, and systemic infection.

2.4. Signs and Symptoms of Salmonellosis

Human salmonellosis is usually characterized by acute onset of fever, abdominal pain, diarrhea, nausea and sometimes vomiting (WHO, 2005). Typically, symptoms of gastroenteritis develop within 6 to 72 hour after ingestion of the bacteria. The symptoms are usually self-limiting and typically resolve within 2 to 7 days. In a small percentage of cases, septicemia and invasive infections of organs and tissues can occur, leading to diseases such as osteomyelitis, pneumonia, and meningitis (CDC, 2001). In some cases, particularly in the very young and in the elderly, the associated dehydration can become severe and life threatening. In such cases, as well as in cases where *Salmonella* causes bloodstream infection, effective antimicrobials are essential drugs for treatment. Serious complications occur in a small proportion of cases (WHO, 2005). Although most cases are self-limiting, the degree to which a person becomes sick depends on his or her health status and the number and virulence of *Salmonella* species ingested. In general, the poorer the individuals health and the more *Salmonella* ingested, the greater the probability for serious illness and death (Mead *et al*., 1999).

2.5. Epidemiology of Salmonellosis

Typhoid cases are stable with low numbers in developed countries, but nontyphoidal salmonellosis has increased globally. Evidently, Typhoid fever, which is caused mainly by *Salmonella* Typhi, continues to be a major problem in developing countries (WHO, 2005). A study estimated that globally there are more than 22 million cases of typhoid fever each year with more than 200,000 deaths, however, the true magnitude is difficult to quantify because the clinical picture is confused with many other febrile illnesses and most typhoid endemic areas lack facilities to confirm the diagnosis (Crump *et al*., 2004). The mortality rates differ from region to region, but can be as high as 5 to 7% despite the use of appropriate antibiotic treatment. On the other hand, nontyphoidal cases account for 1.3 billion cases with 3 million deaths.

In the United States, approximately 2 to 4 million cases of *Salmonella* gastroenteritis occur with about 500 deaths per year. A more accurate figure of salmonellosis is difficult to determine because normally only large outbreaks are investigated whereas sporadic cases are under-reported. Data on salmonellosis are scarce in many countries of Asia, Africa and South and Central America where only 1 to 10% of cases are reported Hanes (2003); Hu and Kopecko (2003).

Typhoid fever is endemic throughout Africa and Asia as well as persisting in the Middle East, some eastern and southern European countries and central and South America. In the US and most of Europe, typhoid is predominantly a disease of the returning traveler. Typhoid incidence in endemic areas is typically low in the first few years of life, peaking in school-aged children and young adults and then falling in middle ages. Most infections occur in childhood especially in Mekong Delta region of Vietnam and are recognizable although often mild (Wray and Davies, 2003).

The most famous outbreak of enteric fever was transmitted by Typhoid Mary. Mary Mallon, a New York City hired household cook, transmitted typhoid fever to at least 22 individuals causing 3 deaths between 1900 and 1907. After being apprehended by public health officials in 1907, she was isolated for 3 years. Even though she was released with the stipulation that she never cooks again, she broke the promise and consequently caused at least 25 more cases of typhoid fever at Manhattan Maternity Hospital when she was employed as a cook in 1915. She was finally isolated until her death in 1938 (Scherer and Miller, 2001; Parry, 2006).

In studies conducted in Jordan on 283 food handlers for potential pathogens in their stool, the rate of isolation of *Salmonella* was 6% (Al-Lahham *et al*., 1990). Another study showed in two hospitals in Winchester, Southern England that Faecal screening of asymptomatic catering staff demonstrated 12.3% *Salmonella* (Dryden *et al*., 1994).

Prevalence of chronic typhoidal *Salmonellae* carriers among food vendors in Kumasi, Ghana showed that Typhoidal *Salmonellae* were isolated from six people out of 258, giving a carriage rate of 2.3% and three of the *Salmonellae* isolated were *S*. Typhi, and the other three were non-typhoidal *Salmonellae* (Feglo *et al*., 2004). Another study done in Nigeria showed that three *S.* Typhi (5.7%), three *S.* Enteritidis (5.7%) and one *S.* Choleraesuis (1.9%) were recovered from seven (13. 2%) of the 53 stool samples processed.

2.6. Transmission of Salmonellosis

Salmonella infection appears to be one of the most common examples of an enteric disease that is transmitted from animals to humans. The transmission occurs both through food products, such as meat, dairy products, and eggs, and by direct contact between animals and humans through the fecal-oral route (Olsvik *et al*., 1985). Foodborne salmonellosis often follows consumption of contaminated animal products such as raw meat, poultry and eggs. Not washing fresh fruits and vegetables before eating them, as well as not thoroughly cleaning work surfaces used to prepare raw meat and other foods in the kitchen can also be source of *Salmonella*. Food can also be contaminated by food handlers who do not thoroughly wash their hands with soap after handling raw meat or after using the bathroom (WHO, 1989). *Salmonella* infections are primarily of foodborne origin but can also occur through contact with infected animals, humans, other feces (Rounds *et al*., 2010).

The main mode of transmission is from food products contaminated with animal products or waste most commonly eggs and poultry but also undercooked meat, unpasteurized dairy products, seafood, and fresh produced. *S*. Enteritidis associated with chicken eggs is emerging as a major cause of foodborne disease. Approximately 1 in 20,000 eggs is thought to be infected with *S*. Enteritidis. Between 1974 and 1994, there was a fivefold increase (from 5% to 25%) in the isolation of *S*. Enteritidis from eggs in the United States; in 1998, the U.S. Department of Agriculture estimated that 80% of all salmonellosis cases were caused by infected eggs (Fuaci and Jameson, 2005).

2.7. Global Prevalence and Incidence of Salmonellosis

Salmonella infection most commonly occurs in countries with poor standards of hygiene in food preparation and handling and where sanitary disposal of sewage is lacking. It mainly occurs in the tropics and sub tropics in Africa, India, Pakistan South East Asia and South America (WHO, 1989; Lanata *et al*., 1990; Al-Lahham *et al*., 1990; WHO, 2003b; Senthikumar and Prabakaran, 2005).

Significant outbreaks of salmonellosis occurred around the world at different times. For instance, in the United States, 164,044 (approximately 32,000 annually) during 1998-2002 (Lynch *et al*., 2006); in China approximately 70% - 80% and during 1992-2005 (Wang *et al*., 2007; Chen *et* *al*., 2008; Liu *et al*., 2008), in Germany, a total of 42,851 (EFSA, 2009). In 2006, a total of 160,649 confirmed cases of human salmonellosis were reported in the EU (Liu, 2010). In many countries, incidence of human *Salmonella* infection has increased drastically over the years.

Salmonellosis is an important global public health problem causing substantial morbidity, and mortality. Although most infections cause mild to moderate self-limiting diseases, serious infections lead to deaths (Jong and Ekdahl, 2006). Besides the importance of this microorganism in public health, another aspect is the cost incurred by human salmonellosis. In 1999, the cost linked to foodborne salmonellosis ranged from 560 million to 2.8 billion in Europe, where *Salmonella* was estimated to be responsible for nearly 166, 000 cases of food-borne salmonellosis (Korsak *et al*., 2006).

With the increasing population in the developing world, there is an increasing demand for meat and meat products which will force the present resource-driven system of livestock production to a demand-driven system (Zessin, 2006) which may in turn increase the disease transmission risks. There is a multifactorial risk of foodborne hazards including salmonellosis in the developing countries due to poor sanitation and inadequate access to potable water (Henson, 2003).

2.8. Prevalence of Salmonellosis in Ethiopia

Salmonella is the most frequently reported cause of foodborne illness (Misganaw and David, 2013). Foodborne salmonellosis often follows consumption of contaminated animal products, which usually results from infected animals used in food production or from contamination of the carcasses or edible organs (Demissie *et al*., 2002). *Salmonella* infection in meat animals arises from intensive rearing practices and the use of contaminated feeds (Gabisa, 2004).

Studies indicated the widespread occurrence and distribution of *Salmonella* in Ethiopia. The number of outbreaks of diseases caused by *Salmonella* in humans has increased considerably in the country (Getnet *et al*., 2008). Salmonellosis in Ethiopia was reported in Tikur Anbessa University Hospital, Addis Ababa, and Jimma University Hospital, South West Ethiopia by Getnet *et al.* (2011), from Addis Ababa by Mogessie and Mesele (1985), and Getnet *et al*. (2011), in Harar, Eastern Ethiopia by Alex Ayalu *et al.* (2011) and by Getnet *et al.* (2008), in their review article conducted on salmonellosis in Ethiopia.

Moreover, high percentages of *S.* Typhi isolates have been found to be resistant for antimicrobial agents (Gizachew *et al*., 2007; Gashaw *et al*., 2008; Bayeh *et al*., 2010). In addition, the very young, elderly and immunocompromized individuals are particularly more susceptible to *Salmonella* infections at a lower infective dose than healthy adults. This is more important in developing countries such as Ethiopia where HIV/AIDS is highly prevalent and *Salmonella* is an important opportunistic infection in HIV/AIDS patients (Catherine *et al*., 2001).

2.9. Public Health and Economic Impacts of Salmonellosis

The incidence of non-typhoidal salmonellosis has doubled in the United States over the past two decades. The center for disease control estimates that there are 2 million cases annually, with 500 to 2000 deaths (CDC, 2001). Although more than 200 serovars of *Salmonella* are considered to be human pathogens, the majority of the reported cases in the United States are caused by *S*. Typhimurium or *S*. Enteritidis (Fuaci and Jameson, 2005). In most parts of the world, countries have seen dramatic and continuous increases in human outbreaks of salmonellosis, caused by infections in animals. In 2004, in the European Union (EU) alone, 192,703 human cases of salmonellosis were reported. These and similar data from other countries almost certainly underestimate the magnitude of the problem, as many cases of salmonellosis are not reported.

In addition to human health implications, it also generates negative economic impacts due to surveillance investigation, and illness treatment and prevention (Gómez-Aldapa *et al*., 2012). Financial costs are not only associated with investigation, treatment and prevention of human illness, fall in to the public and privet sectors and may be surprising, both in terms of the levels of costs incurred and the variety of affected. In the public sector, resources may be diverted from preventive activities in to the treatment of patients and investigation of the source of infection.

Cost estimates per case of human salmonellosis range from approximately US $40 for uncomplicated cases to US$ 4.6 million for cases ending with hospitalizations and deaths (WHO, 2005). The costs of food-borne salmonellosis alone are estimated to reach up to € 2.8 billion annually in EU countries altogether. In Denmark, the annual estimated cost of food-borne salmonellosis is US$ 15.5 million, representing approximately 0.009% of salmonellosis in the country. A *Salmonella* control program has been conducted for several years in the country, and the annual cost of this control program is estimated around US$ 14.1 million (WHO, 2005).

In the Netherlands, annual costs caused by human salmonellosis are estimated between 32 and 90 million Euro (van Pelt and Valkenburgh, 2001). Although few developed countries have managed to report data on the economic cost of *Salmonella*, data related to the cost of food-borne disease are generally not available from developing countries (WHO, 2005).

2.10. Treatment of Salmonellosis

Gastroenteritis caused by *Salmonella* is usually a self-limiting disease (Richards *et al*., 1993; Fuaci and Jameson, 2005) and diarrhea resolves within three to seven days and fever within seventy two hours (Fuaci and Jameson, 2005). Accordingly therapy should be directed primarily to the replacement of fluid and electrolyte losses. Therefore, antimicrobials should not be used routinely to treat uncomplicated non-typhoidal *Salmonella* gastroenteritis or to reduce convalescent stool excretion (Richards *et al*., 1993). However, antimicrobial therapy should be considered for any systemic infection (Parry *et al*., 2002).

Antibiotic treatment usually is not recommended and in some studies has prolonged carriage of *Salmonella*. Neonates, the elderly, and the immunosuppressed (e.g., HIV infected patients) with non-typhoidal *Salmonella* gastroenteritis are especially susceptible to dehydration and dissemination and may require hospitalization and antibiotic therapy (Fuaci and Jameson, 2005). Because of the increasing prevalence of antimicrobial resistance, empirical therapy for life threatening bacteremia or local infection suspected to be caused by non-typhoidal *Salmonella* should include a third generation cephalosporin and a quinolone until susceptibility patterns are known. Amoxicillin and trimethoprim- sulfamethoxazole are effective in eradication of long-term carriage. The high concentration of amoxicillin and quinolone in bile and the superior intracellular penetration of quinolone are theoretical advantages over trimethoprim-sulfamethoxazole (WHO, 2003a).

2.11. Control and Prevention of Salmonellosis

Prevention of salmonellosis by the implementation of hygiene measures is difficult and use of antibiotics may give rise to the emergence of resistance problems (Mastroeni and Menager, 2003). Additional measures to control secondary contamination could be prevention of contamination by cleaning and disinfection, hygiene of personnel and proper processing.

Growth of micro-organisms in meat and poultry products can be controlled by maintaining a cold chain at 10°C, especially for *Salmonella* during transport and storage (Coleman *et al*., 2003).

The use of program aimed at the prevention and control of *Salmonella* and other zoonotic bacteria in primary animal production, can lead to a reduction in the level of contamination of related food products at retail, and thereby also reduce the risk of human exposure to antimicrobial resistant *Salmonella* from those food products. The occurrence of *Salmonella* and antimicrobial resistant *Salmonella* in other food commodities is also likely to be reduced as the risk of cross-contamination is reduced (EFSA, 2008).

There is no effective immunization against infection by *Salmonella*, except against typhoid fever. This is because of large number of *Salmonella* serotypes that would have to be included in vaccines. Typhoid fever, however, is caused by only one serotype and two vaccines are available. One consists of killed cells of *Salmonella* Typhi and is administered by injection. The other vaccine consists of live, attenuated strain of *Salmonella* Typhi. It is administered orally, in the form of capsules that can be swallowed (Pelczar *et al*., 1993).

2.12. Antibiotic Resistance in *Salmonella* spp

Salmonella species are leading causes of acute gastroenteritis in several countries and salmonellosis remains an important public health problem worldwide, particularly in the developing countries (Rotimi *et al*., 2008). The situation is more aggravated by the ever increasing rate of antimicrobial resistance strains (Zelalem *et al*., 2011).

The appearance of both plasmid mediated antibiotic resistant against conventional anti-*Salmonella* drugs and chromosomal resistance to quinolones and fluoroquinolones has reduced therapeutic options for *Salmonella* septicemia in humans (Nor Elmadiena *et al*., 2012).

The first comprehensive study on the serogroup of *Salmonella* spp. in Ethiopia was conducted by Mesele Gedebuo and Alebachew Tassew (1981) in Addis Ababa. From 1974 to 1981 Afeworki Gebre-Yohannes conducted a study to identify the prevalent serotypes and their susceptibility to drugs in Addis Ababa, which serves as a base-line of data for further surveillance of *Salmonella* spp. in Ethiopia (Afeworki, 1985).

He studied 216 *Salmonella* strains which were predominantly isolated from adult patients referred from different hospitals to the Central Laboratory and Research Institute, Addis Ababa between January 1974 and October 1981 and found the existence of 26 different serotypes.

Serogroups B, C, D, E and A were isolated at frequencies of 20.4%, 19.4%, 6.9%, 2.8% and 1.9%, respectively. Out of the 216 *Salmonella* strains, 48.6% were *S*. Typhi. Most of the *Salmonella* isolates were from stool (54.6%).

Daniel (2008) reported that among the 37 *Salmonella* strains collected during the period of 1992-1993 in Tikur Anbesa Hospital, the most common serogroup was group B (81.1%), followed by group D (*S*. Typhi) (10.8%) and group C (8.1%). In a study determining the prevalence of *Shigella* strains and *Salmonella* spp. among the out patients of the Gondar College Teaching Hospital (1994-1996) it was found that out of 7,993 miscellaneous specimens cultured, 147 *Shigella* and 80 *Salmonella* isolates were identified. Of which, serogroup B was the most frequently isolated at 61% (Abrham *et al*., 1997).

According to studies conducted by Behailu and Mogessie (2009), about 70% of the isolates had varying resistance to the tested antibiotics and multiple drug resistance was observed in over 30% of the *Salmonella* isolates. These authors had also indicated that the most frequent resistance pattern was observed for amoxycillin, ampicillin, ciprofloxacin, gentamycin and streptomycin in the case of cattle and poultry.

In the study by Addis *et al*. (2015) on anti-microbial susceptibility patterns of *Salmonellae* Species from food handlers in Addis Ababa University student’s cafeteria, resistant to ampicillin (100%), clindamycin (100%), and erythromycin (100%). Besides, in study by Getachew *et al*. (2014) high frequency of resistance for *Salmonella* isolates was observed to tetracycline 52.5%, co-trimoxazole 37.5% and ampicillin 60%, respectively. In the study by Getnet and Haimanot (2014) in Ethiopia, indicated that all *Salmonella* isolates (16) were resistant against amoxicillin.

3. MATERIALS AND METHODS

3.1. Description of the Study Area

The study was conducted at Dessie Referral Hospital, Dessie, North East Ethiopia. Dessie Town sits at a latitude and longitude of 11^{0}8'N and 39^{0}38'E respectively, with elevations ranging from 2,470 to 2,550 meters above sea level. It is located 401 km North East of the capital city, Addis Ababa. The Hospital is the only Referral Hospital in North East Ethiopia with about 200 beds and 165 health professionals. The pediatric wards have 52 beds and 12 health professionals (2 general practitioners, 1 pediatrician and 9 nurses). There are 16 governmental health institutions (1 Referral Hospital, 1 Primary Hospital, 8 Health centers and 6 Health posts), and 71 private health institutions (3 General Hospitals, 6 Higher clinics, 23 Medium clinics, 15 Pharmacies, and 24 Drug stores) in the town (Asrat *et al*., 2014). Out of these hospitals and health centers, DRH was selected for this study as it has greater patient inflow.

3.2. Study Design

A hospital based cross sectional survey study was conducted to determine the prevalence and drug resistance patterns of *Salmonella* among diarrhoeal patients visiting DRH. Data were collected using pre-tested structured questionnaires prepared in English, and translated to local language Amharic (Appendix I) and laboratory based diagnosis patients from November 2016-January 2017. A pre-tested structured questionnaire was used to collect data regarding sociodemographic factors, clinical features of patients and risk factors to *Salmonella* infection. Standard bacteriological techniques were used at Dessie Regional Laboratory (DRL), to generate data regarding the prevalence of *Salmonella* spp among diarrhoeal.

3.3. Study Population

All patients who came to outpatient department (OPD) of DRH with complaints of diarrhoea were enrolled as the study population. Diarrhoea was considered if the patient had passed three or more loose or watery stools in 24 hours period prior to presentation and dysentery as the presence of blood in the stool (WHO, 2010). The inclusion criterion was all patients visiting DRH with diarrhoeal cases during the study time regardless of sex and age.

In this study, patients less than 15 years of age were considered as children and their stool samples were collected with the help of their parents /care takers.

3.4. Sample Size Determination

The appropriate sample size for a single population based survey was determined by considering the following three factors: i) the estimated prevalence of the disease (i.e. salmonellosis); ii) The desired level of confidence; and iii) The acceptable margin of error. Since there was no previous investigation conducted on the same title in the study area, p value of 0.5 is taken to ensure a sample size large enough to satisfy the precision and confidence constraints. The sample size of a single population was calculated based on the 95% confidence limits and 5% sampling error by using the following formula shown in Bland (1998).

$$n = (Z\alpha/_2)^2\ P\ (1-P)/d^2$$

Where n = required sample size.

$Z\alpha/2$= the standard score corresponding to 95% confidence level, i.e. 1.96.

P = prevalence of *Salmonella* (50%)

d = Margin of error at 5% (standard value of 0.05).

Therefore, the calculated sample size for this study was:

$$n = (1.96)^2 \times 0.5\ (1-0.5)/\ (0.05)^2 = 384$$

3.5. Sampling Method, Specimen Collection, Handling and Transport

A serial sampling method was used where all patients coming to the OPD with diarrhoeal cases were recruited as they came until the required sample size (384) was reached. Before collection of stool samples, patients above 15 years of age and children's parents /care-takers were given orientation on how to take samples and also were provided by a laboratory technician with materials to handle stool specimens.

Following this, about 3 grams of fresh stool sample was collected by the principal investigator and assistant data collectors from each study subject on the same day of enrolment using a wide mouth screw capped sterile container in the laboratory of DRH. Each sample was labeled with a code corresponding to each questionnaire. Finally, the collected stool specimens were delivered to DRL, which is very close to DRH, without using transport media for analysis on the same day. The strain *Escherichia coli* ATCC 25922 were used for determining the quality of media used in the experiment and as a positive control for antibiotic susceptibility tests (NCCLS, 2006).

3.6. Isolation and Identification of *Salmonella* species

All samples collected from DRH were analyzed at DRL with the help of laboratory technicians. Selective enrichment steps to allow the multiplication of *Salmonella* spp. over other enteric bacteria or to allow maximal recovery of *Salmonella* spp. from faecal specimens were carried out as described in Cheesbrough (2006). About 1g of stool sample was added into a tube containing selenite F broth. Selenite broth was incubated aerobically at 37^{O}C for 18 hours.

For optimal isolation of *Salmonella*, two different selective media; general purpose medium with low sensitivity MacConkey agar and a high selective xylose-lysine desoxycholate (XLD) agar (Oxoid, England) were used. A loopful of faecal suspension from enrichment media or selenite broth was sub-cultured on XLD and MacConkey agar. After inoculation, the plates were incubated aerobically at 35-37^{o}C for 18-24 hours. Typical colorless colonies on MacConkey agar and colonies with a black centre and a lightly transparent zone of reddish color due to the color change of the indicator were picked and a series of biochemical tests were done for further identification. In addition to the above, colonies with typical morphology were further identified using, urease test, TSI, LIA, motility, indole and Citrate utilization test.

Urease test (Himedia, India): Urea containing agar slants were inoculated heavily over the entire surface of the slants in Bijou bottles. The caps were loosened and then incubated at 37 ^{o}C for 12 hours. A urease-positive culture producing an alkaline reaction in the medium was evidenced by pinkish-red colour of the medium. Urease-negative organisms do not change the colour of the medium, which is pale yellow-pink. *Salmonella* is always urease negative (Cowan and Steel, 2002).

Triple sugar iron agar (TSI) test (Himedia, India): Each isolate was inoculated by stabbing the butt and streaking the slants of the TSI medium and incubated at 37^{o}C for 24 hours (Cowan and Steel, 2002). Color change of the butt and slant, and gas production were observed to determine negative/positive reactions of the isolate. *Salmonella* forms a red slope (alkaline) and yellow (acid) butt with/out gas or H_2S production (Cheesbrough, 2006).

Citrate utilization test using Simmon's Citrate Agar (oxoid, England): Isolates were inoculated into Simmon's citrate medium slant by stabbing the slopes and aerobically incubated at 37^{o}C for 48 hours to detect color change of the medium according to Cheesbrough (2006). The majority of *Salmonella* spp. is citrate positive as such Simmon's citrate agars slopes were changed from green to deep blue color.

Lysine iron agar (LIA) test: Lysine Iron Agar (oxoid, England) was inoculated by stabbing the butt and streaking the surface of the slant. Then the results were interpreted after incubation for 24 hours at 37°C. On lysine iron agar, *Salmonella* typically shows an alkaline (purple) reaction on the slant and butt and may produce gas and H_2S (blackening of medium).

Motility test (oxoid, England): It was used to determine if the organism is motile or not. The presence of diffuse growth away from the line of inoculation was used to indicate the motility of the organism. *Salmonella* species are always motile.

Indole test (oxoid, England): It was used to identify the bacteria capable of producing indole using tryptophanase. Kovac's reagent was added to the culture. The red layer (positive result) indicates the presence of indole and no color on the layer indicates the absence of indole (negative result). *Salmonella* species are negative for the indole reaction.

3.7. Antibiotic Resistance Test for *Salmonella* Isolates

The antibiotic susceptibility profile of *Salmonella* isolates was determined for the commonly used antibiotics (Oxoid Ltd., Basingstoke, England). Each isolate was tested for the selected antimicrobial agents with their respective concentrations such as Ampicillin (10µg), Tetracycline (30µg), Trimethoprim sulfamethoxole (1.25/23.75µg), Nalidixic acid (30µg) and Ciprofloxacin (100 µg) and following the procedures outlined by Perilla (2003).

The antimicrobial agents mentioned above were drugs commonly prescribed against *Salmonella* infection (WHO, 2003a) and currently in use in the study area. Using a sterile wire loop, 3-5 well isolated colonies were picked and emulsified in 5ml nutrient broth. The prepared turbidity was matched with a turbidity standard (0.5 McFarland) to have an equivalent suspension. Sterile swab was used to inoculate the suspension by streaking on the prepared and dried Mueller Hinton agar plate evenly. It was then allowed to stay for 3-5 minutes. Sterile forceps were used to place the antimicrobial discs on the inoculated plates. Within 30 minutes after applying the disc, the plates were incubated at 35^{o}C for 16-18 hours. After incubation, the diameter of each zone of inhibition was measured in millimeter using a caliper/ruler on the underside of the plate.

3.8. Data Analysis

Questionnaire survey data on risk factors and clinical features of *Salmonella* spp and laboratory data on detection and/or isolation of *Salmonella* species were entered into a computer and analyzed using SPSS version 16 statistical software. The Chi-square test was employed to examine association between prevalence of *Salmonella* spp and clinical features and risk factors. A p-value < 0.05 was considered to indicate statistically significant difference. In addition, descriptive statistics and cross-tabulation of SPSS version 16 were used to analyze the distribution of study subjects by age and sex, and the frequency of positive results under each age and sex category. Zone of inhibition differences between antibiotics used against *Salmonella* isolates were calculated in comparison with the zone of inhibition produced by the positive control strain i.e. *E. coli* (ATTC25922), and this was used to interpret the antimicrobial resistance of *Salmonella* spp.

3.9. Ethical Considerations

Institutional consent was obtained through communications made with Dessie Referral Hospital before conducting the study. The medical Director of the DRH was briefed first about the study before meeting with patients. Then, patients were informed about the objectives of the study. The participation of patients in this study was purely a voluntary activity and their right not to participate was respected. Issues of confidentiality and anonymity were also maintained.

4. RESULTS AND DISCUSSION

4.1. Distribution of the Study Participants

During the 3 months study period, three hundred eighty four (n=384) diarrhoeal patients attending the outpatient department (OPD) of DRH were examined for *Salmonella* spp. The distribution of the study participants by sex and age is shown in Table 1. Since this study has epidemiological value to the study area, age group category was based on Dessie Regional Health Bureau data record system on diarrhoeal diseases prevalence, where 0-4, 4-14 and ≥15 age groups are reported as under five children, young children and adults, respectively.

From the 384 diarrhoeal patients, 203 (52.9%) were females and 181 (47.1%) were males (Table 1). Of the entire study subjects, 180 (46.9%) were younger than five years of age; 120 (31.2%) were between ages 5 and 14 years and 84 (21.9 %) were ≥ 15 years old. During the study period, highest frequency of diarrhoeal diseases in the DRH occurred within the age group of 3 months to four years (Table 1). This was in line with the internationally accepted fact that children suffer from diarrhoeal diseases more than adults do (Blaser, 2000; Workman *et al*., 2006).

In this study, from the total 384 diarrheal patients, 171 (44.5%) came from urban and the remaining 213 (55.5%) from rural areas. With regards to education of patients, 142 (37%) of the samples were collected from literate and the rest 242 (63%) samples were collected from illiterate patients (Table1).

Table 1. Socio- demographic characteristics and prevalence of *Salmonella* spp. among the study participants (n=384)

Socio- demographic characteristics		Total numbers examined	No (%) of positives	P- Values
Age (years)	0.25 – 4	180	12 (60)	0.022***
	5 – 14	120	6 (30)	
	>15	84	2 (10)	
Gender	Male	181	8 (40)	0.371
	Female	203	12 (60)	
Place of residence	Urban	171	7	0.180
	Rural	213	13	
Educational status	Literate	142	12	0.371
	Illiterate	242	8	

4.2. Prevalence of *Salmonella* Species

Three hundred eighty four stool specimens collected from diarrheal patients were examined to determine the prevalence of *Salmonella* isolates (Table 1). The prevalence of *Salmonella* isolates in stool samples of this study, 5.21 % (20/384), is closer to the findings of previous studies made in China by Hengli *et al*. (2008) who reported a prevalence of 5.8% and 4.8% *Salmonella* in the years 2006 and 2007, respectively, and 4.94% Abdulmejid (2012) a study done at Worabe Health Center, Ethiopia.

On the other hand lower prevalence was reported by Mikhail *et al.* (1990) from Djibouti who reported a prevalence rate of 2.9%, 3.3% from Lagos, Nigeria (Ogunsanya ,1994); and 1.08% (Tesfaye *et al*., 2014), 2.5% (Mulatu *et al*., 2014), 3.95% (Yeshiwondim *et al*., 2015) from Ethiopia. However, the prevalence found in this study was found to be lower than the 11.5% *Salmonella* prevalence reported by Alex Ayalu *et al*. (2011) who conducted a study on antibiotic susceptibility patterns of *Salmonella* and *Shigella* isolates in Harar, Eastern Ethiopia and much lower than the 16.7%, 17%, 19% and 20.5% prevalence of *Salmonella* reported by Mussaret *et al.* (2012), Kabir *et al*. (2007), Caprioli *et al*. (1996) and Murugkar *et al.* (2005) in Mexico, Nigeria, Italy and northeastern India, respectively.

The prevalence of *Salmonella* infection by age group and sex in the present study is also shown in Table 1. As can be seen from the table, the prevalence of *Salmonella* infection among male and female patients and the difference was statically insignificant with 0.800 of chi-square value and P = 0.371. This implies that both sexes are equally at risk of *Salmonella* infection. Among the 20 patients infected with *Salmonella* species, 12 (60%) were females and 8 (40%) were males, resulting in an overall male to female ratio of 1:1.5.

As indicated in Table 1, *Salmonella* were isolated in all age groups and statistically significant difference existed between the prevalence of the different age groups (P = 0.022). Out of the total positive cases, more *Salmonella* infection (12/20 or 60%) was observed in children 0-4 years of age than in those young children aged between 5 to 14 years (30%) and adults above 15 years (10%).

4.3. Association between Prevalence of *Salmonella* infection and the Identified Risk Factors in the Study Area

The association between risk factors and the prevalence of *Salmonella* spp. are shown in Table 3. The data generally show that infection with *Salmonella* spp. is significantly associated with the absence of latrine (X^2= 7.200, P= 0.007), drinking unprotected water (X^2= 5.000, P= 0.025), consumption of egg (X^2= 5.000, P= 0.025), co-habitation with domestic animals (X^2= 7.200, P=0.007), consumption of raw milk (X^2= 9.800, P= 0. 002), consumption of raw vegetables and fruits (X^2= 16.200, P= 0.000), consumption of street vended foods (X^2= 12.800, P= 0.000).

Table 2. The association between risk factors and prevalence of *Salmonella* spp.

Factors	*Salmonella* species		X^2	Df	P-value
	Positive (%)	Negative (%)			
Residence					
Urban	7(4.1)	164 (95.9)	1.800	1	0.180
Rural	13(6.1)	200(93.9)			
Educational Status					
Literate	12(8.5)	130(91.5)	0.800	1	0.371
Illiterate	8(3.3)	234(96.7)			
Latrine					
Present	3(1.4)	210(98.6)	7.200	1	0.007***
Absent	17(10)	154(90)			
Drinking Water					
Protected	5(2.5)	194(97.5)	5.000	1	0.025***
Unprotected	15(8.1)	170(91.9)			
Domestic Animals					
Present	14(6.1)	214(93.9)	3.200	1	0.074
Absent	6(3.8)	150(96.2)			
Domestic Animal House					
Separate	4(2.2)	174(97.8)	7.200	1	0.007***
Cohabit	16(7.8)	190(92.2)			
Raw Milk					
Used	17(14.5)	100(85.5)	9.800	1	0.002***
Unused	3 (1.1)	264(98.9)			
Raw Meat					
Used	13(6.1)	200(93.9)	1.800	1	0.180
Unused	7(4.1)	164(95.9)			
Raw Vegetables and fruits					
Used	19(5.9)	302(94.1)	16.200	1	0.000***
Unused	1(1.6)	62(98.4)			
Consumption of street vended foods					
Used	18(8.5)	193(91.5)	12.800	1	0.000***
Unused	2(1.2)	171(98.8)			
Raw egg					
Used	15(6)	234(94)	5.000	1	0.025***
Unused	5(3.7)	130(96.3)			

Df= Degree of freedom, X^2 = chi-square, ***significant at $p<0.05$

In the present study there was a significant correlation between the absence of latrine and infection with *Salmonella*. There was significant association between the risk of contracting salmonellosis and poor living and housing conditions. This finding was typical to the situation, throughout the developing world (Curtis and Cairncross, 2003).

In the present study, there was a significant association between drinking from unprotected water source and the prevalence of salmonellosis (X^2 = 5.000 and P = 0.025). Out of the total positive cases for salmonellosis, patients who used protected water sources were at lower risk of contracting *Salmonella* than those who used unprotected water sources (Table 2). This can be explained by the high level of contamination of water by *Salmonella* from the environment. This finding was in agreement with Guane *et al*. (2000) who reported that the transmission of *Salmonella* Typhi in humans was associated with the ingestion of contaminated water. In addition, other studies have shown that exposure to contaminated water is known to be associated with diarrhea caused by ingestion of microorganisms (Moreira and Lima, 2001).

Salmonella spp. infection was shown to be significantly associated with co-habitation of domestic animals with humans; this result was in line with Dinkineh *et al*. (2014) from Harar, Ethiopia. This finding suggests the fact that *Salmonella* spp. are important human and animal pathogens world-wide and animals are the reservoir for these enteric pathogens (Hoelzer *et al*., 2011).

The consumption of raw milk and raw milk products from a dairy may result in infection by *Salmonella* and many other pathogenic bacteria (CDC, 2007). The present finding also revealed significant association between raw milk consumption and contracting of *Salmonella* infection. Those patients who consumed raw milk were at risk of having salmonellosis compared to those who did not consume raw milk. This is comparable to the findings of Jayarao *et al*. (2006) who reported the transmission of enteric pathogen infections to humans via the consumption of raw milk in Pennsylvania.

In this study, there was a significant association between salmonellosis and the consumption of raw vegetables and fruits from unhygienic sources (Table 2). Different works also showed that *Salmonella* spp. is frequently isolated from raw fruits and vegetables (Robertson and Gjerde, 2001).

The prevalence of *Salmonella* spp. was also highly associated with the consumption of street vended foods which was in line with Feglo *et al*. (2004) report from Ghana, Accra and Dinkineh *et al*. (2014) from Harar, Ethiopia. The possible explanation for this finding is street foods in Dessie Town are sold under unhygienic conditions, with limited access to safe water, sanitary services, or garbage disposal facilities.

Human salmonellosis was initiated by the consumption of contaminated products such as meat and eggs (Foley, 2008 and Mead *et al*., 1999). In this study, significant association (X^2 = 5.000, P = 0.025) was found between consumption of raw eggs and S*almonella* infection. Those patients who consumed raw egg were at risk of having salmonellosis compared to those who did not consume such products.

As indicated in Table 2, in this study, there was no statistically significant association between residence, educational status, presence of domestic animal and raw meat on one hand and infection with *Salmonella* on the other hand.

4.4. Association between Prevalence of *Salmonella* spp. and Clinical Features

The clinical features and their associations with *Salmonella* infections among 384 diarrheal patients are summarized in Table 3. In the present study, from the total *Salmonella* positive patients (20) the majority had abdominal pain followed by vomiting and fever which accounted for 90%, 80% and 70% of the patients, respectively. This result was in agreement with the study conducted in Washington State by Villar *et al*. (1999) who showed that 93% abdominal pain. There was significant association between abdominal pain, vomiting and *Salmonella* infection (X^2 = 12.800, 7.200 and P = 0.000, 0.007, respectively) whereas fever was not significantly associated with *Salmonella* infection (Table 3).

There was also a significant association between consistency of stools, duration of diarrhea and sample positivity for *Salmonella* (X^2 = 13.600, 7.600 and P = 0.004, 0.022 respectively) as the majority (60%) of the stool samples were watery diarrhea in contrast to a study done in Harar by Alex Ayalu *et al*. (2011) who reported that no *Salmonella* species was found from watery diarrhea.

But, the present finding was supported by the study conducted in Addis Ababa which reported that 82.4% of *Salmonella* species were isolated from watery diarrhea samples (Daniel, 2008). The remaining 20%, 10% and 10% of the stool samples were mucoid, bloody and mixed (blood and mucus), respectively (Table 3). This may reflect underlying variation in signs and symptoms with strain differences from place to place.

Most of the positive cases (60%) were noted within 1 to 5 days duration of diarrhea which is in line with Hohmann (2001) who reported that the incubation period for *Salmonella* gastroenteritis is typically from 12 to 72 hrs. Whereas the remaining 30% and10% of the positive cases were 6 to 10 days, 11-15 days duration of diarrhea, respectively. The use of clinical signs and symptoms is therefore very important in helping to identify patients with salmonellosis.

Table 3. Clinical features and their associations with positivity of *Salmonella* species among diarrheal patients.

Symptoms	Positive (%)	Negative (%)	X^2	Df	P-values
Fever					
Yes	14(5.5)	238(94.5)	3.200	1	0.074
No	6(4.5)	126(95.5)			
Vomiting					
Yes	16(6.8)	220(93.2)	7.200	1	0.007 ***
No	4(2.7)	144(97.3)			
Abdominal pain					
Yes	18(6.9)	241(93.1)	12.800	1	0.000 ***
No	2(1.6)	123(98.4)			
Consistency of stool					
Watery	12(6.9)	162(93.1)	13.600	3	0.004 ***
Bloody	2(4.8)	40(95.2)			
Mucoid	4(4)	96(96)			
Mixed (blood and mucus)	2(2.9)	66(97.1)			
Duration of diarrhea(days)					
1-5	12(3.9)	294(96.1)	7.600	2	0.022 ***
6-10	6(9.7)	56(90.3)			
11-15	2(16.7)	10(83.3)			
≥16 days	0.0	4(100)			

X^2=chi-square, Df= degree of freedom, ***significant at $p<0.05$

4.5. Test for antibiotic susceptibility of *Salmonella* Isolates

The antimicrobial susceptibility tests were done on all *Salmonella* isolates using the disk diffusion method and the results are presented in Table 4. Among 20 *Salmonella* isolates, 20(100%), 17(85%), 16(80%) and 3(15%) were found to have developed resistance for Ampicillin, Tetracycline, Trimethoprim-sulphamethoxazole and Nalidixic acid, respectively. None of the isolates were, however, resistant to Ciprofloxacin.

Intermediate resistance was found in 3(15%), 2(10%), 1(5%) and of the tested isolates against Tetracycline, Trimethoprim- sulfamethoxazole and Nalidixic acid, respectively. Furthermore 20(100%), 16(80%) and 2(10%) tested isolates were susceptible to Ciprofloxacin, Nalidixic acid and Trimethoprim-sulphamethoxazole, respectively.

Table 4. The proportions of resistant, intermediate and susceptible *Salmonella* isolates (n=20) to five different antibiotics.

Susceptibility	AMP No. (%)	TRA No. (%)	SXT No. (%)	NA No. (%)	CI No. (%)
R	20(100%)	17(85%)	16(80%)	3(15%)	0
I	0	3(15%)	2(10%)	1(5%)	0
S	0	0	2(10%)	16(80%)	20(100%)

AM = Ampicillin, CI = Ciprofloxacin, SXT = Trimethoprim-sulphamethoxazole, NA = Nalidixic acid, TRA= Tetracycline, S = Sensitive, I = Intermediate, R = Resistance

As can be seen from table 4, high level of *Salmonella* resistance was observed to ampicillin (100%), Tetracycline (85%) and Trimethoprim-sulphamethoxazole (80%) which was comparable to the findings in Harar (100%, 100%, 85.7% respectively) reported by Dinkineh *et al*. (2014); and 100%, 86.8% resistant to Ampicillin and Trimethoprim-sulphamethoxazole reported by Addis *et al*. (2015) and Ali *et al*. (2003) a study from Addis Ababa and Pakistan, respectively. In contrast, all were found to be resistant to Trimethoprim sulfamethoxazole in a study at Mollorca, Spain, during the period 1987-1991 (Reina *et al*., 1994).

The present finding was higher than the previous studies reported by Getachew *et al*. (2014) which showed that high frequency of resistance in *Salmonella* isolates was observed to Tetracycline (52.5%), Cotrimoxazole (37.5%) and Ampicillin (60%). Yeshiwondim *et al*. (2015) also reported that the overall resistance of *Salmonella* spp to Ampicillin was high (80.0%).

The percentages of isolates resistant to Ampicillin and Trimethoprim-sulfamethoxazole in the present study were also higher than those reported from Brazil, where only 88.8% and 56.8% of the isolates were found to be resistant, respectively (Ali *et al*., 2003). But the percentage of resistant isolates to Tetracycline is comparable with our finding (86.4%).

The resistance towards the traditional first line antibiotics such as Ampicillin, Tetracycline, Chloramphenicol and Trimethoprim sulfamethoxazole defines multidrug resistance (MDR) in *Salmonella enterica* (Crump and Mintz, 2010). The high resistance to Ampicillin (100%), Tetracycline (85%) and Trimethoprim sulfamethoxazole (80%) in this study might be due to their easy access and affordability to the public.

In this study ciprofloxacin and nalidixic acid were found still to have high potency against *Salmonella* isolates in the study area, where 100% and 80% of *Salmonella* isolates were shown to be susceptible to Ciprofloxacin and Nalidixic acid, respectively. The present finding was in line with a study conducted in Harar by Dinkineh *et al*. (2014).

Table 5. Comparison of the antimicrobial resistance profile of *Salmonella* isolates from Dessie Referral Hospital with those of selected previous reports from other parts of Ethiopia.

	Study area with respective study periods						
Antibiotic	Jimma[1]	Addis Ababa[2]	Harar[3]	Jimma and Addis Ababa[4]	Harar[5]	Butajira[6]	Dessie[*]
AM	59.3	81.3	100	82.3	100	60	100
CI	Nd	Nd	Nd	0.9	28	2.5	0.0
SXT	40.7	75.7	Nd	80.5	Nd	37.5	80
NA	8.5	37.8	Nd	8.0	3	5	15
TRA	59.3	94.5	71.4	39.8	100	52.5	85

1 = Abebe (2002), 2 = Daniel (2008), 3 = Alex Ayalu *et al*. (2011), 4 = Getnet *et al*. (2011), 5 = Dinkineh *et al*. (2014), 6 = Getachew *et al*. (2014), * = the present study, Nd = no data, AM= Ampicillin, CI= Ciprofloxacin, SXT= Trimethoprim -sulfamethoxazole, NA= Nalidic acid, TRA= Tetracycline.

Table 5 shows a comparison of findings from different regions and periods, such as central Ethiopia (Addis Ababa), Southern Ethiopia (Butajira), southwest Ethiopia (Jimma), Eastern Ethiopia (Harar) and Dessie (the present study). The data revealed that the highest percentages of Ampicillin and Tetracycline resistant *Salmonella* isolates were reported from Harar (100% each), Addis Ababa (81.3%, 94.5%) in 2008 and Dessie (100%, 85%). Similarly, the highest prevalence of Trimethoprim-sulphamethoxazole resistance was documented in Jimma and Addis Ababa (Getnet *et al*., 2011), Dessie (the present study) and Addis Ababa (Daniel, 2008), where 80.5%, 80% and 75.7% of the respective isolates have been shown to be resistant to this antibiotic, respectively. Though relatively lower, resistance against Trimethoprim sulphamethoxazole, Tetracycline was reported from Jimma (Abebe, 2002) and Jimma and Addis Ababa (Getnet *et al*, 2011) where 40.7% and 39.8% of their isolates, respectively, were found to be resistant to those antibiotic.

5. SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

5.1. Summary

The relative contribution of *Salmonella* infection to the overall burden of diarrheal diseases in the study area was unknown. Thus, a cross sectional descriptive survey study was designed to determine the prevalence of *Salmonella* species and their antibiotic resistance patterns among diarrheal patients visiting DRH, Dessie, North East Ethiopia from November 2016 to January 2017.

Three hundred eighty four stool samples were collected using sterile stool cups at the laboratory of Dessie Referral Hospital after collecting all the structured questionnaires filled by the patients. The samples were delivered to Dessie Regional Laboratory within 1-2 hours of collection. About 1g of stool sample was added in tube containing selenite F broth. Selenite F broth was incubated aerobically at 37^{o}C for 18 hours. Then inoculated on low sensitivity MacConkey agar and a high selective xylose-lysine desoxycholate (XLD) agar and incubated aerobically at 35-37^{o}C for 18-24 hours. Typical colorless colonies on MacConkey agar and pink to red colonies on XLD agar were picked and a series of biochemical tests were done for further identification. Antimicrobial susceptibility tests were done for the isolates of *Salmonella* species using Muller-Hinton Agar following the single disc diffusion technique.

Three hundred eighty four stool specimens collected from diarrheal patients were examined to determine the prevalence of *Salmonella*. A total of 20 *Salmonella* isolates were obtained from the stool specimens giving a prevalence of 5.21% in all age groups. Out of the total positive cases, more *Salmonella* infection (12/20 or 60%) was observed in children 0-4 years of age than in those young children aged between 5 to 14 years (30%) and adults above 15 years (10%).

The most common symptoms and signs were abdominal pain (90%), vomiting (80%) and fever (70%), Watery (60%), Mucoid (20%), Mixed (10%) and Bloody (10%), 1 to 5 days duration of diarrhea (60%).

Lack of latrine, cohabitation with domestic animals, raw milk consumption, consumption of raw vegetables and fruits, unprotected water, raw egg and consumption of Street vended foods were the risk factors that were associated with contracting *Salmonella* species. This study has also shown that all *Salmonella* isolates were sensitive to Ciprofloxacin while at the same time they showed highest level of antibiotic resistance for Ampicillin (100%) followed by Tetracycline (85%) and Contrimoxazole (80%).

5.2. Conclusion

The findings of this research indicated that the prevalence of salmonellosis among diarrheal patients who visited DRH, Dessie, North East Ethiopia from November to January 2017 was 5.21%. This prevalence rate of *Salmonella* species might be the result of the drinking water from unprotected source, consumption of raw vegetable and fruits, consumption of raw meat and absence of latrine in the households. Relatively, its prevalence was lower for those study subjects that had access to safe and adequate drinking water, proper excreta disposal systems.

Prevention of salmonellosis caused by *Salmonella* relies primarily on measures that prevent spread of the organism within the community and from person to person. These include: hand-washing with soap, ensuring the availability of safe drinking water, safe disposal of human waste, safe handling and processing of foods. These measures will not only be useful for reducing the prevalence of salmonellosis, but also for preventing infection with other diarrheal diseases as well. In all cases, health education and the cooperation of the community in implementing control measures are essential to reduce the prevalence of salmonellosis.

This study has also shown that ciprofloxacin may be used as the drug of choice for empirical treatment. On the other hand, tetracycline, ampicillin, contrimoxazole, may not be used as drugs of choice for the treatment of *Salmonella* infection unless culture and sensitivity tests are done prior to treatment. Therefore, *Salmonella* spp. should receive significant attention in the diagnosis and control of diarrhoeal disease.

5.3. Recommendations

Based on the findings of the study, the following recommendations are made:

- The present study did not differentiate *Salmonella* isolate into species level. Therefore, further studies should be conducted on the prevalence of serogroups and serotypes of *Salmonella* isolates in the future.
- This cross sectional study was conducted in a short period of time from November to January 2017. Since the prevalence and patterns of antimicrobial resistance of *Salmonella* species vary with season, further studies should be carried out to assess the seasonal variation in prevalence and antimicrobial resistance patterns of *Salmonella* species so that the extent of *Salmonella* infection will be well established.
- Raw eggs, raw vegetables and fruit and milk should not be consumed in order to limit the spread of salmonellosis.
- Pretreatment of water obtained from unprotected sources prior to consumption, the need to improve the hygienic conditions of street vended foods and discouraging the cohabitation of animals with humans in order to limit the spread of salmonellosis.
- Ciprofloxacin may be used as a drug of choice for empirical treatment whereas Tetracycline, Ampicillin and Contrimoxazole should not be used as a drug of choice for the treatment of *Salmonella* infection without making sensitivity tests prior to treatment.

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7. APPENDICES

7.1. APPENDIX I

English version questionnaire and Consent form.

Participant Information Sheet

Dear respondents,

Good morning/ Good afternoon

My name is **_______________________**

Title of the Study: Prevalence and Antibiotic Susceptibility Profile of *Salmonella* Isolates among Diarrhoeal Patients Visiting Dessie Referral Hospital, North East Ethiopia

Duration of the Study: - November - January, 2016/17.

The purpose of this Study is to generate information about the relative contribution of *Salmonella* infection to the overall burden of diarrheal diseases and to pin point appropriate therapeutic drug. In order to attain this goal, I request your honest and genuine participation. No individual response will be passed to a third person; it is used only for the purpose of this study. It is your full right not to participate, to discontinue or to participate in the study. If you do not like to participate you are not obliged to answer the questionnaire. There will be no negative impact on you whatever your decision. But your honest participation will have contribution to generate information that can be used in the fight against salmonellosis. So, please take a few minute to answer the questionnaire. If you have question or complaints don't hesitate to ask the contact person by the following address.

Thank you so much for your cooperation in responding this questionnaire!!!

Samuel Chane

Tele: 0931129027

Mail: samuelchane19@gmail.com

Do you want to participate in the study?

Yes, I want to participate

No, I do not want to participate

CONSENT FORM

I the undersigned have been informed and understood that the purpose of this particular research project is to find out the prevalence and antibiotic susceptibility profile of *Salmonella* among diarrheal patients visiting Dessie Referral Hospital. It is your full right not to participate, to discontinue or to participate in the study. If you do not like to participate you are not obliged to answer the questionnaire. There will be no negative impact on you whatever your decision. Hence, with this understanding, your honest participation will have contribution to generate information that can be used in the fight against Salmonellosis.

Signature of the patients or children parent's/ care taker's: ____________

Date: _____________

Code no.__________

PART I: Personal profile and Socio demographic Characteristics

1. Age______________

2. Sex A) Male B) Female

3. Place of residence: A) Urban B) Rural

4. Educational status:

A) Literate B) Illiterate

5. Where is the source of drinking water? A) Protected water B) Unprotected water

6. Latrine: A) Present B) Absent

7. Is there domestic animals in your house: A) Yes B) No

8. If your answer for question number 7 is yes, where do the animals spend the night?

A) In their own separate enclosure B) Together with family in one roof

9. Do you /your child consume raw milk?

A) Yes B) No

10. Do you/your child consume raw meat?

A) Yes B) No

11. Do you/your child consume raw vegetables and fruits?

A) Yes B) No

12. Do you/your child consume street foods?

A) Yes B) No

13. Do you/your child consume egg?

A) Yes B) No

Part II: Signs and Symptoms

No	Symptoms	
1	Presence of fever	Yes /No
2	Vomiting	Yes /No
3	Abdominal pain	Yes /No
4	Duration of diarrhea	1-5 days / 6-10 days / 11-15 days / >16 days
5	Consistency of stool	Watery/Bloody/Mucoid/Mixed(blood & mucus)

7.2. APPENDIX II

Amharic version of questionnaire and Consent form

<u>የስምምነት ማረጋገጫ ቅፅ</u>

ከታች ፊርማዬን ያኖረኩት የጥናቱን አላማ ተነግሮኝ በሚገባ ተረድቻለሁ፡፡ እኔ የምሰጠው መረጃ ለዚህ ጥናት አገልግሎት ብቻ የሚውል መሆኑን እና በሚስጥር እንደሚጠበቅ እንዲሁም ማንነቴ እንደማይገለፅ ተነግሮኛል፡፡ በተጨማሪም በጥናቱ መሳተፍ አለመሳተፍ ወይም በማንኛውም ጊዜ ከጥናቱ ተሳታፊነት አቋርጬ መውጣት እንደምችል ሙሉ መብት እንዳለኝ ተረድቻለሁ፡፡ ስለዚህ ይን መጠይቅ ለመመለስ እና የራሴን ሰገራ ወይም የልጄን ሰገራ ናሙና ለመስጠት ፍቃደኛ መሆኔን በፊርማዬ አረጋግጣለሁ፡፡

ፊርማ----------------------------

ቀን---------------------------------

የመጠይቁ ኮድ -----------------------------

ክፍል አንድ ፡- **<u>ማህበራዊ እና ዲሞግራፊያዊ ሁኔታዎች</u>**

1. ጾታ ሀ) ወንድ ለ) ሴት

2. እድሜ

3. የመኖሪያ አድራሻ ሀ) ከተማ ለ) ገጠር

4. የትምህርት ሁኔታ ሀ) ተምሬያለሁ ለ) አልተማርኩም

5. ለመጠጥ የምትጠቀሙበትን ውሃ ከየት ታገኛላችሁ?

6. የመጸዳጃ ቤት ሀ) አለ ለ) የለም

7. በቤት ውስጥ የቤት እንስሳ አለ ? ሀ) አለ ለ) የለም

8. እንስሳው/ ቶቹ ማደሪያ ሀ) እራሱን የቻለ ማደሪያ አላቸው ለ) ከቤተሰብ ጋር በአንድላይ ይኖራሉ

9. ያላተፈላ ወተት እርስዎ /ልጆት ተጠቅመዋል? ሀ) አዎ ለ) አልተጠቀምኩም/ አልተጠቀመም

10. ጥሬ ስጋ እርስዎ /ልጆት ተመግበዋል? ሀ) አዎ ለ) አልተመገብኩም/ አልተመገበም

11. ያልበሰለ አትክልት እርስዎ /ልጆት ተመግበዋል? ሀ) አዎ ለ) አልተመገብኩም/ አልተመገበም

12. ከጎዳና እርስዎ /ልጆት ምግብ ገዝተው ተመግበዋል? ሀ) አዎ ለ) አልተመገብኩም/ አልተመገበም

13. እርስዎ /ልጆት እንቁላል ተመግበዋል? ሀ) አዎ ለ) አልተመገብኩም/ አልተመገበም

ክፍል ሁለት፡- ስለ ህመሙ ሁኔታ

1. ትኩሳት	አለው/ የለውም
2. ትውከት	አለው/ የለውም
3. የሆድ ህመም	አለው/ የለውም
4. ማስቀመጥ ከጀመርዎት ምን ያህል ጊዜ ሆነ	ከ1-5 ቀን /ከ6-10ቀን/ ከ11-15ቀን/ ከ16 ቀን በላይ
5. የተቅማጡ አይነት	ውሃማ/ ደም የተቀላቀለበት/ ንፍጥ የተቀላቀለበት/ ንፍጥ እና ደም ያለው

7.3. APPENDIX III

Plate 1. *Salmonella* colonies on XLD and Mac Conkey agar

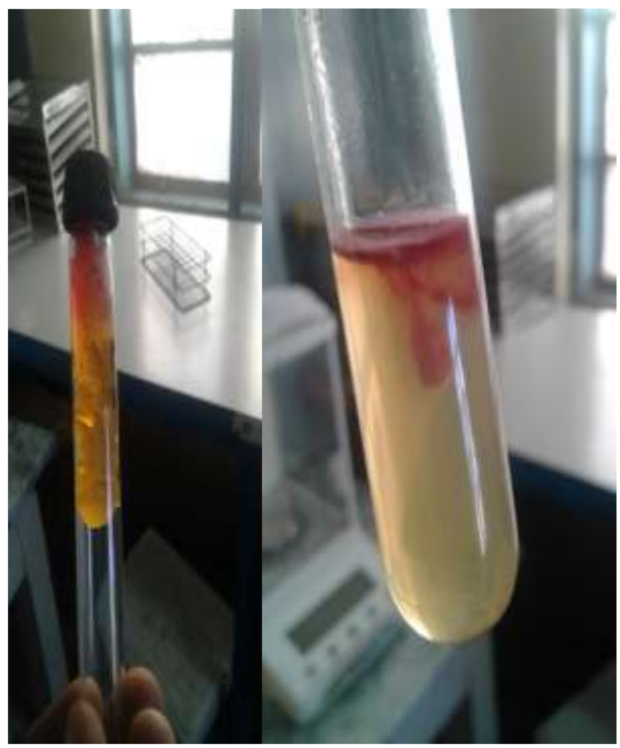

Plate 2. *Salmonella* appearance in TSI and motility test after 18 hours incubation.

Plate 3. Antibiotic sensitivity test by disk diffusion method