ISOLATION AND ANTIMICROBIAL SUSCEPTIBILITY PROFILE OF SHIGELLA AND SALMONELLA SPECIES FROM CHILDREN WITH ACUTE DIARRHOEA IN MEKELLE HOSPITAL AND SEMEN HEALTH CENTER, TIGRAY, ETHIOPIA

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TABLE OF CONTENTS **PAGES**

LIST OF TABLES **PAGES**

LIST OF FIGURES **PAGES**

ABBREVIATIONS

API	Analytical Profile Index
AST	Antimicrobial Susceptibility Testing
ATCC	American Type Culture Collection
CDC	Center for Disease Control and Prevention
CLSI	Clinical Laboratory Standards Institute
DHS	Demographic Health Survey
DMIP	Department of Microbiology, Immunology & Parasitology
FDRE	Federal Democratic Republic of Ethiopia
FMOH	Federal Ministry of Health
KIA	Kligler Iron Agar
LPS	Lipopolysaccharide
MDG	Millennium Development Goal
MDR	Multi- Drug Resistant
NTS	Non-typhoidal *Salmonella*
ONPG	Ortho Nitro-Phenyl-β-D-Galactopyranosidase
OPD	Out Patient Department
TRHB	Tigray Regional Health Bureau
WHO	World Health Organization
XLD	Xylose Lysine Deoxycholate

ABSTRACT

Background: Diarrhoea and acute gastroenteritis are among the leading causes of illnesses and deaths in infants and children throughout the world, especially in developing countries. Globally, *Salmonella* and *Shigella* remain the major contributors to acute enteric infections and diarrhoea. Emergence and spread of antimicrobial resistance to newer and more potent agents used in treatment have been described for *Salmonella* and *Shigella* species.

Objectives: To isolate and determine the antimicrobial susceptibility pattern of *Salmonella* and *Shigella* species from children with acute diarrhoea in Mekelle Hospital and Semen Health Center.

Materials and Methods: A cross sectional study was conducted among 260 children with acute diarrhoea in Mekelle, from November 2011 to March 2012.

Results: Out of the 260 study participants 145 (55.8%) were males and 115 (44.2%) were females. Majority of the patients (44.2%) were children under five years of age. A total of 120 enteropathogens were isolated. The frequency of isolation was 19 (7.3%), 18 (6.9%) and 83 (31.9%) for *Salmonella* species, *Shigella* species and intestinal parasites respectively. Most of the *Shigella* isolates were resistant to ampicillin (88.9%), Tetracycline (77.8), cotrimoxazole (55.6%) and chloramphenicol (55.6%). Among the *Salmonella* isolates highest resistance was observed to ampicillin (89.5%), Tetracycline (89.5%), chloramphenicol (78.9%) and cotrimoxazole (57.9%). Multi-drug resistance was noted in 19 (100%) and 16 (88.9%) of *Salmonella* and *Shigella* species respectively.

Conclusion and recommendation: *Shigella* and *Salmonella* are still challenging pathogens in children < 5 years of age. High antibiotic resistance was observed among both isolates to ampicillin, tetracycline, chloramphenicol and cotrimoxazole. Ciprofloxacin, norfloxacin and ceftriaxone were effective for all isolates. To reduce the incidence of these enteropathogens, improving personal and food hygiene has to be strengthened. The establishment of antibiotic policy and treatment guideline are recommended based on the susceptibility profile.

Key words: Diarrhoea, *Salmonella*, *Shigella*, Antimicrobial susceptibility, Ethiopia

CHAPTER I: INTRODUCTION

1. 1. General Introduction

Diarrhoea and acute gastroenteritis are among the leading causes of illness and deaths in infants and children throughout the world, especially in developing countries (Nelson *et al.*, 2003) in situations where water supplies are contaminated and sanitation is poor (Cheesbrough, 2006). An inadequate water supply, both in quantity and quality, poor sanitation, overcrowding and malnutrition are the main factors implicated in the occurrence, spread and severity of diarrhoeal disease (Alper, 2003). This is so in Asia, Africa and Latin America, where an estimated 2.5 million deaths occur each year in children, resulting in over a quarter of all childhood deaths (Nelson *et al.*, 2003). Diarrhoea is a major cause of childhood morbidity and mortality especially in socio economically developing countries, which remains the second leading cause of death among children under five globally *(*Reither *et al*., 2007, WHO, 2009).

World Health Organization (WHO) has estimated that 1.5 billion episodes of diarrhoea occur every year in developing countries, resulting in 3 million deaths (Alper, 2003). It accounts for an estimated 12,600 deaths each day in children in Asia, Africa, and Latin America (Alper, 2003). Despite the wide range of treatment and prevention modalities that are available, diarrhoea still remains a major contributor to infant mortality worldwide. This is an obstacle to the achievement of Millennium Development Goal(MDG) number 4 (WHO, 2009). In Ethiopia according to 2000 Ethiopia DHS report 24 percent of children under 5 years of age experienced diarrhoea in the two weeks prior to the survey and the FDRE MOH facility-based surveillance system reports that in 2002-03, the proportions of diarrhoea attributable causes of under-5 mortality have been estimated as 20%. Diarrhoeal diseases kill more children than malaria, HIV/AIDS and measles combined (FMOH, 2005).

Diarrhoea can be caused by different agents such as; bacteria, parasites and virus *(*Vargas *et al*., 2004). The main etiology of the diarrhoea is related to a wide range of bacteria (such as *Campylobacter jejuni*, *Escherichia coli*, *Salmonella species*, *Shigella species*, *Vibrio cholera*, *Yersinia enterocolitica*, and *Aeromonas species*), enteroparasites (*Giardia lamblia*,

Cryptosporidium species and *Entamoeba histolytica*), and viruses (adenovirus, Norwalk virus, and rotavirus) (Vargas *et al.*, 2004). The severity of the illness is mediated by different factors related to both the patient (nutritional status, presence of concomitant illness and human immunodeficiency virus status) and the etiological agent (specific bacterial virulence and antimicrobial resistance (Okeke *et al*., 2003).

Salmonella species and *Shigella* species remain among the bacteria most frequently isolated from stool samples obtained from diarrhoeal patients, especially in rural areas from developing countries (Okeke *et al*., 2003). Globally, *Salmonella* and *Shigella* remain the major contributors to acute enteric infections and diarrhoea. The common route of infection by these pathogens is the ingestion of contaminated foods and drinks (CDC, 2001). The problem of antimicrobial resistance in bacterial pathogens causing diarrhoeal diseases continues to be alarming. Emergence and spread of antimicrobial resistance to newer and more potent agents used in treatment have been described for *Salmonella* and *Shigella* species (Kansakar *et al*., 2011).

Antimicrobial resistance has complicated the selection of antibiotics for the treatment of enteric bacterial pathogens, particularly to commonly used antimicrobial agents such as ampicillin, tetracycline and trimethoprim–sulfamethoxazole (Isenbarger *et al*., 2002). Information concerning enteric pathogens in each country is essential in terms of epidemiology, surveillance and management of patients (Kansakar *et al*., 2011). Since most diarrhoeal diseases are treated empirically, it is important to know the susceptibility pattern of the prevalent pathogens (Taneja *et al*., 2004). In Ethiopia there is a great need to establish the identity and antibiotic susceptibility patterns of different bacterial agents which cause enteric infections in order to introduce effective treatment for diarrhoeal illness (Asrat, 2008).

According to the Ethiopian census data population statistics projections on 2007 G.C. (1999/2000 E.C.) in Mekelle the total number of under 15 years of age children was 78,770, out of a total population of 215,546 which indicates 36.5 % were children under 15 years of age. According to TRHB annual report from Hospitals in 2010, diarrhoea was one of the top

ten in Under 5 Children OPD visits, admissions and deaths (TRHB, 2010). Each year Mekelle has an estimated 133,000 episodes of diarrhea in children aged two months to five years and 13% of deaths in Mekelle is due to dysentery (Giorgi and Krishnan, 2009). In Mekelle, there is lack of adequate information on bacterial enteric pathogens and their antimicrobial resistance trend. Hence this study aimed to isolate and determine the antimicrobial susceptibility profiles of *Shigella* and *Salmonella* species from children with diarrhoea in Mekelle.

1.2. LITERATURE REVIEW

1.2.1. Salmonella

1.2.1.1. General characteristics

Salmonella species are Gram-negative, motile, facultative anaerobic bacilli characterized by O, H and Vi antigens (Finegold and Giannella, 2000). Members of the genus *Salmonella* can cause a variety of diseases, including gastroenteritis and enteric (typhoid) fever. Currently, all strains of *salmonella* are grouped in a single species; *S. enterica*, which has approximately 2500 different serotypes, or serovars including the clinically significant serotypes *typhimurium* and *typhi*. Most strains of *Salmonella* produce acid and gas during fermentation of glucose. They also produce H_2S from sulfur-containing amino acids except *Salmonella Paratyphi A* and *Salmonella typhi,* which are weak producer (Harvey *et al*., 2007).

1.2.1.2. Epidemiology

Members of the genus *Salmonella* are widely distributed in nature. Serotype *typhi* is exclusively human pathogen, whereas other strains are associated with animals and foods (for example eggs and poultry). They are mainly transmitted by fecal-oral route. People are often infected when they eat contaminated foods of animal origin such as meat or eggs and may involve chronic carriers (Finegold and Giannella, 2000). They are endemic in many countries and cause most serious disease in the young. Children and immunocompromised patients are susceptible to invasive complications (Vugia *et al*., 2004). Non-typhoidal *Salmonella* is one of the principal causes of food poisoning worldwide with an estimated annual incidence of 1.3 billion cases and 3 million deaths each year (Vugia *et al*., 2004). More than 95% of *Salmonella* infection cases are food-borne, and salmonellosis accounts for 30% of deaths resulting from food borne illness in the United States (Mead *et al*., 1999).

Salmonella species were one of the predominant enteropathogens causing gastroenteritis, as reported from different corner of the world. Iran, Japan, Tanzania, Egypt showed prevalence of *5.8%,* 14.03%, 14%, 7.4% respectively (Wasfy *et al*., 2000, Savadkoohi and Ahmadpour-Kacho, 2007, Temu *et al*., 2007).

In Ethiopia, a number of studies were conducted; a study conducted in Jimma Hospital, South West Ethiopia, from March to July 2000, showed a total of 59 *Salmonella* strains were isolated from 384 pediatric outpatients with diarrhoeal illness with frequency of isolation 15.4% (Mache, 2002). Of these, Serogroup A comprised 5 (8.5%), B 17 (28.8%), C 13 (22%), D 8 (13.6%, other than *S.* Typhi), E 3 (5.1%) and *S.* Typhi 13 (22%). The most frequently isolated serogroup was B (28.8%) while the least frequent was group E (5.1%). Mache (2002) reported that, of all *Salmonella* isolates, 78 % (46/59) belonged to non-Typhi serogroups, which in turn indicates that these serogroups are responsible for the majority of diarrhoea in children (Mache, 2002).

Another study from Harar, Eastern Ethiopia, in Hiwot Fana and Misrak Arbegnoch teaching hospitals from January 2007 to February 2007, showed a total of 28 species of salmonella were isolated from 244 diarrhoeal stool samples with proportion of 11.5% (Reda *et al*., 2011). Another study in Addis Ababa also showed that the most commonly isolated *Salmonella* serogroup was group B, followed by group D (*S. typhi*) and group C (Asrat, 2008), but in Jimma the most commonly isolated Salmonella serogroup was group B, followed by group C and D (Mache, 2002).

1.2.1.3. Virulence factors

The outcome of a *Salmonella* infection is determined by the status of the host and status of the bacterium. The status of the bacterium is determined by the virulence factors. These virulence factors are virulence-plasmids, toxins, fimbriae, flagella and the pathogenicity islands of Salmonella are the major contributing factors of salmonella pathogenesis (Asten and Dijk, 2005).

1.2.1.4. Pathogenesis

Salmonella invade epithelial cells of the small intestine. Disease may remain localized or become systemic, sometimes with disseminated foci. The organisms are facultative, intracellular parasites that survive in phagocytic cells (Harvey *et al*., 2007). Infections begin with the ingestion of organisms in contaminated food or water. After leaving the stomach, *Salmonella* must traverse the mucosal layer overlaying the epithelium of the small intestine. After crossing the mucosal layer overlaying the intestinal epithelium, *Salmonella* interacts with both enterocytes and Microfolds cells (M cells) (Francis *et al*., 1992). Once it crosses the intestinal epithelium, *Salmonella* serotypes that cause systemic infections enter macrophages, and migration of infected macrophages to other organs of reticuloendothelial systems probably facilitates the dissemination of bacteria in the host (Parry *et al*., 2002). Production of Salmonella enterotoxin, local inflammation and illness manifestation usually begins suddenly with diarrhea and vomiting, accompanied in some cases by high fever. In cases of massive diarrhoea, symptoms may be observed that result from the loss of water and electrolytes (Kayser, 2005).

1.2.1.5. Drug resistance profile

In Africa and most other developing countries, multidrug resistance, particularly to commonly available antibiotics, remains a major challenge for the healthcare system (Bonfiglio *et al*., 2002).

A study conducted in Egypt showed *Salmonella* isolates were sensitive to amikacin, aztreonam, ceftriaxone and nalidixic acid. But, more than 70% of all isolates were resistant to streptomycin, erythromycin, ampicillin, chloramphenicol, sulphamethoxazole-trimethoprim, and tetracycline (Wasfy *et al*., 2000). Another study from Nigeria showed the organism was sensitive to amoxicillin, cotramoxazole, nitrofurantoin, gentamicin, ofloxacin and tetracycline although it was resistant to nalidixic acid and augmentin (Omololu–Aso *et al.*, 2010).

Salmonellosis caused by drug resistant *Salmonella* strains is a major health problem, especially among children in developing countries like Ethiopia (Mache, 2002, Asrat, 2008). A study conducted to determine the prevalent *Salmonella* serogroups and resistance pattern of the isolates to commonly used antibiotics among children in Jimma, South West Ethiopia, showed that, all the isolates were 100% susceptible for polymyxin B and gentamicin except one strain in serogroup D and B respectively, while 91% of the isolates were susceptible to nalidixic acid (Mache, 2002). The susceptibility of all strains in each serogroup to ampicillin and tetracycline was below 48%. Between 52.5% to74.6% of the isolates was sensitive to the other antibiotics. Gentamicin, polymyxin B and nalidixic acid were found to be the most effective antimicrobials, at least in vitro, where as tetracycline, ampicilln and cephalothin were the least effective for all serogroups (Mache, 2002). Ninety three percent of the isolates were found to be resistant to one or more drugs. Multiple resistances were frequently encountered to ampicillin, tetracycline, cephalothin, trimethoprim-sulfamethoxazole and kanamycin. In this report, resistance to ampicillin and tetracycline combinations were dominant, at the same time polymixin B, gentamicin and carbenicillin were found to be the drugs of choice (Mache, 2002).

Another study from Harar showed sensitivity of the *Salmonella* isolates were 0.0% to ampicillin and amoxicillin; 14.2% to tetracycline; 28.6% to chloramphenicol; 89.3% to norfloxacin; and 92.8% to gentamicin but in contrast to study from Addis Ababa, the results of this study demonstrated that gentamicin and norfloxacin are drugs of choice for treating diarrhoea caused by salmonella species (Reda *et al*., 2011).

A study conducted in Addis Ababa also showed that among all antibiotics tested for *Salmonella* species, isolated during the period of 1992-1993, the highest resistance was observed with ampicillin (81.2%), cephalothin (86.4%), chloramphenicol (83.7%), erythromycin (100.0%) gentamicin (75.6), sulfonoamide (81.1%), tetracycline (94.5%) and trimethoprim-sulfamethoxazole (75.7%). But it was 100.0% susceptible to norfloxacin (Asrat, 2008).

1.2.1.6. Diagnosis

In developing countries, *Salmonellosis* is frequently diagnosed solely on clinical grounds. However, isolation of the causative organism is necessary for a definitive diagnosis, for performing antimicrobial susceptibility testing, and for further characterization (Beyene, 2008). The diagnosis of *Salmonella* species can be conveniently divided into four phases: initial isolation, screening identification, definitive identification and typing. The diagnostic method of choice is detection of the pathogens in cultures. Selective/ indicator mediums are used to isolate *salmonella* from stool and other specimens in case of enteric fever. Identification is done using metabolic patterns or biochemical tests. Serovar classification is determined with specific antisera in the slide agglutination test. Molecular techniques can also be used (Kayser, 2005).

1.2.1.7. Treatment

In Africa and most other developing countries, multidrug resistance, particularly to commonly available antibiotics, remains a major challenge for the healthcare system (Bonfiglio *et al*., 2002). 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 (WHO, 2003). 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, 2003).

1.2.1.8. Prevention

The main method of effective prevention of salmonella infection is accomplished by proper sewage disposal; correct handling of food, and good personal hygiene. Three vaccine alternatives are available: Typhoid fever vaccinations for travelers to endemic areas can be achieved with the oral attenuated vaccine Virotif Ty 21a (Kayser, 2005).

1.2.2. Shigella

1.2.2.1. General characteristics

They are small Gram-negative rods, non- or late lactose fermenters, fermenting sugars without gas production. Although non-motile using conventional tests, recent studies have shown the presence of flagellar genes and expression of a motile phenotype under certain conditions (Gillespie and Hawkey, 2006). The genus *Shigella* contains four species: *Sh. dysenteriae, Sh. flexneri, Sh. boydii* and *Sh. sonnei*. The species are differentiated on the basis of simple biochemical tests and serology of their LPSs: *Sh. dysenteriae* are non-mannitol fermenters and their *O*-polysaccharide LPS is unrelated antigenically to the other shigellas. *Sh. flexneri* and *Sh. boydii* ferment mannitol, but the latter are antigenically distinct from *Sh. flexneri*. All *Sh. flexneri* O-specific polysaccharides of the LPS antigens have a common rhamnose-containing tetrasaccharide (Gillespie and Hawkey, 2006). *Sh. sonnei* differs from the other members of the genus in that it is a late lactose fermenter, which can be detected with ONPG, unlike other members of the genus which are non-lactose fermenting (Gillespie and Hawkey, 2006).

1.2.2.2. Epidemiology

Shigellosis is a global human health problem and becoming one of the leading causes of diarrhoeal morbidity and mortality in children under five years of age worldwide (Herwana *et al*., 2010). It is endemic throughout the world and among the most common causes of bacterial diarrhoeal diseases. As a global human health problem it is more severe than other forms of gastroenteritis (Savadkoohi and Ahmadpour-Kacho, 2007). Globally, it is estimated that shigellosis causes about 1,100,000 deaths per year, two- thirds of the patients being children under 5 years of age (Yismaw *et al*., 2006). In the United state *Shigella* species infect approximately 450,000 persons annually, resulting in over 6,000 hospitalizations (Mead *et al*., 1999).

It is still an important public health problem, especially in developing countries, where there is substandard hygiene and unsafe water supplies (Niyogi *et al*., 2001). Humans are the only natural hosts for *Shigella*. The predominant mode of transmission is by fecal-oral contact, and the low infectious inoculum makes *Shigellae* highly contagious (fewer than 200 viable organisms are sufficient to cause disease). Hence, secondary cases within a household are common, particularly under conditions of crowding or poor sanitation (Kayser, 2005). Persons symptomatic with diarrhoea are primarily responsible for transmission. Less commonly, transmission is related to contaminated food and water or fomites; however, the organism generally survives poorly in the environment. In certain settings where disposal of human faeces is inadequate, houseflies can serve as a mechanical vector for transmission (Kayser, 2005).

The forty serotypes of shigella are organized into four groups (A, B, C and D) based on the serologic relatedness of their polysaccharide O antigens. Group D (*Sh. sonnei*) is the serogroup found most commonly in the United States (Harvey *et al*., 2007). In developing countries including Ethiopia, *Sh. flexneri* and *Sh. dysenteriae* are most frequently isolated, whereas *Sh. sonnei* and *Sh. flexneri* predominate in developed countries (Yismaw *et al*., 2006, Temu *et al*., 2007).

A study conducted in United states from 1999 to 2002 showed Among the *Shigella* isolates identified (80%) were *Shigella sonnei*, (18%) were *Shigella flexneri*, (1%) were *Shigella boydii*, and (0.4%) were *Shigella dysenteriae (*Sivapalasingam *et al., 2006)*. According to reports from Tanzania, Djibouti and Yemen where it represents respectively 14%, 15% and 13% of enteropathogenic agents, Shigella was a major cause of childhood diarrhoea in Africa (Banajeh *et al*., 2001, Temu *et al*., 2007, Manirakiza *et al*., 2010).

In Ethiopia a cross sectional study conducted in Jimma Hospital and Jimma Health center, South West Ethiopia, from March to July 2000, showed a total of 77 *Shigella* strains were isolated from 384 pediatric outpatients with diarrhoeal illness with proportion of 20.1%. Of these, Serogroup A comprised (29.9 %), B (40.3%), C (19.5%) and D (10.4%). The most frequently isolated serogroup was B (40.3%) while the least frequent was group D (10.4%)

(Mache, 2001). In Gondar University Hospital, northwest Ethiopia a five year study on antimicrobial resistance pattern of *Shigella* species between September 2001 and August 2005, showed a total of 214 *Shigella* species were isolated from 2891 stool specimens with proportion of 7.4% (Yismaw *et al.*, 2006). Further study done in the same study area from August 2006 to February 2008 showed a total of 90 *Shigella* isolates were found from 1200 diarrhoeal stool specimens with frequency of isolation 7.5%. Of these, Serogroup A comprised 9 (10 %) isolates, B 65 (72.2%), C 8 (8.9%) and D 8 (8.9%). The most frequently isolated serogroup was B (72.2%) while the least frequent was group C and D (8.9. %) (Tiruneh, 2009). Those Studies from Jimma and Gondar showed the predominant *Shigella* serogroups isolated was *S.flexinery* (B), *S.dysentriae* (A), *S.boydii* (C) and *S.sonnei* (D) in order of their frequency of isolation (Mache, 2001, Tiruneh, 2009), but study in Addis Ababa, Tikur Anbassa and Ethio-Swedish Children's Hospital showed *S.sonnei* (D) was frequently isolated than *S.boydii* (C) (Asrat, 2008).

1.2.2.3. Virulence factors

Invasiveness and enterotoxin are the main virulence factors of Shigellosis. It is thought that *Shigella* first transit the mucous membrane of the colon by passing through M cells (M cells are phagocytic cells in the mucous membrane whose function is to sample microbes from the intestinal lumen and pass them on to the lymphoid tissue of the Peyer's patch in order to activate the immune defenses against intestinal microbes). Once across the mucosa, *Shigella* uses invasins to enter the epithelial cells from the underside (Gillespie and Hawkey, 2006). Once inside, they escape from the vacuole into the cytoplasm and multiply. Then they move through the host cell and spread to adjacent host cells by a unique process called actin-based motility whereby actin filaments polymerize at one end of the bacterium producing comet-like tails that propel the *Shigella* through the cytoplasm of the host cell. When they reach the boundary of that cell, the actin filaments push the *Shigella* across that membrane and into the adjacent cell. As the *Shigella* grow and spread within the epithelial cells, those epithelial cells die and provoke a strong inflammatory response leading to the symptoms of dysentery. *S. dysenteriae* produce Shiga toxin that disrupts host cell protein synthesis resulting in damage to the intestinal epithelium (Gillespie and Hawkey, 2006).

1.2.2.4. Pathogenesis

Shigellae invade and destroy the mucosa of the large intestine. Infection rarely penetrates to deeper layers of the intestine, and does not lead to *Shigella* bacteremia. An exotoxin (Shiga toxin) with enterotoxic and cytotoxic properties has been isolated from these organisms, and its toxicity may play a secondary role in development of intestinal lesions (Harvey *et al*., 2007). The toxin inhibits protein synthesis in eukaryotic cells by splitting the 23S rRNA at a certain locus. Shigatoxin contributes to the colonic epithelial damage, the small intestine diarrhoea with watery stools at the onset of shigellosis and with less frequent it causes hemolytic-uremic syndrome(HUS) (Kayser, 2005). *Shigella* causes classic bacillary dysentery, characterized by diarrhoea with blood, mucus, and painful abdominal cramping. Because of delay in humoral responses, complication and mortality rate due to shigellosis in children is most severe than other age groups (Savadkoohi and Ahmadpour-Kacho, 2007).

1.2.2.5. Drug resistance profile

Frequency of antibiotic resistance among *Shigella* species is growing and has been reported in various studies globally (Isenbarger *et al*., 2002).

A study from United States showed among the *Shigella* isolates tested (64%) were resistant to two or more agents (multidrug resistant). Common resistance patterns included the combination of ampicillin, streptomycin, sulfamethoxazole and tetracycline resistance (31%), ampicillin, streptomycin and sulfamethoxazole resistance (14%) and ampicillin and streptomycin resistance (10%). Resistance to the combination of ampicillin, chloramphenicol, streptomycin, sulfamethoxazole and tetracycline was seen in (8%) isolates. *Shigella* isolates in the United States remain susceptible to ciprofloxacin and ceftriaxone (Sivapalasingam *et al*., 2006). According to study from Iran, 91% of isolates were resistant to one or more antimicrobial agent(s) which had similarities with studies reported from Gondar (Mashouf *et al*., 2006, Yismaw *et al*., 2006). In majority of studies done in different countries most *Shigella* isolates showed minimal resistance to nalidixic acid, but in Iran emerging resistance against nalidixic acid (42.3%) was observed (Mashouf *et al*., 2006).

In Tanzania all isolates were fully susceptible to ciprofloxacin, nalidixic acid, erythromycin,cefuroxime and gentamycin (Temu *et al*., 2007). However, all isolates showed high resistance to ampicillin, tetracycline, trimethoprim-sulphamethoxazole and chloramphenicol. Reports from Yemen and Tanzania showed all isolates were susceptible to nalidixic acid (Banajeh *et al*., 2001, Temu *et al*., 2007).

The unwise use of antibiotics by patients and physicians alike in many developing countries such as Ethiopia has lead to an increased antibiotic resistance and resulted in reduced therapeutic efficacy in these countries (Okeke *et al*., 2003, Asrat, 2008). A study conducted to determine the prevalence of *Shigella* serogroups and resistance pattern of the isolates to commonly used antibiotics among children in Jimma, South West Ethiopia, showed that, the susceptibility of all the isolates in each serogroup to ampicillin and tetracyclines was below 47% , while less than 50% of serogroup A strains were susceptible to chloramphenicol. Sixty to seventy five percent of the isolates were susceptible to trimethoprime-sulphamethoxazole and carbenicillin in each serogroup, while more than 90% of the isolates were found to be susceptible to gentamicin, polymyxin B and nalidixic acid (Mache, 2001).

Another study conducted in Gondar University teaching hospital from September 2001 to August 2005 to determine the pattern of antimicrobial sensitivity of *Shigella* species to commonly used antibiotics, showed the overall sensitivity of *Shigella* species in the area was 92.1% to gentamicin, 91.1% to ciprofloxacin, 47.2% to chloramphenicol, 26.6% to cotrimoxazole, 20.1% to ampicillin and 14% to tetracycline. About 46% of the isolates were found to be resistant to at least three commonly used drugs, while 1.4% were found to be resistant to all the commonly used drugs (Yismaw *et al*., 2006). Further study in the same area from August 2006 to February 2008, all *Shigella* isolates showed the highest resistance rates to tetracycline (90%), co-trimoxazole (84.6%). ampicillin (78.9%) and chloramphenicol (67.8%), and lowest resistance rates to gentamicin (12.2%), ciprofloxacin (2.2%) and norfloxacin (1.1%). All the isolates were sensitive to nalidixic acid and ceftriaxone (Tiruneh, 2009).

Another study conducted in Tikur Anbassa and Ethio-Swedish Children's Hospital, Addis Ababa, Ethiopia *Shigella* species showed that most strains were resistant to ampicillin (78.7%), cephalothin (86.7%), chloramphenicol (74.7%), erythromycin (100.0%), sulfonamide (54.7%), tetracycline (97.3%) and TMP–SXT (45.3%), but susceptible to gentamicin (100%), nalidixic acid (97.3%) and norfloxacin (100.0%) (Asrat, 2008).

1.2.2.6. Diagnosis

Isolation of *Shigella* in the clinical laboratory typically involves an initial streaking for isolation on differential/selective media with aerobic incubation to inhibit the growth of the anaerobic normal flora. Commonly used primary isolation media include MacConkey, Hektoen Enteric Agar, Xylose Lysine Deoxycholate (XLD) and Salmonella-Shigella (SS) Agar. These media contain bile salts to inhibit the growth of other Gram-negative bacteria and pH indicators to differentiate lactose fermenters (Coliforms) from non-lactose fermenters such as *Shigella*. There is no suitable enrichment media for *Shigella* species (Finegold and Giannella, 2000, WHO, 2005). A few strains of *shigella* grow poorly on inhibitory media, and it is advisable to use MacConkey agar and to examine any non-lactose-fermenting colonies after overnight incubation (Gillespie and Hawkey, 2006). Following overnight incubation of primary isolation media at 37°C, colorless, non-lactose-fermenting colonies are streaked and stabbed into tubed slants of Kligler's Iron Agar or Triple Sugar Iron Agar. In these differential media, *Shigella* species produce an alkaline slant and an acid butt with no bubbles of gas in the agar. This reaction gives a presumptive identification, and slide agglutination tests with antisera for serogroup and serotype confirm the identification (Finegold and Giannella, 2000, WHO, 2005).

Some *E coli* biotypes of the normal intestinal flora closely resemble *Shigella* species (i.e. they are nonmotile, delayed lactose fermenters). These coliforms can usually be differentiated from *Shigella* by the ability to decarboxylate lysine. However, some coliforms cause enteroinvasive disease because they carry the Shigella-like virulence plasmid, and these pathogens are conventionally identified by laborious serological screening for EIEC serotypes (Finegold and Giannella, 2000). Sensitive and rapid methodology for

identification of both EIEC and Shigella species utilizes DNA probes that hybridize with common virulence plasmid genes or DNA primers that amplify plasmid genes by polymerase chain reaction (PCR). Enzyme-linked immune sorbent assay (ELISA) using antiserum or monoclonal antibody recognizing Ipa proteins can also be used to screen stools for enteroinvasive pathogens (Finegold and Giannella, 2000, Kayser, 2005).

1.2.2.7. Treatment

Early diagnosis and drug therapy in children is crucial because of high tendency for occurrence of complications. Over the past decades, *Shigella* species have become progressively resistant to most of the widely used and inexpensive antibiotics (Mashouf *et al*., 2006). Resistance has emerged even to newer, more potent antimicrobial agents. Moreover, a change in the incidence of *Shigella* subgroups from time to time makes it difficult to formulate a drug of choice for Shigellosis (Mashouf *et al*., 2006). The antimicrobial resistance patterns of *Shigella* species vary according to geographic region and in the same place over time, leading to a therapeutic problem (Niyogi *et al*., 2001). Patients treated with an ineffective antibiotic may have more complications than if they had not been treated, because the antibiotic is likely to affect the normal intestinal flora, thus actually favoring the growth of the resistant *Shigella* (WHO, 2001). Selection of an antimicrobial should be based on the sensitivity patterns of strains recently isolated in the area (WHO, 2008). Ceftriaxone, Ciprofloxacin or azithromycin can reduce the duration of illness and the period of shedding organisms (WHO, 2008, Harvey *et al*., 2007).

1.2.2.8. Prevention

Prevention of *Shigellosis* 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, safely disposing of human waste, breastfeeding of infants and young children, safe handling and processing of food, and control of flies (WHO, 2005).

1.3. Significance of the Study

According to the 2009 World Health Organization (WHO) bulletin, diarrhoeal diseases account for an estimated annual 1.5 million deaths among children under five years of age in the world (WHO, 2009). Despite the wide range of treatment and prevention modalities that are available, diarrhoea still remains a major contributor to infant mortality worldwide. This is an obstacle to the achievement of Millennium Development Goal (WHO, 2009). Diarrhoeal illnesses account for an estimated 12,600 deaths each day in children in Asia, Africa, and Latin America (Alper, 2003). In Ethiopia, 20% of deaths in children aged under five years are due to diarrhoeal diseases (FMOH, 2005). An epidemiological study of an infectious disease in a community is an initial step toward the introduction of the proper interventions for controlling the disease, because the features of the disease vary from place to place depending on the local meteorology, geography, and socioeconomic elements (Yamashiro *et al*., 1998).

According to TRHB Annual Report from Hospitals in 2010, diarrhoea was one of the top ten in under five Children OPD visits, admissions and deaths in the region including Mekelle (TRHB, 2010).

Though the treatment of choice for acute diarrhoea is fluid and electrolyte replacement, antibacterial agents are often recommended for treatment of suspected shigellosis and invasive salmonellosis. Since most diarrhoeal diseases are treated empirically, it is important to know the susceptibility pattern of the prevalent pathogens (Taneja *et al*., 2004). The problem of antimicrobial resistance in bacterial pathogens causing diarrhoeal diseases continues to be alarming. Emergence and spread of antimicrobial resistance to newer and more potent agents used in treatment have been described for *Salmonella* and *Shigella* (WHO, 2009).

In order to ensure appropriate treatment, continual surveillance is required to determine which antibiotics are still active (WHO, 2001), because, choice of antibiotics should be guided by local data and knowledge of local patterns of resistance is essential to optimize guidelines for empirical antimicrobial treatment (Savadkoohi and Ahmadpour-Kacho,

2007). In Ethiopia, *Salmonella* and *Shigella* have been reported to be resistant to first line antibiotics such as ampicillin, tetracycline and chloramphenicol (Mache, 2002, Yismaw *et al*., 2006, Asrat, 2008, Tiruneh, 2009). The continuous surveillance of multidrug resistant strains is very important in order to know the changing antibiotic susceptibility pattern as well as the cyclical change of the serogroup from time to time as the resistance pattern also changes with the change in the serogroup. Periodic analysis and reporting of antibiotic susceptibility is an important measure to guide antibiotic treatment (Srinivasa *et al*., 2009).

Therefore information concerning enteric pathogens in each country and localities is essential in terms of epidemiology, surveillance, and management of patients(Kansakar *et al*., 2011). Currently, there is lack of adequate information on prevalence of diarrhoea causing enteropathogens and their antimicrobial resistance in Northern Ethiopia. Hence this study aimed to isolate and determine the antimicrobial susceptibility profile of common enteric bacterial pathogens; *Salmonella* and S*higella* species from children with acute diarrhoea in Mekelle, Northern Ethiopia.

1.4. OBJECTIVES

1.4.1. General objective

- To isolate and determine the antimicrobial susceptibility pattern of *Shigella* and *Salmonella* species from children with acute diarrhoea in Mekelle Hospital and Semen Health Center, Mekelle, Tigray, Ethiopia.

1.4.2. Specific objectives

- To determine the distribution of *Shigella* and *Salmonella* species in different age groups from children with acute diarrhoea
- To assess the antibiotic susceptibility patterns of *Salmonella* and *Shigella* Species to the commonly used antimicrobial agents in the treatment of children with acute diarrhoea in Mekelle.

CHAPTER II: MATERIALS AND METHODS

2.1. Study design and area

A descriptive cross-sectional study was conducted from November 2011 to March 2012 in Mekelle Hospital and Semen Health Center, Mekelle, Northern Ethiopia. Mekelle town is located 787 kilometers Northern from Addis Ababa. According to the Ethiopian Census data population statistics projections on 2007 (1999/2000 E.C.) in Mekelle the total number of children under 5 and under 15 years of age was 26,536 and 78,770, respectively (Giorgi and Krishnan, 2009).

2.2. Study population

The study included all children with acute gastroenteritis from pediatric inpatient and outpatient departments who visit Mekelle Hospital and Semen Health Center with in the study period. Participants who met the following inclusion criteria were included in the study: acute diarrhoea; (the passage of loose stool by an individual, at least three times a day, for <14 days in duration), age 6 month to 14 years, no anti-infective therapy 48 hours prior to recruitment and informed written consent from the children's parents or guardians. Children with antibiotic treatment with in 48 hrs before recruitment, whose parents did not agree to participate, age less than 6 month and greater than 14 years were excluded.

2.3. Sample size determination

The sample size was determined based on the prevalence rate of *Shigella* species done by Mache on children in Jimma (Mache, 2001) and calculated by considering a 95% level of confidence, with a 5% margin of error and a 5% contingency.

$$n = \frac{(Z\alpha/_2)^2 \times P(1-P)}{d^2} = \frac{(1.96)^2 \times 0.201(1-0.201)}{(0.05)^2} = 246.78$$

Where; n = sample size; z = confidence interval; α = level of significance; d = tolerable error and p= proportion (20.1%)

A total of 260 study participants were included in the study.

2.4. Sample collection, handling and transport

Two hundred and sixty stool specimen were collected from all study participants that presented with acute diarrhoea (defined as three or more loose stools per day) using dry, clean, leak proof and wide mouth stool containers. Stool specimens were collected according to WHO standard procedure. In brief, the specimens were collected in labeled, leak-proof and clean plastic stool cups (WHO, 1991) after informed consent was obtained from all the participants through their legal and competent guardians or parents and transported into the Microbiology laboratory of Mekelle Regional Health Research Laboratory using Cary Blair transport media within 2-4 hours of collection.

2.5. Processing of stool specimens

2.5.1. Microscopic examination

Microscopic examination of stool specimens was performed using saline and iodine solutions. Direct microscopy of the smears in saline (0.85% Nacl solution) was performed for the detection of ova, larvae, trophozoites and cysts of intestinal parasites (WHO, 1991).

2.5.2. Culture and identification

a. Direct inoculation

Stool samples were directly inoculated using sterile cotton swabs onto MacConky agar (Oxoid; UK) and Xylose Lysine Deoxycholate (XLD) agar (Oxoid; UK) and the plates were incubated aerobically at 37°C for 24 h.

b. Enrichment broth

The same stool samples were also inoculated onto Selenite F broth enrichment media (Mast Diagnostics, UK) and incubated at 37°C for 24 hrs, which is intended for the best recovery

of *Salmonella* species. Following the incubation of Selenite F broth, sub- cultures were done onto both MAC and XLD plates and incubated at 37°C for 24 h.

The growth of *Salmonella* and *Shigella* species was detected by their characteristic appearance on MAC (NLF, smooth, colorless colonies, sometimes with black centered) and XLD agar (small red colonies and black-centered colonies). Confirmatory identification was done by the pattern of biochemical reactions using a standard bacterial identification system (API 20E, BioMerieux, Marcy-l'Etoile, France) (see appendix IV) and polyvalent (Poly O and H) antiseras for *Salmonella* species and Vi for *S.typhi* (WHO, 2002, CLSI, 2006).

2.5.3. Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed for all *Salmonella* and *Shigella* isolates using the disk diffusion method. In brief, a McFarland 0.5 standardized suspension of the bacteria in 0.85% sterile saline was prepared and swabbed over the entire surface of Mueller Hinton agar (Oxoid, UK) with a sterile cotton swab (CLSI, 2006). The inoculated plates were left at room temperature to dry for 3-5 minutes and a set of 9 antibiotic discs (Mast Diagnostics, UK) was then delivered onto the inoculated surface of Muller-Hinton plate with the following concentrations of the discs; ampicillin (AP) (10 µg), chloramphenicol (C) (30 µg), gentamycin (CN) (10 µg), norfloxacin (NOR) (10 µg), cotrimoxazole (TS) (25 µg), nalidixic acid (NA) (30 µg), ceftriaxone (CRO) (30 µg), ciprofloxacin (CIP) (5 µg) and tetracycline (T) (30 µg) (Mast Diagnostics, UK). The criteria used to select the antimicrobial agents tested were based on their availability and frequent prescriptions especially in the study area for the management of children with acute diarrhoeal infections.

After overnight incubation at 37°C, clear zones produced by antimicrobial inhibition of bacterial growth were measured in mm using a straight line ruler. The diameter of the zone was read using an interpreting chart for zone sizes. Findings of antibiotic resistance testing were recorded as susceptible, intermediate and resistant (CLSI, 2006).

2.5.4. Reference Strain

E. coli (ATCC 25922) was used as quality control throughout the study for culture and antimicrobial susceptibility testing. This strain was obtained from Ethiopian Health and Nutrition Research Institute (EHNRI).

2.6. Variables

Table 2.1. Study variables

Dependent variables	Independent variable
Distribution of *Shigella* species in different age group and sex of study participants	Age
Distribution of *Salmonella* species in different age groups and sex of study participants	Sex
Antimicrobial susceptibility status of *Salmonella* and *Shigella* species	Consistency of stool (watery, bloody, mucoid and mixed).

2.7. Statistical analysis

Data was entered and analyzed using SPSS version 16.0 software. Statistical analysis was focused on the relationships between antimicrobial resistance patterns with *Salmonella* and *Shigella* species of the patients. The distribution of *Shigella* and *Salmonella* species in relation to age, sex and consistency of the stool was also analyzed. *P* values were based on two-tailed test results and *P* values of <0.05 was considered statistically significant.

2.8. Ethical clearance

The M.Sc research project was given ethical clearance by the Department Research and Ethical Review Committee (DREC) and approved by Department of Microbiology, Immunology and Parasitology, School of Medicine, Addis Ababa University. Official permission to conduct the study from the study sites (Tigray Regional Health Bureau, Mekelle Hospital and Semen health Center) was obtained. Written informed consent was obtained from study participants' parents/ guardians (see Appendix III). The results of study participants with positive for enteropathogens were communicated with the attending physician for the management of the cases.

CHAPTER III: RESULTS

3.1. Study participants

The age and sex distribution of the study participants investigated for salmonellosis, shigellosis and intestinal parasites are summarized in Figure 3.1. A total of 260 children with acute diarrhoea visiting pediatric departments of Mekelle Hospital and Semen Health Center between November 2011 and March 2012 were included in this study. The age ranges from 6 month to 14 years. Majority of the patients 115 (44.2%) were children under 5 years of age. One hundred forty five (55.8%) of the study participants were males and 115 (44.2%) were females resulting in an overall male to female ratio of 1.3:1. Of these 260 patients, 115 (44.2%) were from Mekelle Hospital and 145 (55.8%) were from Semen Health Center.

The nature of the diarrhoeal stool specimens was watery 163 (62.7%), bloody 12(4.6%), mucoid 76 (29.2%) and mixed 9 (3.5%). The most common appearance of diarrhoea was mucoid with or without blood for both *Salmonella* 9 (47.3%) and *Shigella* 9 (50%).

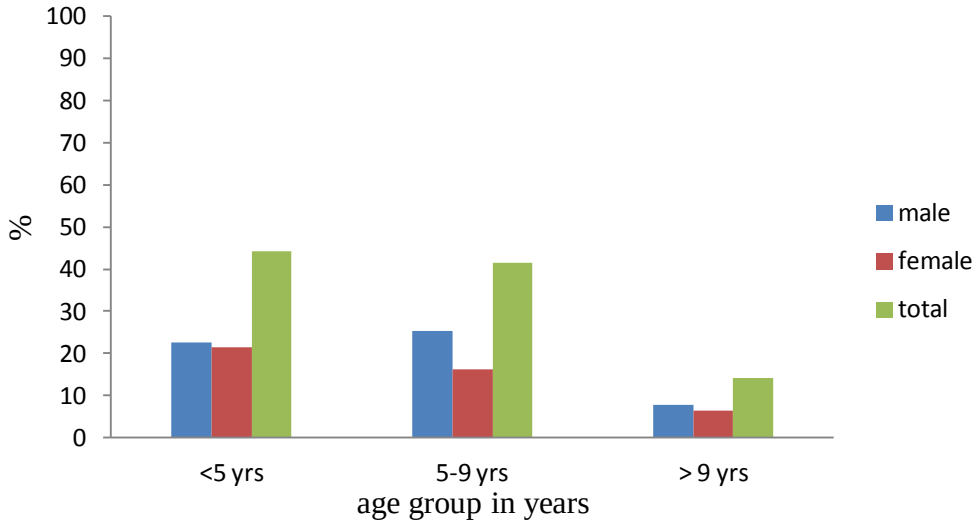

Figure 3.1: Age and sex distribution of 260 children investigated for *Salmonella*, *Shigella* and intestinal parasites in Mekelle Hospital and Semen Health Center, Nov. 2011 to March 2012.

3.2. Clinical Features

The clinical features of children with acute diarrhoea investigated for enteric pathogens summarized in Table 3.1. Illness symptoms included were fever 146 (56.2%), tenesmus 94 (36.2%), abdominal pain 116 (44.6%), headache 26 (10%) and nausea 129 (49.6%).

Table 3.1. Clinical findings and nature of diarrhoea of the 260 study participants in Mekelle Hospital and Semen Health Center, Nov. 2011 to March 2012

	Age group in years			
Clinical findings	< 5 n = 115 No. (%)	5-9 n = 108 No. (%)	>9 n = 37 No. (%)	Total n = 260 No. (%)
Tenesmus	32 (27.8)	44 (40.7)	18 (48.6)	94 (36.2)
Nausea	66 (57.4)	52 (48.1)	11(29.7)	129 (49.6)
Abdominal pain	22 (19.1)	65 (60.2)	29 (78.4)	116 (44.6)
Fever	73 (63.5)	59 (54.6)	14 (37.8)	146 (56.2)
Headache	2 (1.7)	14 (13.0)	10 (27.0)	26 (10.0)
Nature of diarrhoea				
Watery	69 (60)	65 (60.2)	29 (78.4)	163 (62.7)
Mucoid	32 (27.8)	36 (33.3)	8 (21.6)	76 (29.2)
Bloody	11 (9.6)	1 (0.9)	0 (0.0)	12 (4.6)
Mixed	3 (2.6)	6 (5.6)	0 (0.0)	9 (3.5)

3.3. Detection of enteropathogens from stool samples

The number and percentage of detection of enteropathogens in diarrhoeal stool samples obtained from pediatrics patients by culture and microscopic examination is presented in Table 3.2. A total of 120 enteropathogens were isolated. Of the total 120 identified enteropathogens, 19 *Salmonella* species, 18 *Shigella* species and 83 intestinal parasites were identified.

Among the 18 children who were positive for *Shigella*, abdominal pain was the commonest clinical findings 10/18 (55.6%), followed by tenesmus 9 (50%), nausea 7/18 (38.9%), fever 7/18 (38.9%) and headache 3/18 (16.7%). Among the 19 children who were positive for *Salmonella*, nausea was the commonest clinical findings 11/19 (57.9%), followed by abdominal pain 9/19 (47.4%), tenesmus 9/19 (47.4%) and headache 1/19 (5.3%).

Table 3.2: Frequency of isolation of enteropathogens from the 260 children with acute diarrhoea in Mekelle Hospital and Semen Health Center, Nov. 2011 to March 2012.

Enteropathogens	**Age group in years**			
	<5 No. (%)	5-9 No. (%)	>9 No. (%)	Total No. (%)
Salmonella				
Salmonella species (non typhi and paratyphi A)	11 (78.6)	3 (21.4)	0(0.0)	14 (100)
Salmonella typhi	0 (0.0)	1 (50.0)	1 (50.0)	2 (100)
Salmonella paratyphi A	3 (100)	0 (0.0)	0 (0.0)	3 (100)
Total	**14 (73.7)**	**4 (21.0)**	**1 (5.3)**	**19 (100)**
Shigella				
Shigella species	12 (75.0)	4 (25.0)	0 (0.0)	16 (100)
Shigella sonnei	0 (0.0)	0 (0.0)	2 (100)	2 (100)
Total	**12 (66.7)**	**4 (22.2)**	**2 (11.1)**	**18 (100)**
Intestinal parasites				
Entameoba histolytica	17 (43.6)	14 (35.9)	8 (20.5)	39 (100)
Giardia lamblia	17 (50.0)	14 (41.2)	3 (8.8)	34 (100)
Hymenolepis nana	0 (0.0)	4 (80.0)	1 (20.0)	5 (100)
Taenea species	0 (0.0)	1 (100)	0 (0.0)	1 (100)
Trichuris trichuria	0 (0.0)	1 (100)	0 (0.0)	1 (100)
Schistosoma mansonni	1 (50.0)	1 (50.0)	0 (0.0)	2 (100)
Entrovious vermicularis	1 (100)	0 (0.0)	0 (0.0)	1 (100)
Total	**36 (43.4)**	**35 (42.2)**	**12 (14.4)**	**83 (100)**

3.3.1. *Salmonella species*

A total of 19/260 (7.3%) *Salmonella* species were isolated from children with diarrhoea, of which 2 (10.5%) *Salmonella typhi,* 3 (15.8%) *Salmonella paratyphi A* and 14 (73.7%) *Salmonella species* were isolated.

The age distribution of salmonella were 14 (73.7%), 4 (21.1%) and 1 (5.3%) in the age group <5, 5-9, >9 years respectively as shown in Figure 3.2. *Salmonella* infections were more common among children < 5 years of age group than the other age groups (p = 0.007) as presented on table 3.3. The sex distribution of the isolates were 12 (63.2%), 7 (36.8%), in male and female respectively. Sex has no statistically significance association (p = 0.501). Among the 19 children who were positive for *Salmonella* species 9 (47.4%), 7 (36.8%), 1 (5.3%), 2 (10.5%) were isolated from watery, mucoid, bloody and mixed diarrhoea respectively. Isolation of Salmonellosis were not statistically associated with nature of diarrhoea (p >0.05).

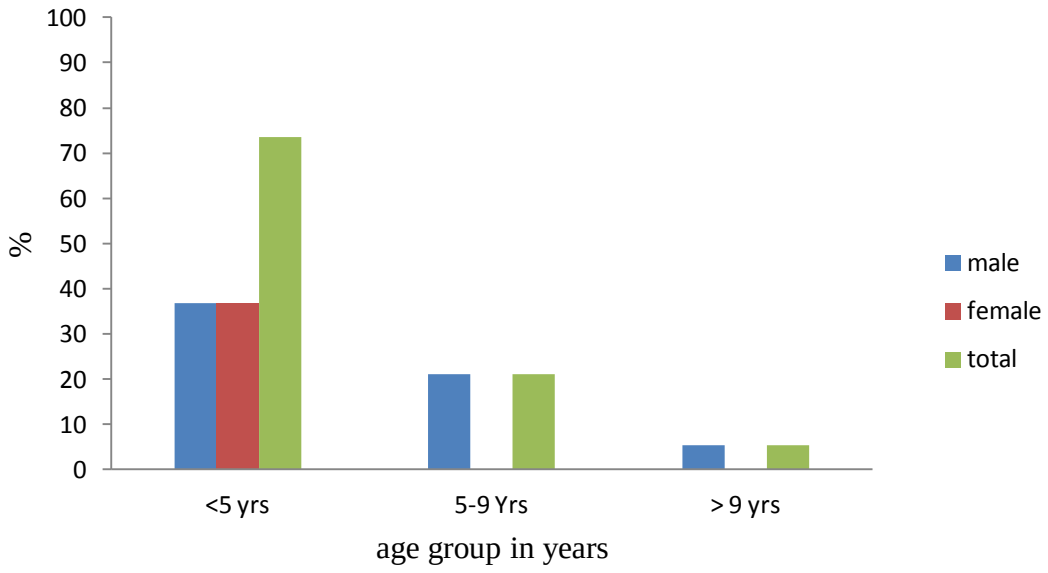

Figure 3.2. Age and sex distribution of children who were positive for *Salmonella* species in Mekelle Hospital and Semen Health Center, Nov. 2011 to March 2012.

Table 3.3. Association of culture positive and negative *Salmonella* species with age group and nature of diarrhea in Mekelle Hospital and Semen Health Center, Nov. 2011 to March 2012

	Positive (%) (*Salmonella* spp.) n=19	Negative (%) (*Salmonella* spp.) n=241	Total (%) n=260	P- value
Age group in years				
<5	Yes 14 (73.7) No 5 (26.3)	101 (41.9) 140 (58.1)	115 (44.2) 145 (55.8)	0.007
5-9	Yes 4 (21.1) No 15 (78.9)	104 (43.2) 137 (56.8)	108 (41.5) 152 (58.5)	0.06
>9	Yes 1 (5.3) No 18 (94.7)	36 (14.9) 205 (85.1)	37 (14.2) 223 (85.8)	0.245
Nature of diarrhoea				
Watery	Yes 9 (47.4) No 10 (52.6)	154 (63.9) 87 (36.1)	163 (62.7) 97 (37.3)	0.151
Mucoid	Yes 7 (36.8) No 12 (63.2)	69 (28.6) 172 (71.4)	76 (29.2) 184 (70.8)	0.449
Bloody	Yes 1 (5.3) No 18 (94.7)	11 (4.6) 230 (95.4)	12 (4.6) 248 (95.4)	0.889
Mixed	Yes 2 (10.5) No 17 (89.5)	7 (2.9) 234 (97.1)	9 (3.5) 251 (96.5)	0.080

3.3.2. *Shigella species*

A total of 18/260 (6.9%) *Shigella* species were isolated from children with diarrhoea, of which 2 (11.1) *Shigella sonnei* and 16 (88.9%) *Shigella* species were isolated. The distribution of *Shigella* isolates in different age groups were 12 (66.7%), 4 (22.2%) and 2 (11.1%) in the age group < 5, 5-9, > 9 years respectively as shown in figure 3.3. *Shigella* infections were more common among children < 5 years of age than the other age groups (p= 0.047). The sex distribution of the isolates were 8 (44.4%), 10 (55.6%), in male and female respectively. Sex has no statistically significance (p = 0.316). Among the 18 children who were positive for *Shigella* species 6 (33.3%), 8 (44.4%), 3 (16.7%), 1 (5.6%) were isolated from watery, mucoid, bloody and mixed diarrhoea respectively. Isolation of shigellosis were statistically associated with bloody diarrhoea (p= 0.012) as indicated on table 3.4.

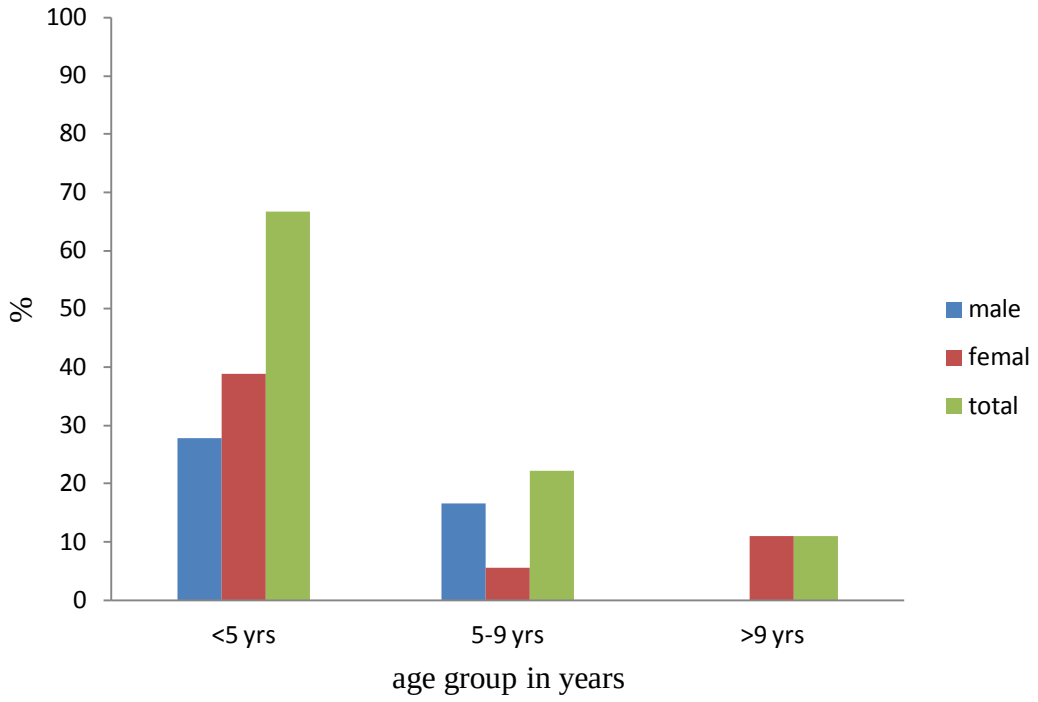

Figure 3.3. Age and sex distribution of children who were positive for *Shigella* species in Mekelle Hospital and Semen Health Center, Nov. 2011 to March 2012.

Table 3.4. Association of isolation of *Shigella* species with age group and nature of diarrhea in Mekelle Hospital and Semen Health Center, Nov. 2011 to March 2012.

	Positive (%) (*Shigella* spp.) n=18	Negative (%) (*Shigella* spp.) n=242	Total (%) N=260	P- value
Age group in years				
<5	Yes 12 (66.7) No 6 (33.3)	103 (42.6) 139 (57.4)	115 (44.2) 145 (55.8)	0.047
5-9	Yes 4 (22.2) No 14 (77.8)	104 (43) 138 (57)	108 (41.5) 152 (58.5)	0.085
>9	Yes 2 (11.1) No 16 (88.9)	35 (14.5) 207 (85.5)	37 (14.2) 223 (85.8)	0.695
Nature of diarrhoea				
Watery	Yes 6 (33.3) No 12 (66.7)	157 (64.9) 85 (35.1)	163 (62.7) 97 (37.3)	0.08
Mucoid	Yes 8 (44.4) No 10 (55.6)	68 (28.1) 174 (71.9)	76 (29.2) 184 (70.8)	0.141
Bloody	Yes 3 (16.7) No 15 (83.3)	9 (3.7) 233 (96.3)	12 (4.6) 248 (95.4)	0.012
Mixed	Yes 1(5.6) No 17 (94.4)	8 (3.3) 234 (96.7)	9 (3.5) 251 (96.5)	0.614

3.3.3. Intestinal Parasites

The total intestinal parasites isolated from the study participants are presented in Table 3.2. Among the 83 parasites isolated from the stool of children who had complained of diarrhoea, the parasites isolated were *Entrovious vermicularis* (1, 0.4%), *Hymenolopis nana (5, 1.9%), Schistosoma mansoni* (2, 0.8%), *Giardia lamblia* (34, 13.1%), *Entamoeaba histolytica* (39, 15%), *Taenia species* (1, 0.4%), *Trichuris trichuria* (1, 0.4%).

Out of the total 83 identified parasites, *Entamoeaba histolytica and Giardia lamblia* were the most frequently identified enteric parasites with prevalence rate of 15% and 13.1% respectivelly.

The distribution of these isolates in different age groups was 36 (43.4%), 35 (42.2%) and 12 (14.4%) in the age group <5, 5-9, >9 years respectively. Intestinal parasitic infections had no statistical significance difference among age groups ($p > 0.05$). The sex distribution of the isolates were 52 (62.6%), 31 (37.4%), in male and female respectively. Sex has no statistically significance ($p = 0.513$).

Table 3.5. Age and sex distribution of children positive for intestinal parasites in Mekelle Hospital and Semen Health Center, Nov. 2011 to March 2012.

Age group in year	**Sex no. (%)**		
	Male	**Female**	**Total**
<5	20(24.1)	16(19.3)	36(43.4)
5-9	23(27.7)	12(14.5)	35(42.2)
>9	9(10.8)	3(3.6)	12(14.4)
Total	**52(62.6)**	**31(37.4)**	**83(100)**

3.3.4. Co-infections

Co-infections were found in 18 (6.9%) of the patients. Of these, three were bacteria/ parasite/parasite, five were bacteria/parasite and ten were parasite/parasite co-infection. There was no bacteria/bacteria coinfection. *Giardia lamblia* and *Entameaba histolytica* were found to be involved in co-infection including with *Salmonella* and *Shigella* isolates.

3.4. Antimicrobial susceptibility pattern

3.4.1. *Salmonella Species*

The antimicrobial susceptibility testing was done on all *Salmonella* isolates using disk diffusion method and the results are presented in Table 3.6. Among the 19 *Salmonella* isolates, the overall rates of resistance were ampicillin 17 (89.5%), tetracycline 17 (89.5), chloramphenicol 15 (78.9%), cotrimoxazole 11 (57.9%), nalidixic acid 6 (31.6%), gentamicin 3 (15.8%), ceftriaxone 2 (10.5%) and norfloxacin 1 (5.3%). All isolates were sensitive to ciprofloxacin.

Table 3.6. Antimicrobial Susceptibility Profile of *Salmonella species* (n=19) isolated from children in Mekelle Hospital and Semen Health Center, Nov. 2011 to March 2012.

Antimicrobial agents	**Susceptible No. (%)**	**Intermediate No. (%)**	**Resistance No. (%)**
Ampicillin	2(10.5)	0(0.0)	17(89.5)
Co-trimoxazole	7(36.8)	1(5.3)	11(57.9)
Chloramphenicol	3(15.8)	1(5.3)	15(78.9)
Norfloxacin	17(89.5)	1(5.3)	1(5.3)
Tetracycline	2(10.5)	0(0.0)	17(89.5)
Gentamicin	16(84.2)	0(0.0)	3(15.8)
Ceftriaxone	17(89.5)	0(0.0)	2(10.5)
Nalidixic acid	12(63.1)	1(5.3)	6(31.6)
Ciprofloxacin	19(100)	0(0.0)	0(00.0)

3.4.2. *Shigella Species*

The results of the antimicrobial susceptibility pattern of *Shigella* isolates are summarized in Table 3.7. Among the 18 *Shigella* isolates, the overall rates of resistance were ampicillin 16 (88.9%), tetracycline 14 (77.8%), chloramphenicol 10 (55.6%), cotrimoxazole 10 (55.6%), nalidixic acid 5 (27.8%) and gentamicin 5 (27.8%). All isolates were 94.4-100% susceptible to ciprofloxacin, ceftriaxone and norfloxacin. Resistance to one or more antimicrobial agent(s) was noted in 17 (94.4%) of the isolates.

Table 3.7. Antimicrobial Susceptibility Profile of *Shigella species* isolated from children in Mekelle Hospital and Semen Health Center, Nov. 2011 to March 2012.

Antimicrobial agents	**Susceptible No. (%)**	**Intermediate No. (%)**	**Resistance No. (%)**
Ampicillin	0(0)	2(11.1)	16(88.9)
Cotrimoxazole	7(38.9)	1(5.6)	10(55.6)
Chloramphenicol	8(44.4)	0(0.0)	10(55.6)
Norfloxacin	18(100)	0(0.0)	0(0.0)
Tetracycline	4(22.2)	0(0.0)	14(77.8)
Gentamicin	12(66.7)	1(5.5)	5(27.8)
Ceftriaxone	17(94.4)	1(5.6)	0(0.0)
Nalidixic acid	10(55.5)	3(16.7)	5(27.8)
Ciprofloxacin	18(100)	0(0.0)	0(0.0)

3.4.3. Multi-Drug Resistance (MDR)

Multi-drug resistance (resistance to two or more drugs) was observed in 19/19 (100 %) and 16/18 (88.9%) of *Salmonella* and *Shigella* isolates as shown in Table 3.8.

Table 3.8. *Salmonella* and *Shigella* isolates resistance to two or more antibiotics in Mekelle Hospital and Semen Health Center, Nov. 2011 to March 2012.

***Salmonella* species**	No. of isolates	Resistance to antibiotics	No.	***Shigella* species**	No. of isolates	Resistance to antibiotic	No.
	19				**18**		
		AP,T	2			AP,T	2
		AP,T,C	2			AP,CN	1
		AP,T,TS	3			C,T,NA	1
		AP,T,C,TS	3			AP,T,TS	1
		AP,T,C,NA	1			AP, T, C	2
		T,C,TS,CRO	1			AP,T,C,TS	2
		AP,C,TS,CRO	1			AP,T,TS,CN	2
		T,C,NA,NOR	1			AP,C,TS,NA	1
		AP,T,C,TS,CN	1			AP,T,C,TS,CN	1
		AP,T,C,CN,NA	1			AP,T,C,TS,NA	2
		AP,T,C,TS,NA	2			AP,T,C,TS,CN, NA	1
		AP,T,C,TS,CN, NA	1				

AP = ampicillin, T = tetracycline, C = chloramphenicol, CN = gentamicin, TS = cotromoxazole, NA = nalidixic acid, CRO = ceftriaxone, NOR= norfloxacillin, CIP = ciprofloxacin

CHAPTER IV: DISCUSSION

Despite the wide range of treatment and prevention modalities that are available, diarrhoea still remains a major contributor to childhood mortality and morbidity worldwide. This is an obstacle to the achievement of Millennium Development Goal (MDG) number 4, which aims to reduce 2/3 of childhood mortality by 2015 (WHO, 2009). Diarrhoeal diseases account for an estimated annual 1.5 million deaths among children under five years of age in the world (WHO, 2009). It can be caused by many etiological agents, but mainly by enterobacteria such as *Salmonella* species, *Shigella* species, *Escherichia coli*, *Campylobacter jejuni* and *Vibrio cholera*. Intestinal parasites such as *Entamoeba histolytica* and *Giardia lamblia*, and some viruses (adenovirus, Norwalk virus, and rotavirus) are also important agents (Okeke *et al.*, 2003).

Among the bacteria causing diarrhoeal diseases *Salmonella* and *Shigella* continue to be a major public health problem (Alkizim *et al*., 2011). In Ethiopia, salmonellosis and shigellosis resistant to first line antibiotics such as ampicillin, tetracycline and chloramphenicol have been reported as a major challenge (Mache, 2002, Yismaw *et al*., 2006, Asrat, 2008, Tiruneh, 2009).

Since most diarrhoeal diseases are treated empirically, it is important to know the local susceptibility pattern of the prevalent enteropathogens to ensure appropriate treatment and effective control measures (WHO, 2001, Taneja *et al*., 2004). Because, administration of antibiotics should be guided by local data and knowledge of local patterns of resistance is essential to optimize guidelines for empirical antimicrobial treatment (Savadkoohi and Ahmadpour-Kacho, 2007). To our knowledge this is the first study conducted in Mekelle which detected a total of 120 enteropathogens from Children with acute diarrhoea.

The overall prevalence of *Salmonella species* in this study was 7.3%. This is in agreement with a study conducted in Yemen, 6.8% (Banajeh *et al*., 2001) and higher than the findings reported in Addis Ababa, 3.8% (Asrat *et al*., 1999) and Djibouti, 2.9% (Mikhail *et al*., 1990). But was lower than the 15.4% isolation rate reported from Jimma (Mache, 2002).

The variability of isolation of Salmonella may be attributable to the difference in study areas and period, because the features of the disease vary from place to place and time to time depending on the local meteorology, geography, and socioeconomic elements (Yamashiro *et al*., 1998).

The relatively lower isolation rate of s*almonella* in this study compared to the previous study in Jimma could be due to increased awareness of the community about personal and environmental hygiene from the continuous intervention made by the health extension workers in Mekelle.

According to this study the isolation rate of Salmonellosis was higher in children less than 5 years of age, which is in accordance with previous study done in Iran (Prakash, 2008). This might be due to the reason that, children are less likely than adults to wash their hands after defecating, more likely to put their fingers or dirty objects into their mouth and also more likely to play in soil where they may come into contact with faeces.

In this study, majority of *Salmonella* isolates were resistant to ampicillin (89.5%), Tetracycline (89.5%), chloramphenicol (78.9%) and co-trimoxazole (57.9%). This observation is in contrast with findings from Jimma (Mache, 2002) and Brazil (Diniz-Santos and Silva, 2006) where most of the isolates were sensitive to ampicillin (40.7%), chloramphenicol (64.4%), tetracycline (40.7%) and co-trimoxazole (59.3%).

Compared to previous report from Jimma (Mache, 2002), greater than fourfold increase in resistance to gentamicin from 1.7% to 15.8% was observed in the present study. This increase might be because of the relatively increased irrational use of the antibiotics in the study area. In developing countries like Ethiopia antibiotics are carelessly used by patients and physicians, where it is a common practice that antibiotics can be purchased without prescription, which leads to misuse of antibiotics by the public thus contributing to the emergence and spread of antimicrobial resistance (Okeke *et al*., 2003, Asrat, 2008).

Resistance to two or more drugs was observed in 100% of the isolates in this study. The organisms seems to have increased their resistance to the drugs from lower levels reported earlier (Mache, 2001) to levels of more than 90% in reports by Asrat (2008). This is similar to the pattern across the globe where the organisms are consistently increasing their resistance to these commonly used first line drugs. This is a sharp increase from earlier reports indicating the aggravating problem of drug resistance by these microbes over the years. Multi-drug resistant *Salmonella* isolates have become an issue of worldwide concern (Okeke *et al*., 2003, Kariuki *et al*., 2006).

The isolation of *Shigella* species (6.9%) in this study is in agreement with those reports from Harar 6.9% (Reda *et al*., 2011), Gondar 7.5% (Tiruneh, 2009), including Brazil 7.1% (Diniz-Santos and Silva, 2006). But lower than findings in Addis Ababa 11.7% (Asrat *et al*., 1999) and Jimma 20.1% (Mache, 2001) from similar study participants. However, greater than the 5.8% (Beyene and Haile-Amlak, 2004) from Jimma and 0.8% in Palestine (Elamreen *et al*., 2007). This variation may be attributable to the differences in sample size, study time and place.

Higher isolation rate (66.7%) of the *Shigella* isolates was found in children less than 5 years of old. This might indicate that Shigellosis was the problem of children in less than 5 years of age in Mekelle, which is in agreement with studies conducted in Gondar and Iran (Ranjbar *et al*., 2008, Tiruneh, 2009).

Mucus in the stool, with or without blood, was the main characteristic of *Shigella* infection, found in 50% (9/18) of patients, although shigellosis was statistically associated only in patients with bloody diarrhoea, which is in agreement with reports from Harar, Ethiopia (Reda *et al*., 2011). However, in contrast with a study conducted in Addis Ababa, where in addition to mucoid (8.4%) and bloody (6.8%) diarrhoea, the majority (82.4%) of the diarrhoeal samples in which *Salmonella* and *Shigella* were isolated, had watery nature (Asrat, 2008). This may reflect underlying geographic variations in strain patterns from place to place. In developing countries *Sh. dysenteriae* and *Sh. flexneri* are prevalent species causing mucoid to bloody diarrhoea, while in developed countries *Sh. sonnei* and *Sh. boydi*

species predominate. Shigellosis is commonly associated with mild watery diarrhoea. While *Sh. dysentriae* are consistently associated with dysentery, it is less common for *Sh. flexneri* to cause bloody diarrhoea (Kasper *et al.*, 2005). The fact that *Sh. flexneri* made up 54% of the isolates in Addis Ababa (Asrat, 2008) may clarify the watery diarrhoea in this result. These findings may indicate the need for strain identification in our study area, in order to better understand the prevalent strains and their clinical manifestations. Because, the severity and antimicrobial resistance patterns also changes with the change in the serogroup (Srinivasa *et al*., 2009).

Among all antibiotics tested for *Shigella species* the highest resistance was observed with ampicillin (88.9%), tetracycline (77.8%), chloramphenicol (55.6%) and cotrimoxazole (55.6%). These findings are in agreement with the previous Ethiopian studies conducted in different places and time (Yismaw *et al*., 2006, Asrat, 2008, Tiruneh, 2009, Reda *et al*., 2011). This marked resistance pattern observed in this study also agrees with reports from other parts of the world, South India (Mamatha *et al*., 2007), Palestine (Elamreen *et al*., 2007). Compared to previous studies reported in Ethiopia (Mache, 2001, Yismaw *et al*., 2006, Asrat, 2008, Tiruneh, 2009, Reda *et al*., 2011), *Shigella* isolates had a higher level of resistance to gentamicin (27.8%). This may be due to the indiscriminate overuse of the drug in the community.

In agreement with studies conducted in United States (Sivapalasingam *et al*., 2006), Indonesia (Herwana *et al*., 2010) and Harar (Reda *et al*., 2011) all *Shigella* isolates in this study are highly sensitive to norfloxacin, ciprofloxacin and ceftriaxone.

We detected a high level of susceptibility to ciprofloxacin against reports from Gondar (Tiruneh, 2009) and Manipal, South India (Mamatha *et al*., 2007) who reported resistance levels of 8.9% and 30% respectively. This high susceptibility of *Shigella* organisms for ciprofloxacin in this study was in agreement with studies from Awasa (Roma *et al*., 2000), Kenya (Brooks *et al*., 2003) and Brazil (Diniz-Santos and Silva, 2006).

In agreement to studies conducted in Jimma, Gondar and Addis Ababa (Mache, 2001, Yismaw *et al*., 2006, Asrat, 2008, Tiruneh, 2009) including neighboring Kenya (Brooks *et*

al., 2003), *Shigella* isolates were highly resistant to ampicillin. However, there seems to be a lower pattern of resistance to the drug in studies reported from Brazil (Diniz-Santos and Silva, 2006, Mamatha *et al.*, 2007). This could be due to the fact that ampicillin has been used in the country for a long time and because of its easy availability and potential for misuse. The antimicrobial resistance patterns of *Shigella* species vary according to geographic region and in the same place over time, leading to a therapeutic problem. Such differences are never stable and may change rapidly especially in places where antibiotics are used excessively, particularly in developing countries (Leslie *et al*., 1998, Niyogi *et al*., 2001).

Of the *Shigella* isolates, 17 (94.4%) were found to be resistant to one or more antimicrobial agent(s) and 16 (88.9%) were multi-drug resistant, which is comparable with findings from Gondar, where 90.8% and 87.8% of the isolates were resistant to one or more antimicrobial agent(s) and multi-drug resistant respectively (Yismaw *et al*., 2006).

Several factors may contribute to resistance by pathogens causing gastroenteritis in the setting of a developing country like Ethiopia. These include frequent overuse, misuse and factors related to the potency and quality of antimicrobials and the distribution of resistant strains (Sharma *et al*., 2005, Asrat, 2008). In addition, syndromic diagnosis and diagnostic imprecision usually force physicians to adopt for broad spectrum antibiotics such as amoxicillin and tetracycline, over prescribing; and less antibiotic diversity which lead to the emergence and spread of antimicrobial resistance (Okeke *et al*., 2003). Since non-typhoidal gastro-enteritis is usually self-resolving, antibiotic treatment is not commonly recommended (Okeke *et al*., 2003). Fortunately, there seems to be limited resistance to the drugs ciprofloxacin norfloxacine and ceftriaxon. However, given current trends in the country, unless intensive efforts are made to stem the unrestricted use of antimicrobials in the area, it will not probably be long before the microbes develop resistance to these expensive drugs and complicate effective treatment of gastroenteritis (Okeke *et al*., 2003).

The overall prevalence of intestinal parasites in this study was 31.9%. This is in agreement to study conducted in Addis Ababa, 27.5% (Adamu *et al*., 2005). But lower than the findings reported in Southwest Ethiopia on children, 54.7% (Hileamlak, 2005). The relatively lower prevalence rate in our finding could be due to the simple microscopic techniques we used compared with the concentration techniques used by others and also could be due to increased awareness of the community about personal and environmental hygiene from the continuous intervention made by the health extension workers in Mekelle.

Of the Seven different intestinal parasites isolated in the present study, *E. histolytica* and *G. lamblia* were the dominant parasites isolated from children with acute diarrhoea, with prevalence rates of 15% and 13.1% respectively. This could be due to the fact that these two parasites are well known etiologic agents of diarrhoea (Vargas *et al*., 2004).

LIMITATION OF THE STUDY

It was not possible to conduct *Shigella* and *Salmonella* serogrouping/serotyping due to lack of antisera.

CONCLUSION AND RECOMMENDATIONS

This study gives a brief overview of the burden and distribution of *Shigella*, *Salmonella* and intestinal parasite related diarrhoeal disease in children. The overall prevalence of Salmonellosis, Shigellosis and intestinal parasitosis was 7.3%, 6.9% and 31.9% respectively with greater than 2/3 of the Salmonellosis and Shigellosis cases found in children less than 5 years of age. Accordingly *Shigella* and *Salmonella* were still challenging in children especially less than 5 years of age. High antibiotic resistance was observed among both isolates particularly to ampicillin, tetracycline, chloramphenicol and cotrimoxazole. Resistance to two or more antibiotics was observed in 100% and 88.9% of *Salmonella* and *Shigella* species respectively. Only ciprofloxacin, norfloxacin and ceftriaxone were effective for both isolates.

These findings reinforce the need for continues surveillance program and strengthen infection control system to reduce the rate of infection and to apply appropriate guidelines for the use of therapeutic antibiotics. Based on these findings the following recommendations are forwarded:-

1. There is a need for a continuous surveillance program and periodic analysis and reporting of antibiotic susceptibility as an important measure to produce updated information on local pathogens which guides antibiotic treatment especially for resistant bacteria to provide the basis of empirical therapy.

2. The establishment of antibiotic policy and treatment guideline is recommended based on the susceptibility profile.

3. Further study on the serogroup distribution of the isolates of *Shigella* and *Salmonella* is recommended. Additional investigations are recommended in order to: identify prevalent serotypes in the region, enhance epidemiological study of *Salmonella* and *Shigella* like phage typing and plasmid analysis.

4. There is a need for resource allocation to implement the surveillance program and to improve the diagnostic capability of the laboratory.

5. In this study area, since ceftriaxone, norfloxacin and ciprofloxacin were the most effective antibiotics, these antibiotics may be used for empirical therapy of Shigellosis and Salmonellosis before culture and sensitivity result is available.

6. To reduce the incidence of Salmonellosis and Shigellosis public health measures such as improving personal and food hygiene and intensive health education has to be taken.

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APPENDIX I: Questionnaire

Questionnaire to isolate and determine the antimicrobial susceptibility profile of *Salmonella* and *Shigella* species associated childhood diarrhoea among patients attending Mekelle Hospital and Semen Health Center, Tigray, Ethiopia.

I. Patient Identification

Participant name_____ Serial number_____ sex___
age____ card no_____ address_____ Date_____

II. Clinical data

	Yes	No
Diarrhoea	☐	☐
If yes, specify duration _____		
Nausea	☐	☐
Tenesmus	☐	☐
Abdominal pain	☐	☐
Headache	☐	☐
Fever	☐	☐
If yes, specify _____ and duration _____		
History of antibiotic treatment within the last month	☐	☐

If yes, name of antibiotics _____, duration _____

Clinical Diagnosis_____

III. Laboratory Data

Consistency of stool	Yes	No
Watery	☐	☐
Mucoid	☐	☐
Bloody	☐	☐
Mixed	☐	☐

Others specify

Microscopic examination of the stool specimen: for intestinal parasites

Name of parasite identified ……..

Culture and bacterial identification

Name of the bacteria isolated……

Serogrouping result……

Antimicrobial susceptibility testing

	S	**I**	**R**
• Ampicillin	-----	-----	-----
• Ceftriaxone	------	-----	-----
• Norfloxacin	------	-----	-----
• Chloramphenicol	------	------	-----
• Co-trimoxazole	------	------	-----
• Gentamicin	------	-----	-----
• Nalidixic acid	-----	------	-----
• Ciprofloxacin	------	------	-----
• Tetracycline	------	------	-----

Comments_____ Name of investigator _____

Signature _____Date _____

APPENDIX II: Patient Information Sheet for parents /guardians

(English version)

Your Children are kindly invited to participate in this study, which involves about 260 Children from Mekelle Hospital and Semen Health Center. The main aim of this study is to isolate and determine the antimicrobial susceptibility pattern of *Salmonella* and *Shigella* species among children with acute diarrhoea.

a. Purpose: The purpose of this study is to assess and determine the antimicrobial susceptibility pattern of *Shigella* and *Salmonella* species to the commonly prescribed antibiotics among children in Mekelle Hospital and Semen Health Center, Mekelle, Ethiopia.

b. Duration: the duration of this study depend upon the availability of study participants it can probably take about four months.

c. Procedures to be carried on: In order to undertake the aforementioned study stool specimen will be taken from children who have diarrhoea for culture and susceptibility testing. The study subjects are expected to give stool samples, and information related to their illness.

d. Risk and discomfort: There will no any risk associated during sample collection. your child/adopted child will not face any risk.

e. Expected benefits: If your child participates in this research, he/she will be examined by qualified health worker and you will be informed his/her result if positive for further treatment.

g. Confidentiality: All information that all be collected from the study participants will be kept confidential. Any information about the participant that will be collected from the study will be stored in a file that will not bear a name on it, but only a number assigned to it instead.

h. Compensation: No compensation will be provided by participating in this study.

g. Termination of the study: Participation in the study is voluntary, and refusal to participate involves no penalty or loss of benefits to which you are otherwise entitled. The study participants have a right to

- Keep hold information
- Decline to cooperate in the study
- To refuse provision of specimens

I would also like to inform you that this study will be approved by Department Ethical and Review Committee and ethically cleared by Institutional Review Board (IRB), School of Medicine, College of Health Science, Addis Ababa University. If you have any question about the right of the study participant the address is:

School of Medicine, College of Health Science, Addis Ababa University

Office of Associate Dean, Postgraduate Programs and Research

P.O. Box 9086. Addis Ababa, Ethiopia

Tel. 251-011-551-28-765

If you have question about the study the address of the principal investigator is:

G/Michael G/Egziabher

Department of Microbiology, Immunology and Parasitology

School of Medicine, College of Health Science, Addis Ababa University

P.O.Box. 9086, Addis Ababa, Ethiopia

Tel: 0914169600

ለወላጆች/ ኣሳዳጊዎች የተዘጋጀ የጥናት ማብራርያ ቅጽ (ትርጉም በአማርኛ)

በትግራይ ክልል መቀሌ ሆስፒታል ለህክምና ከሚመጡ ዕድሚያቸዉ ከ ኣስራ ኣራት ዓመት በታች ከሆኑና ተቅማጥ በሽታ ካለባቸዉ ልጆች ላይ ሽገላ እና ሳልሞኔላ በተባሉት ባክተርያ በሚደረገዉ ጥናት ተሳታፊ ለሚሆኑት ልጆች ለወላጆቻቸዉ የተዘጋጀ ማብራርያ ቅፅ

ሀ. የጥናቱ ዓላማ: የዚህ ጥናት ዓላማ በልጆች ላይ የኣንጀት ተሰህቦ እና ተቅማጥ መንስኤ የሆኑትን የሺገላና ሳልሞኔላ ስርጭት እና በነዚህ የሚመጣዉን በሽታ ለማከም የሚረዳንን የመድሀኒት ዓይነት ለማዎቅ የሚያስችል ነዉ።

ለ. የሚፈጀዉ ጊዜ፡ ይህ ጥናት እስከ ኣራት ወር ሊፈጅ ይችላል።

ሐ. የስራዉ /ኣካሄድ ቅደም ተከተል፡ ጥናቱ ለማካሄድ ዕደሚያቸዉ ከ ኣንድ እስከ ኣስራኣራት ዓመት ከሆኑና የተቅማጥ በሽታ ካላቸዉ ልጆች የሰገራ ናሙና በመዉሰድ የተለያዩ የቤተሙከራ ምርመራዎች ይካሄዳሉ። ሌሎችም ከበሽታዉ የተዛመዱ መረጃዎች ይወሰዳሉ።ስለዚህ የጥናቱ ተሳታፊዎች የሰገራ ናሙና እንዲሁም ኣንዳንድ መረጃዎችን በመስጠት የጥናቱ ተባባሪ መሆን ይጠበቅባቸዋል።

መ. ሊደርስ የሚችል ኣደጋ፡ በዚህ ጥናት ዉስጥ ኣደጋ የሚያደረስ ድርጊት የለም።

ሠ. የሚገኝበት ጥቅም፡ ልጅዎ በዚህ ጥናት ቢሳተፍ/ብትሳተፍ በሽታውን የሚያመጣዉ ባክተርያ ተለይቶ ይታወቃል።መድሃኒቱም ስለሚታወቅና ዉጤቱም ለህኪም ሰለሚሰጥ ልጅዎ ትክክለኛ ሕክምና ያገኛል። በተጨማሪም ወደፊት እነዚህ በሽታዎች በቁጥጥር ስር ለማድረግ ጥናቱ የበኩሉ ሚና ስለሚጫወት የዜግነት ግዴታዎ ሊወጡ ይችላሉ።

ረ.ሚስጥራዊነት፡ በዚህ ጥናት የሚገኝ ማንኛዉም መረጃ በሚስጥር የሚያዝ ይሆናል።ለእያንዳንዱ የጥናቱ ተሳታፊዎች የተለየ ቁጥር በመስጠት የሚሰበሰበዉ መረጃ ሁሉ በስም እንዳይሆን ይደረጋል።

ሰ. ፍቃደኝነትን ስለማቋረት፡ የጥናቱ ተሳታፊዎች መረጃ ያለመስጠት፣በጥናቱ ለመሳተፍ ፍቃደኝነት የማሳየት የማሳየት እንዲሁም ናሙና ያለመስጠት መብታቸዉ የተጠበቀ ነዉ።

ኣድራሻ ማወቅ ካስፈለግዎ፡- ህክምና ት/ቤት ፤ኣዲስ ኣበባ ዩኒቨረሲቲ የድህረ ምረቃ ፕሮግራምና ምርምር የተባባሪ ዲን ቢሮ የ.መ.ሳ.ቁ. 9086 ኣዲስ ኣበባ; ስልክ: 251-011-551-28-765

የዋናዉ ተመራማሪ ኣድራሻ፤ ገ/ሚካኤል ገ/ኣብሄር

የማይክሮባዮሎጂ፤ኢሚኖሎጂና ፓራሲቶሎጂ ት/ክፍል

ህክምና ት/ቤት፤ ኣዲስ ኣበባ ዩኒቨረሲቲ የ.መ.ሳ.ቁ. 9086 ኣዲስ ኣበባ; ስልክ. 0914169600

APPENDIX III: Consent Form for parents/guardians

(English version)

Name……..…….. Card no…..……. Ward……. Serial no……

Date of admission …… Reason of admission……

I have been informed that the objective of this study is to isolate and determine the current antimicrobial susceptibility pattern of *Shigella* and *Salmonella* species to the commonly used antibiotics in Children with diarrhoea. The aim of the study is explained to me (the guardian accompanying the infants on admission). The results of this study have importance to treat children who have diarrhoea and according the result profile, which guide the pediatrician to manage the patient. I have also informed about the confidentiality of the questionnaires. Therefore, with full understanding of the importance of the study, I agreed voluntarily to allow my child the requested samples in the above for clinical investigation in the study and I benefit only from the free laboratory investigation result.

I_____ hereby give my consent for giving of the requested information and stool specimen as the doctors find best for my child.

Signature: _____ Date_____

ቅፅ 2

ለወላጆች/ ኣሳዳጊዎች የተዘጋጀ የስምምነት መግለጫ (ትርጉም በአማርኛ)

የተሳታፊ ስም_____መለያ ቁጥር_____

ካርድቁጥር _____ ዋረድ/ኦፒዲ _____

የወላጅ/ኣሳዳጊ ስም_____ የተመረመረበት/ችበት ቀን_____
የተኛበት/ችበት ቀን_____ የተኛበት/ችበት ቀን_____

የጥናቱ ዓላማ በልጆች ላይ የተቅማጥ መንስኤ የሆኑትን የሽገላና ሳልሞኔላ ስርጭትና በነዚህ የሚመጣዉን በሽታ ለማከም የሚረዳንን መድሃኒት ለመለየት የሚያስችል መሆኑ ተገልፆልኛል፡፡የጥናቱ ዉጤት የተቅማጥ ችግር ላለባቸዉ ሕፃናት ለማከም የሚያስችል ነዉ፡፡ጥናቱ ዉስጥ ለመሳተፍ ፍቃደኛ ከሆኑ ሕፃናት የሰገራ ናሙና የሚወሰድ ሲሆን በተሳታፊዎች የሚያሰከትለዉ ችግር እንደሌለና ከተሳታፊዎች የሚገኝ ማንኛዉም መረጃም በሚሰጥር እንደሚያዝ ተገልፆልኛል፡፡ስለዚ የትናቱ ጥቅም በመገንዘብ በፍቃደኝነቴ ከልጄ የሰገራ ናሙና እና ኣስፈላጊዉ መረጃ ለመስጠት ፍቃደኛ ነኝ፡፡ኣሁን የማገኘዉ ጥቅም ደሞ ነፃ የላብራቶሪ ኣገልግሎት ነዉ፡፡

እኔ_____ ከላይ የተገለፀዉን ኣደምጬ ለልጄ የሚጠቅም መሆኑ ስለተረዳሁ ከልጄ የሰገራ ናሙና እና ኣስፈላጊዉ መረጃ ለመስጠት በፍቃደኝነቴ ተስማምቼለሁ፡፡

የወላጅ/ኣሳዳጊ ፊርማ_____ቀን_____

የተመራማሪዉ ፊረማ_____ቀን_____

ቅጥዒ 3

ንወለዲ/መዕበይቲ ዝተዳለወ ናይ ስምምዕነት መግለፂ (ትርጉም ብትግርኛ)

ናይተሳታፊስም_____ መፍለይቁፅሪ_____

ካርድ ቑፅሪ_____ ዋረድ/ኦፒዲ_____

ናይ ወላዲ/መዕበይ ሥም_____ ዝተመርመረሉ/ትሉ ዕለት_____

ዝደቀሰሉ ዕለት _____ ዝደቀሰሉ ምኽንያት _____

ዕላማ እዚ መፅናዕቲ ኣብ ህፃናት ናይ ተቐማጥ መንቀሊ ዝኾኑ ሽገላና ሳልሞኔላ ስርጭትና በዚኣም ዝመፁ ሕማማት ንምሕካም ዘገልግሉ ናይ መድሓኒት ዓይነት ንምፍላይ ዘኽእል ምኻኑ ተሓቢሩለይ እዩ። ዉፅኢት እዚ መፅናዕቲ ድማ ናይ ተቐማጥ ፀገም ንዘለዎም ህፃናት ንምሕካም ዘኽእል እዩ። ኣብዚ መፅናዕቲ ንምስታፍ ፍቓደኛ ካብ ዝኾኑ ህፃናት ናይ ሰገራ ናሙና ከም ዝዉሰድን ኣብ ተሳተፍቲ ዘስዕቦ ፀገም ከም ዘየለን ከምኡእዉን ካብ ተሳተፍቲ ዝርከብ ዝኾነ ዓይነት ሓበሬታ ብሚሽጥር ዝተሓዝ ምኻኑ ተሓቢሩለይ እዩ።ኣብዚ መፅናዕቲ ምስታፍ ድማ ብፍቓደኛነት ወይም ብድሌት ምኻኑ ተሓቢሩለይ እዩ።ስለዚ እዚ መፅናዕቲ ዘለዎ ጥቕሚ ብምርዳእ ብድሌተይ ካብ ዉላደይ ናይ ሰገራ ናሙናን ኣድላይ ሓበሬታን ንምሃብ ፍቓደኛ እየ። ክረኽቦ ዝኽእል ጥቕሚ ድማ ነፃ ናይ ላብራቶሪ ምረመራ ምኻኑ እዉን ተረዲኤ ኣለኹ።

ኣነ _____ ኣብ ላዕሊ ዝተገለፀ ድሕሪ ምድማፅ ንዉላደይ ዝጠቅም ምኻኑ ስለተረዳእኹ ብድሌተይ ካብ ዉላደይ ናይ ሰገራ ናሙናን ኣድላይ ሓበሬታን ንምሃብ ተስማዕሚዐ ኣለኹ።

ናይ ወላዲ/መዕበይ ፊርማ_____ዕለት_____

ናይ ተመራማሪ ፊርማ_____ዕለት_____

APPENDIX IV: Laboratory procedure for API test

API 20E is a standardized identification system for Enterobacteriaceae and other non-fastidious, Gram negative rods. This strip consists of 20 micro tubes containing dehydrated substrates. These tests are inoculated with a bacterial suspension that reconstitutes the media. During incubation, metabolism produces color changes that are either spontaneous or revealed by the addition of reagents. The reactions are read according to the reading table and the identification is obtained by referring to the Analytical Profile Index or using the identification software.

Procedure

Oxidase test

The oxidase test must be performed according to the manufacturer's instructions for use. The result should be recorded on the result sheet as it is an integral part of the final profile (21st identification test).

Preparation of the strip

1. Prepare an incubation box (tray and lid) and distribute about 5 ml of distilled water or demineralized water [or any water without additives or chemicals which may release gases (e.g., Cl2, CO2, etc.)] into the honeycombed wells of the tray to create a humid atmosphere.

2. Record the strain reference on the elongated flap of the tray. (Do not record the reference on the lid as it may be misplaced during the procedure.)

3. Remove the strip from its packaging.

4. Place the strip in the incubation box.

Preparation of the inoculums

1, Open an ampule of API NaCl 0.85 % Medium (5 ml) or an ampule of API Suspension Medium (5 ml) or use any tube containing 5 ml of sterile saline or sterile distilled water, without additives.

2. Using a pipette remove a single well isolated colony from an isolation plate. It is

recommended to use young cultures (18-24 hours old).

3. Carefully emulsify to achieve a homogeneous bacterial suspension.

This suspension must be used immediately after preparation.

Inoculation of the strip

1. Using the same pipette, fill both tube and cupule of the tests CIT, VP and GEL with the bacterial suspension.

2. Fill only the tube (and not the cupule) of the other tests.

3. Create anaerobiosis in the tests ADH, LDC, ODC, H2S and URE by overlaying with mineral oil.

4. Close the incubation box and Incubate at 36°C ± 2°C for 18-24 hours.

Reading the strip

• After the incubation period, read the strip by referring to the Reading Table.

• If 3 or more tests (GLU test + or –) are positive, record all the spontaneous reactions on the result sheet and then reveal the tests which require the addition of reagents:

- TDA Test: add 1 drop of TDA reagent. A **reddish brown** color indicates a **positive** reaction to be recorded on the result sheet.

- IND Test: add 1 drop of JAMES reagent. A **pink** color developed in the whole cupule indicates a **positive** reaction to be recorded on the result sheet.

- VP Test: add 1 drop each of VP 1 and VP 2 reagents. Wait at least 10 minutes. A **pink** or **red** color indicates a **positive** reaction to be recorded on the result sheet.

If a slightly pink color appears after 10 minutes, the reaction should be considered **negative**.

Interpretation

Identification is obtained with the **numerical profile**.

• Determination of the numerical profile:

On the result sheet, the tests are separated into groups of 3 and a value 1, 2 or 4 is indicated for each. By adding together the values corresponding to positive reactions within each group, a 7-digit profile number is obtained for the 20 tests of the API 20 E strip. The oxidase reaction constitutes the 21st test and has a value of 4 if it is positive.

• Identification:

This is performed using the database (V4.0) or with the Analytical Profile Index:

- Look up the numerical profile in the list of profiles.

- With the identification software:

- Enter the 7-digit numerical profile manually via the keyboard.

DECLARATION

I, the undersigned, declare that this M.Sc. thesis is my original work, has not been presented for a degree in any other University and that all sources of materials used for this thesis have been duly acknowledged.

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Signature: _____

Date and place _____

Addis Ababa, Ethiopia