KWAME NKRUMAH UNIVERSITY OF SCIENCE & TECHNOLOGY,

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SALMONELLA CARRIAGE AMONG FOOD HANDLERS AND PATIENTS ATTENDING ST. JOSEPH'S HOSPITAL, JIRAPA, UPPER WEST REGION

BY

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DECLARATION

I hereby declare that this submission is my own work towards the M.Sc. and that, to the best of my knowledge, it contains no material previously published by another person, nor material which has been accepted for the award of any other degree of the University, except where due acknowledgement has been made in the text.

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DEDICATION

To my wife Mrs. Joyce Nsiah Gyansah and daughter Miss Michelle Aseda Senwah Gyansah.

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ABSTRACT

Typhoid fever is ranked seventh (7th) among common infections affecting the people of Jirapa. However, the source of spread of the infections is not known. This study was conducted to determine if food vendor carriage constitute a major source of spread of the disease in the community. The study was conducted from January to May 2015. One hundred and seventy (170) stool samples obtained from asymptomatic food handlers were tested for *Salmonella*. 182 stool and 112 blood samples were collected from patients and tested for Salmonella. Salmonella was recovered from 4.1% (7/170) food handlers and 10.4% (31/294) of patients. Stool samples yielded more *Salmonella* isolates 12.1% (22/182) than blood samples 7.1% (8/112). Among the *Salmonella* isolates obtained from food handlers, *Salmonella* Typhi accounted for 71.4% (5/7) whereas 28.6% (2/7) were other *Salmonella* species. There were 31 *Salmonella* isolates obtained from patients. Of the 31, *Salmonella* Typhi recorded 9 (29.0%) and *Salmonella* species recorded 22 (71.0%). There was a total of 38 Salmonellae isolated from the study group.

Abdominal pains, headache and diarrhea were the common complaints presented by patients. Most *Salmonella* isolates showed high resistant pattern towards the common antimicrobials used including ampicillin, tetracycline and chloramphenicol. All the *Salmonella* isolates were however, susceptible to ciprofloxacin, ceftriaxone, cefotaxime and ceftaxidime. *Salmonella* isolates were tested for ESBL and they were all not ESBL producers. About 4.1% of food handlers in Jirapa were carriers of Salmonellae. These food handlers constitute a potential source for the transmission of the *Salmonella* pathogen, and may account for one of the reasons for the high cases of *Salmonella* infections in Jirapa.

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LIST OF ABBREVIATION

AFLP - Amplified fragment length polymorphism

API – Analytical Profile Index

AST - Antimicrobial Susceptibility Tests

BD – Becton Dickinson

CDC - Centre for Disease Control

CHRPE – Committee for Human Research Publications and Ethics

CLSID – Clinical Laboratory standards Institute

CSN - Central Nervous System

ESBLs - Extended-spectrum beta-lactamase

MDR - Multi drug resistant

MIC - Minimum Inhibitory Concentration

MLST - Multilocus sequence typing

NTS - Non-typhoidal *Salmonella*

OPD - Outpatient department

PCR - Polymerase chain reaction

PFGE - Pulsed-field gel electrophoresis

RAPD - Randomly amplified polymorphic DNA

TSI – Triple Sugar Iron

UK – United Kingdom

USA - United States of America

WHO - World Health Organization

CHAPTER ONE

1.0 INTRODUCTION

Food borne diseases are a major public health problem worldwide. The problem is worsened in developing countries due to difficulties in attaining standard hygienic food handling practices and poor sewage disposal. Most people in both developing and developed countries suffer from food borne diseases each year (WHO, 2007). These diseases are caused by different kinds of bacteria, parasites and viruses. Chemicals from poisonous mushrooms and harmful toxins of some bacteria can also cause these diseases (CDC, 2005). Among the enteric pathogens, *Salmonella* species are of particular concern as causes of enteric fevers, food poisoning and gastroenteritis (Benson, 2001).

Enteric fever is a common cause of death and disease in many parts of the world. About 22 million cases are thought to occur worldwide each year, with 200,000 deaths as a result (Bhutta, 2006). The greatest burden of the disease is found in infants and children in Southeastern Asia (Crump *et al.*, 2004, Darton *et al.*, 2014). In sub-Saharan Africa and countries in Southeastern Asia, typhoid fevers outbreaks are frequently reported (Muyembe *et al.*, 2009).

There are over 2500 different serotypes of *Salmonella* (Bell and Kyriakides 2002; Crum-Cianflone 2008). Most human infections are caused by a serotype of *Salmonella enterica* subspecie *enterica* (subspecies I), which infects warm-blooded animals (Christenson, 2013). Some *Salmonella* serotypes are host-adapted and can infect only one or a few animal species. For instance, *Salmonella* Choleraesius is mainly found among pigs and *Salmonella* Dublin in cattle (WHO, 2013). Also, *S*. Typhi and *S*. Paratyphi specifically cause human infections. On the other hand, others such as *S*. Typhimurium are able to cause infections in humans and many animal species (Jay *et al.*, 2003; Wallis 2006).

Infections caused by the *Salmonella* bacteria are referred as Salmonellosis. This can be grouped into typhoidal *Salmonella* and non-typhoidal *Salmonella* (Abdullahi, 2010). Typhoidal Salmonellae are usually invasive and are made up of S. Typhi and S. Paratyphi A, B and C which cause enteric fevers. (Darby and Sheorey, 2008). They are responsible for significant morbidity and mortality in developing countries. (Christenson, 2013).

Non- typhoidal *salmonella* are made up of all other serotypes including *S*. Enteritidis, *S*. Typhimurium, *S*. Dublin, and *S*. Choleraesius. Non-typhoidal *Salmonella* (NTS) infection is a common food borne disease worldwide. Though the disease is usually uncomplicated and self-limiting, immune compromised individuals are at risk for severe disease such as bacteremia, meningitis, and osteomyelitis and among others (Christenson, 2013).

Salmonella infection is acquired from the consumption of food or water which has been contaminated with the faecal material of an infected person (Madigan *et al.*, 2009). Close contact with infected animals such as poultry, pigs, goats, cattle, dogs and cats and eating fresh fruits and vegetables that have not been washed well are also frequent risk for acquisition (Christenson, 2013).

Salmonellae are non-fastidious and are commonly isolated from foods and drinks such as milk and dairy products, eggs, poultry and processed meats and other substrate. An infective dose of about 10^5 *Salmonella* per gram of food and10^4 *Salmonella* per litre of water is needed to initiate an infection. *Salmonella* species are able to overcome the defense mechanism and invade the epithelial tissue of the small and large intestines,

which results in inflammation and diarrhea. Some strains of *Salmonella* also produce heat liable cytotoxins which cause damage to the surface of the intestinal mucosa resulting in characteristic symptoms of enteric fever (Jay *et al.,* 2003, Burrows and Renner, 1999).

About 2-5% of individuals recovering from typhoid fever become temporary or permanent salmonella carriers. These individuals harbour the bacteria in the gallbladder, biliary tract, or rarely in their intestines (WHO, 2010). The bacterium, intermittently reaches the lumen of the bowel and it is excreted in the stool, which may contaminate water and food (Lesser and Miller, 2001, Miller and Pagues, 2000).

In places with low environmental sanitation, human *Salmonella* carriers, especially food handlers may contribute to the spread of the disease. Many studies have implicated food handlers as *Salmonella* carriers and thus serve as a potential source of salmonella infections. Mensah *et al.,* (1997) reported a *Salmonella* prevalence of 3.2% among 176 food vendors in Accra, Ghana. In Nigeria, Salmonellae were isolated from 7 (13.2%) out of the 53 stool samples from food handlers tested, of which three (5.7%) were *S*. Typhi, three (5.7%) were *S*. Enteritidis and one (1.9%) was *S*. Choleraesius (Smith et al., 2008). Another study done on 206 apparently healthy food handlers working in Bukas in Lagos, Nigeria showed that 17% of food handlers were infected *Salmonella* with S. Typhi *S. and S.* Enteritidis accounting for 6.8% and 5.3% of the infection respectively (Smith *et al.*, 2009).

In Namakkal, India, out of the 35 stool samples from asymptomatic food handlers processed, 6 (17.1%) yielded positive *Salmonella* growth. Of the total 17 isolates, 5 were found to be multidrug resistant strains (Senthilkumar *et al.*, 2005). This study indicated that, food handlers can be a source of drug resistant strains which is a serious public health problem.

Jirapa, like most district capitals in Ghana still has a problem in environmental sanitation as most homes do not have their own private toilets. The few Public toilets in the town are used by both the food handlers and their customers, but these toilets lack soap and water for hand washing.

The people of Jirapa are predominantly farmers. They engage in farming activities including rearing of animals such as pigs, cattle, poultry and livestock using traditional methods. They also have close contacts with pets such as dogs and cats. All these animals are known to be natural reservoirs for most *Salmonella* species (Boyle *et al.,* 2007). Close contact with these colonized animals; put them at a great risk of acquiring *Salmonella* infections.

1.1 *Salmonella* infections in Ghana

In Ghana, typhoid fever is among the leading causes of morbidity and mortality. In 2010, it was rated 10th among the top ten diseases seen at the OPD. The total number of typhoid fever cases reported in Ghana for the year 2011 was 103,353 with 793 (CFR = 0.77%) deaths (Ghana Health Service Annual Reports, 2010, 2011).

Epidemiological data on invasive non-typhoidal *Salmonella* infections is limited in Ghana. However, few studies have reported that the disease burden is highest in children.

A recent study by Larbi *et al.,* (2014) in Accra, Ghana, reported a higher prevalence of non-typhoidal *Salmonella* bacteremia (63.5%) than typhoidal *Salmonella* bacteremia (36.5%). They observed that NTS bacteremia was highest in children under 5 years. Another study by Wilkins *et al.,* (1997) in Ghana, found out that, of the 24 (21.6%) children with *Salmonella* bacteraemia, 59% (14) was due to *Salmonella spp* and 25% (6) was due to *Salmonella* Typhi. Evans *et al.,* (2004) also reported a co-infection of NTS and malaria in Kumasi, Ghana. Marks *et al.,* (2010) demonstrated that invasive *Salmonella* infections constitute a significant problem in Ghana. Out of the 389 bacterial culture positives, 16.5 %(64) were positive for S. Typhi, with majority (56.8%) of infections occurring in children less than 15 years of age.

In a study done by Saba *et al.,* (2013) in the Northern Region of Ghana, four (4) serotypes of *Salmonella* were identified out of the ninety-one (91) fecal specimens obtained from both out patients and in patients. The serotypes identified in this study were *S.* Urbana*, S.* Ouakam, *S*. Stanleyville, and *S*. Senftenberg.

1.2 Problem Statement

Typhoid fever is among the widespread diseases affecting the population of Jirapa and has been rated seventh (7th) among the common infections. In 2013, a total of 971 representing 1.9% typhoid fever cases were reported at the St. Joseph's Hospital. Other *Salmonella* related diseases such as diarrheal and septicemia were reported as 699 (1.3%) and 315 (0.6%) respectively (St. Joseph's Hospital's Annual Report, 2013). The prevalence and the source of spread of the Salmonellae infecting the community are not known. Additionally, epidemiological data on *Salmonella* infections in the Upper West Region are not readily available. These concerns have necessitated this study.

1.3 Justification

Food handlers play important role in ensuring food safety. However, in Jirapa, the proportion of certified food handlers and their *Salmonella* carrier status is not known. Screening of food handlers for *Salmonella* infections is not usually done hence the risk posed by food handlers in the spread of the *Salmonella* infection is not known in Jirapa.

The aim of this study is to determine the prevalence of *Salmonellae* carrier status of food handlers at Jirapa. It also aims to test patients reporting to the St. Joseph's Hospital in Jirapa for *Salmonella* with the hope of determining the enormity of the *Salmonella* prevalence in Jirapa.

Based on the study results, appropriate recommendations for the improvement of food safety and sanitary conditions in that area would be made.

1.4 Aim

To determine the prevalence of *Salmonella* infections among Food Handlers, and patients reporting to the St. Joseph's Hospital, Jirapa.

1.5 Objectives

- To isolate *Salmonella* species in stool samples of food handlers in Jirapa
- To isolate *Salmonella* species in blood and stool samples of patients reporting to the St. Joseph's Hospital with complaints of enteric fever and gastroenteritis.
- To determine the antimicrobial sensitivity pattern of the isolates and also to determine if the isolates produce ESBL.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 The organism Salmonella

Salmonella is a Gram-negative, non-spore forming rod-shaped bacterium, belonging to the family *Enterobacteriaceae.* It causes enteric fever and gastroenteritis (Jay *et al.,* 2003).

2.1.1 History

Salmonella was first discovered from pigs in 1880 by Daniel Elmer Salmon, an American veterinary pathologist and Theobald Smith. In 1890, the organism was named after D.E. Salmon to honour him (Ziprin, 1994).

2.1.2 Classification and Nomenclature

The genus *Salmonella* consist of two species; *S. bongori* and *S. enterica*, which are grouped further into subspecies based on their biochemical and genomic characteristics. The Kaufman-White typing system, classifies *Salmonella enterica* into six subspecies (Brenner *et al.,* 2000). Over 2500 different serovers of *Salmonella* exist (Bell and Kyriakides 2002; Crum-Cianflone 2008).

The nomenclature of *Salmonella* is complicated, since it involves the genus, subspecies and the serover (Tindal, 2005). The serover name is started with a capital letter, but not italicized (Chengappa *et al.,* 1993). For instance, *Salmonella enterica* subspecies enterica serovar Dublin. However, referring *Salmonella* species by only their genus and serover names, (such as *Salmonella* Dublin) is accepted (Chengappa *et al.,* 1993, CDC, 2010).

Table 2.1: Species and subspecies in the *Salmonella* genus (Card, 2009)

Salmonella Species	Sub species	Number of Serovers
S. enterica	enterica	1,478
	salamae	498
	arizonae	94
	diarizonae	327
	houteane	71
	indica	12
S. bongori		21
Total		2,501

2.1.3 Microbiology

Salmonella are Gram negative facultative anaerobic rods bacteria. Most *Salmonella* species possess peritrichous flagellae which make them motile (Bell *et al.,* 2002, Guthrie, 1991). They do not form spores (Varnam and Evans, 1991). Apart from *S.* Typhi, *S*. Paratyphi C and S. Dublin, *Salmonella* are non-capsulated (Cheesbrough, 2000, Helmuth, 2000). They require a temperature of 8°C to 45°C for growth, and most strains can resist a pH between 4 to 9. The biochemical characteristics of *Salmonella* show that most species are non-lactose fermenters and are able to utilize citrate as the only carbon source. On TSI agar, most *Salmonella* species produce both gas and hydrogen sulfide (Bell *et al.,* 2002, Molbak *et al.,* 2006).

Three antigens, O, H, and Vi antigens characterize *Salmonella* strains serologically. The O antigens which are the outer polysaccharides of the cell wall are used to divide salmonellae into groups (Giannella, 2002). H antigens are found in the flagella and help the bacterium to survive host immune response. There are two forms of H antigens, phases 1 and 2. The Vi (virulence) antigens are located in the capsular polysaccharide of

some serovers like *S*. Typhi, *S*. Paratyphi C and *S*. Dublin. The Kauffman- White scheme is a scheme used worldwide for the classification of *Salmonella* serovars based on their antigenic properties (Helmuth, 2000). The antigenic properties of each serovar are summarized and described in the antigenic formulae (Mortimer, 2004, Wattiau, 2008)

Table 2.2: Antigenic formulae of some *Salmonella* serotypes (Card, 2009)

Serotype	Subgroup	Somatic Antigen O	Flagella Antigen H	
			Phase 1	Phase 2
S. Paratyphi A	A	1,2,12	a	(1,5)
S. Typhimurium	B	1,4, (5),12	i	1,2
S.Agona	B	4,12	f,g,s	-
S. Derby	B	1,4, (5),12	f,g	(1,2)
S. Typhi	D	9,12, (Vi)	c	1,2
S. Enteriditis	D	1,9,12	g,m	(1,7)

2.2 Pathology of *Salmonella* infection

2.2.1 Mode of transmission

Salmonella infection is acquired from the consumption of food or water which has been contaminated with the faecal material of an infected person (Madigan *et al.,* 2009). Close contact with infected animals such as poultry, pigs, goats, cattle, dogs and cats and eating fresh fruits and vegetables that have not been washed well are also frequent risk for acquisition (Christenson, 2013).

2.2.2 Infective Dose

Salmonella are non-fastidious and can live on foods and drinks such as milk and dairy products, eggs, poultry and processed meats and other substrate. An infective dose of about 10^5 *Salmonella* per gram of food (Jay *et al.,* 2003) and 10^4 *Salmonella* per litre of water (Burrows and Renner, 1999) is needed to initiate an infection. The inoculum required for infection depends on the type of strain and the physiological wellbeing of the host (Wannissorn, 2001). For instance individuals with low gastric acidity such as the elderly and people who use antacids regularly may reduce the infective dose to 10^3 cells. On the other hand, the infective dose can increase to 10^9 cells in individuals who have been vaccinated against *Salmonella* (Raffatellu *et al.,* 2006).

2.2.3 Incubation Period

The incubation period for non-typhoidal *Salmonella* infections is 6 to 12 hours (Christenson, 2013). In typhoidal infections the incubation period is 7 to 14 days for typhoid fever and 1 to 10 days for paratyphoid fever (Pui *et al.,* 2011).

2.2.4 Virulence and infectivity

Salmonella species possess a number of structural and physiological features which enable them to cause infections (Jay *et al.,* 2003). The length of the O side chains is directly related to the ability of the bacterium to withstand the lytic action of complement (Jay *et al.,* 2003). Another important virulent feature is the presence and type of fimbriae. The fimbriae help *Salmonella* species to adhere to the epithelium cells of the host (Jones, 2005). *Salmonella* species are able to resist the low pH of the stomach, adhere to the intestinal epithelial cells and produce heat labile toxins such as enterotoxin

and cytotoxins, which causes diarrhea (loss of intestinal fluids) and enteric fever respectively (Jay *et al.,* 2003).

2.2.5 Pathogenesis of *Salmonella* Infections

Non-typhoidal *Salmonella* strains are rarely invasive because they do not extend past the intestinal lymphatic system. Their interactions with host cells release proinflamatory cytokines, which lead to accumulation of neutrophils in that area resulting in gastroenteritis (Lahiri *et al.,* 2010).

Typhoidal isolates such as *S*. Typhi have specialized fimbriae that help them to attach to epithelial cells over the peyer patches in the ileum ensuring penetration through the intestinal mucosa. The bacteria is engulfed by macrophages and translocated into draining lymphatic nodes which results in bacteremia and subsequent dissemination. The organisms survive and multiply within the host cells in a Salmonella-containing vacuole, and invade the liver, spleen, and bone marrow. The bacteria continue to multiply and are released into the blood stream which invades other parts of the body including the gall bladder (Parry *et al.,* 2002). The bacteria enter the gastrointestinal tract again and infect the peyer patches and are subsequently shed in stools (Parry *et al.,* 2002).

2.3 Clinical forms of *Salmonella* infections in humans

2.3.1 *Salmonella* gastroenteritis

S*almonella* gastroenteritis, also known as salmonellosis is usually caused by non-typhoidal serotypes, especially *Salmonella* Enteritidis. Gastroenteritis usually begins with nausea and vomiting and the progresses to abdominal pain and diarrhea, which can vary from mild to severe and with or without blood (WHO/FAO 2002; Darby and

Sheorey 2008). Salmonellosis usually lasts a few days, self-limited and do not require medications except in the very young and the very old (Christenson, 2013).

2.3.2 Enteric fever

Enteric fevers are caused by *S*. Typhi and *S*. Paratyphi A, B and C (Darby and Sheorey 2008). *S*. Typhi causes typhoid fever, whereas *S*. Paratyphi A, B and C cause paratyphoid fever (Jay *et al.,* 2003).

Fever, gastrointestinal symptoms (such as vomiting, severe diarrhea, abdominal distension, and pain), cough, relative bradycardia, rose spots (pink macules frequently observed on the abdomen and chest), and splenomegaly are frequently regarded as features of typhoid and paratyphoid fever (Christenson, 2013).

2.3.3 *Salmonella* bacteremia

Bacteremia is common with *Salmonella* infections. The frequent symptoms with Salmonellae bacteremia include chills, high fever and anorexia. *Salmonella* becomes present throughout the body resulting in persistent and focal or metastatic infection to many organs in the body. This can lead to life-threatening conditions such as endocarditis, meningitis, urinary tract infections, septic arthritis, and osteomyelitis (Hohmann, 2001; Percival *et al.,* 2004). In pregnancy, *salmonella* infection (especially *S.* Typhi) may lead to abortion and foetal death, transplacental infection of the foetus and maternal death (Carroll and Williams, 2008).

2.3.4 Asymptomatic carriers

Even after complete recovery, about 3% of typhoidal infections and 0.1% of non-typhoidal infections become chronic carriers (Giannella, 2002). About 2-5% of

individuals recovering from typhoid fever become temporary or permanent carriers, harboring the organisms in the gallbladder, biliary tract, or rarely in their intestines (Vandepitte *et al.,* 2003). The organism intermittently reaches the lumen of the bowel and it is excreted in the stool, which may contaminate water and food (Lesser *et al.,* 2001, Miller *et al,* 2000).

2.4 Epidemiology of *Salmonella* infections

2.4.1 Non – Typhoidal *Salmonella* infections

Salmonella gastroenteritis is a global public health problem. The world burden is estimated at 93.8 million illnesses, with 155,000 deaths each year. *Salmonella* Enteritidis is the most common isolated subspecies because it is responsible for 65% of these infections, followed by *S.* Typhimurium at 12% (Christenson, 2013). In the United States, an estimated 1 million foodborne illnesses occur each year, resulting in 350 deaths (Chai *et al.,* 2012).

In Australia, Salmonellosis is a common disease in all states and territories. In 2012, salmonellosis had a notification rate of 49.8 cases per 100,000 populations (11,273 cases) (EFSA 2013).

In developed countries, Non-typhoidal *Salmonella* infections mostly present as gastrointestinal disease and are rarely associated with systemic diseases, except in immunocompromised individuals (Ekdahl *et al.,* 2005). These strains, however, create a major problem in Sub-Sahara Africa and are commonly isolated from the blood of patients presenting with fevers (Marks *et al.,* 2004). *S*. Typhimurium and *S*. Enteritidis are the main causes of invasive non-typhoidal *Salmonella* infection (iNTS). In 2012,

invasive non-typhoidal *Salmonella* infections were reported to have a case fatality rate of 20–25% in Africa. (Feasey *et al.,* 2012).

In Ghana, Larbi *et al.,* (2014) reported a higher prevalence of non-typhoidal *Salmonella* bacteremia (63.5%) than typhoidal *Salmonella* bacteremia (36.5%). Also, Commey *et al.,* (1994) observed that, most of the isolates from the blood culture of Ghanaian children were non-typhoidal *Salmonella* strains.

2.4.2 Typhoidal *Salmonella* infections

Enteric fever is a common cause of death and disease in many parts of the world. About 22 million cases are thought to occur worldwide each year, with 200,000 deaths as a result (Bhutta, 2006). The greatest burden of the diseased is found in infants and children in Southeastern Asia (Crump *et al.,* 2004, Darton *et al.,* 2014). In sub-Saharan Africa and countries in Southeastern Asia, typhoid fevers outbreaks are frequently reported (Muyembe *et al.,* 2009, Baddam *et al.,* 2012). Travelers to endemic regions are at risk. Most cases in the United States have been linked with international travel. Travelers visiting friends and relatives are at the highest risk of infection (Christenson, 2013).

The total number of typhoid fever cases reported in Ghana for the year 2011 was 103,353 with 793 (CFR = 0.77%) deaths (GHS Annual Report, 2011). A study done by Uwe Grob et al, (2009) in Ghana found out that *Salmonella enterica* serovar Typhi was the most prevalent specie among the 24 *Salmonella* isolates from blood culture.

2.4.3 Age and gender-specificity of salmonella infections

Salmonella infections have a bimodal age distribution with peaks occurring in children and the elderly. In South East Asia, several reports have indicated that the prevalence of *Salmonella* infections is highest in children below 5 years, with high cases of

complications and hospitalization (Siddiqui *et al.,* 2006, Ochiai *et al.,* 2005). Feasey *et al.,* (2010), also observed a significant gender difference in the age at which adults acquired invasive non-typhoidal *Salmonella*, being a median of 5 years younger in women than men. In a report by Olsen *et al.,* (2001), a higher prevalence of *Salmonella* infections in males than in females was observed among the elderly. They also observed that, more middle-aged women were infected than their male counterparts (Olsen *et al.,* 2001).

2.4.4 Risk factors for *Salmonella* infections

Risk factors for salmonellosis include individual who use antacids or proton pump inhibitors frequently, infants and the elderly (Christenson, 2013). Also, conditions that impair cell-mediated lymphocyte function, such as HIV/AIDS, malnutrition, corticosteroid therapy, and post-transplantation immunosuppressive therapy, are major risk factors. Additionally, an overload of the reticuloendothelial system with iron or hemoglobin, such as in patients with sickle cell anemia, hemolytic anemia, thalassemia, and malaria, may increase the likelihood of severe disease. Diseases such as leukemia and lymphoma also impair the reticuloendothelial system function (Christenson, 2013).

2.4.5 Seasonality of occurrence

Seasonal variations affect the epidemiology of *Salmonella* infections (Mohanty *et al.,* 2006). The seasonality in the occurrence and prevalence of *Salmonella* infections was reported by Larbi *et al.,* (2014) in Ghana. They stated that *Salmonella* Typhi infections follow the rainfall pattern with the highest number of cases occurring during March to August. The number of cases of non-typhoidal *Salmonella* infections was fairly distributed across the months (Larbi *et al.,* 2014). In two different studies (2005 and

2006), Kariuki *et al.,* also observed a high number of cases in May and June (it mostly rains during these months). They suggested that it might be due to reduced sanitary conditions in homes and the environment in which children live and play (Kariuki *et al.,* 2005 and 2006). In India, Mohanty *et al.,* (2006) observed that the highest cases of typhoid fever occurred during April to June in the dry season.

2.4.6 *Salmonella* occurrence in food

Salmonella is one of the most common causes of food borne diseases worldwide (Carlos, 2012). *Salmonella* is found in the gastrointestinal tract of warm and cold-blooded vertebrates, with many animals being asymptomatic. The environment (crops, plants, soil, rivers and lakes) is contaminated by the fecal shedding of *Salmonella* species by these asymptomatic animals (Carlos 2012). Food stuffs such as vegetables and fruits (which have been contaminated by sewage) and animal products are the major sources of *Salmonella* infections (ICMSF 1996; Jay *et al.,* 2003, Crum Cianflone, 2008). *Salmonella* species have been associated with many outbreaks of foodborne diseases worldwide.

In France, *Salmonella* Agona was associated with two consecutive outbreaks among infants, through the consumption of powdered infant formula (Brouard *et al.,* 2007).

In New Mexico, nut butter was implicated in an outbreak of *Salmonella* Bredeney in the fall of 2012. This outbreak occurred in twenty states and resulted in hospitalization of 10 out of the 42 people that were infected (CDC 2012).

2.4.7 Food handlers

According to the Food Standard Agency of UK, the term 'food handler' refers to people who directly touch open food as part of their work (Food Standard Agency of UK, 2004). A major risk of contamination lies with the food handlers. A food handler may pose as a hazard potential in the transmission chain of *Salmonella* infections in three ways; as patient (infected and actively transmitting the organism), as a passive transmitter (not infected but passively transmitting the *Salmonella* from infected source such as poultry to food by such means as unwashed hands) and as a carrier (Cruickshank and Humphrey, 1987).

A number of studies have implicated food handlers as *Salmonella* carriers and thus serve as a potential source of infection of enteric fever. Feglo *et al.,* (2004), reported that, 2.3% of food vendors were *Salmonell*a carriers in Kumasi, Ghana. Mensah *et al.,* (1997) also reported a prevalence of 3.2% involving 176 food vendors in Accra Ghana.

In Nigeria, *Salmonella* were isolated from 7 (13.2%) out of the 53 stool samples from food handlers tested, of which three (5.7%) were S. Typhi, three (5.7%) were S. Enteritidis and one (1.9%) was S. Choleraesuis (Smith *et al.,* 2008).

In Namakkal, India, out of the 35 stool samples from asymptomatic food handlers processed, 6 (17.1%) yielded positive *Salmonella* growth. Of the total 17 isolates, 5 were found to be multidrug resistant strains (Senthilkumar *et al.*, 2005). This study indicates that food handlers can be a source of drug resistant strains which is a serious public health problem.

Food handlers, who are chronic carriers, can spread *Salmonella* for more than a year. A classical case is “Typhoid Mary” who caused uproar in the United States. A cook called Mary Mallon over a ten year period cooked for eight different families. Denying she ever had the disease, “Typhoid Mary” is known to have infected fifty-four people; three of whom died (Arnold, 1989; Shanson, 1989; Atlas, 1995).

2.5 Laboratory diagnoses

The diagnosis of *Salmonella* infections is based on the isolation of the bacteria from clinical specimens such as blood, stool, urine, bone marrow, bile and other body fluids through conventional culture methods. In enteric fever, blood culture is the procedure most likely to reveal the organism during the first two weeks of illness (Geddes, 1974). However, this method requires 7-14 days to obtain a result; also, the irrational use of antibiotics has lowered the sensitivity of this method.

The Widal test is a common serological test which detects antibodies of O and H antigens of *S*. Typhi, but has low sensitivity and specificity. Also, it cannot detect many *Salmonella* strains (Ali *et al.,* 2008).

Serum IgM, IgG, and IgA antibodies to *Salmonella* can be measured through enzyme immunoassay (EIA). In post-infectious complications when the organism is often difficult to recover, the EIA can be used to detect *Salmonella* antibodies (Isomäki O *et al.,* 1989).

Polymerase chain reaction (PCR) has proved better than these conventional methods. It has successfully been used in identifying *Salmonella* species in both clinical and environmental specimens (Ali *et al.,* 2008). Several methods are used to extract

Salmonellae DNA from target specimens. These include Multiplex PCR, Real time PCR, Isothermal PCR and pulsed-field gel electrophoresis (PFGE) (Higuchi *et al.,* 1993, Hu *et al.,* 2002, Maurer, 2006, Jung *et al.,* 2010).

2.5.1 Culture and Isolation of Salmonella from clinical specimen

2.5.1.1 Specimen required

The suitable specimens required for the isolation of *Salmonella* species include blood, urine and faeces /rectal swab. In the event of suspected outbreak, food and water samples could also be tested for the presence of *Salmonella* species. Samples from sterile sites such as cerebrospinal fluid (C.S.F.) and other body fluids may be required for the isolation of *Salmonella* species if the disease is suspected to be systemic (Murray *et al.,* 1999).

2.5.1.2 Culture media for the isolation of *Salmonella* species from fecal specimen

Isolation of *salmonella* from fecal specimens involves the use of four main media types. First, the non-selective pre-enrichment, such as alkaline peptone water, is used to reduce the chances of obtaining a false negative result (Gracias and McKillip, 2004). The second type of media used is selective enrichment medium such as selenite cysteine broth or tetrationate broth (TT). This is followed by plating on selective or differntial medium such as Salmonella-Shigella agar (SS), Hektoen enteric agar, MacConkey agar, Xylose Lysine Deoxycholate (XLD) agar, Bismuth sulphite agar, Mannitol Lysine Crystal Violet Brilliant Green agar, or Deoxycholate-citrate agar (DCA) (Hohmann, 2001). Finally, identification media such as Triple Iron Sugar agar (TSI), Motility Indole Urea agar (MIU) and Citrate agar are used to distinguish them from other enteric bacteria (Hohmann, 2001).

2.5.1.3 Identification of *Salmonella* species

2.5.1.3.1 Morphological Identification

Salmonella colonies are 2-4 mm in diameter and are colourless (non-lactose fermenters) on MacConkey Agar after 24hr incubation. On Deoxycholate-citrate agar and Salmonella-Shigella agar, most *Salmonella* strains produce clear colonies with distinct black centres (hydrogen sulphide: H_2S) (Hohmann, 2001). *Salmonella* species are observed under the microscope as Gram negative short rods after Gram staining.

2.5.1.3.2 Biochemical identification

The common biochemical tests used in identifying *Salmonella* species include triple sugar iron (TSI), Urea, citrate, L-lysine decarboxylase, β-galactosidase (ONPG), Voges Proskauer, Indole and motility tests. These tests are used to differentiate *Salmonella* species from other enteric bacteria such as *Proteus* colonies, which have similar characteristics (Global Salm-Surv, 2003).

On TSI agar, most *Salmonella* species form alkaline slant and acid butt and produce both hydrogen sulphide and gas. They also produce lysine decarboxylase, are citrate positive, motile, and are urea negative (Ewing, 1986). However, not all *Salmonella* species exhibit the same biochemical properties. For instance, *S*. Paratyphi A does not produce hydrogen sulphide (H_2S) (*Salmonella* case definition summary, 2000).

The API 20E has 23 miniaturised biochemical tests and it is used to identify Gram-negative organisms, particularly those in the family *Enterobacteriaceae*. This method is convenient to use, but it is expensive as compared to conventional biochemical tests (Global Salm-Surv, 2003). Other kits and automated machines such as Vitek

(bioMerieux, Marcy- l'Etoile, France), MicroStation (Biolog, Heyward, USA) and Cobas (Becton Dickinson Diagnostic Instrument Systems, Sparks, USA) are also available for the identification of *Enterobacteriaceae* (Salmonella case definition summary, 2000).

2.5.1.3.3 Serological identification of *Salmonella* species

Serological identification involves the use of polyvalent and monovalent antisera for somatic (O), flagellar (H) and capsular (Vi) antigens. Isolates which show agglutination to O or Vi antisera and a positive reaction to H antiserum are identified as *Salmonella* species (Nikitin *et al.,* 1986). Specified antisera are subsequently used for serotyping and identifying the serovar. However, serotyping is not usually done in a routine clinical laboratory, since it is costly to maintain a full set of antisera to perform a complete serological identification of *Salmonella* isolates. *Salmonella* Isolates are normally sent to reference laboratories for serotyping. Special public health laboratories perform definitive serotyping of the O, H and Vi antigens for epidemiological purposes. (rapidmicrobiology.com).

2.6 Antimicrobial susceptibility testing

Antimicrobial susceptibility test (AST) is essential in helping the clinician to select the most appropriate agent for treating that disease (Atlas, 1995). There are many methods used in AST, these include disk diffusion, broth dilution and agar dilution tests, the E – test, automated AST systems, mechanized specific tests and genotypic methods (Atlas, 1995). A number of guidelines such as National Committee for Clinical Laboratory Standards (NCCLS) are available for antimicrobial susceptibility testing and subsequent interpretative criteria (Bager, 2000; Craig, 1993).

2.6.1 Disk diffusion method

The disk diffusion method involves the use of disks, tablets or strips that have been impregnated with antibiotic with specified concentration to determine whether a particular bacterium is susceptible or resistant to the antibiotic. After inoculating to the organism on a solid culture media (preferably Mueller-Hinton agar), the disk is placed aseptically onto the media. The antibiotic in the disk diffuse into the culture medium in decreasing amount the further it is away from the disk. If the bacterium is inhibited by the concentration of the antibiotic, there will be no growth in the immediate area around the disk. This area is called the zone of inhibition and establishes the organism as either sensitive or resistant to the antibiotics used (Finegold *et al.,* 1978, Atlas, 1995). The disc diffusion method is commonly used in most Laboratories because it is convenient, efficient and less expensive (Atlas, 1995).

2.6.2 Broth dilution

The broth dilution method is often referred as the “gold standard”. In this method, standardized microbial inoculum is tested against different concentrations of an antimicrobial agent (usually doubling dilutions) in a standardized liquid medium (Atlas, 1995). This method can be done by using either macrodilution or microdilution. Macrodilution is done in tubes with at least 2ml of broth, whereas microdilution is done in small micro titration plates containing broth volume of 0.05 to 0.1ml (Balows *et al.,* 1991; Craig, 1993). The lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation is referred as the Minimum inhibitory concentration or MIC (Balows *et al.,* 1991).

2.7 Treatment of *Salmonella* infections

Antimicrobial treatment of uncomplicated salmonellosis in healthy individuals may not be necessary since the disease is usually self-limiting. Treatment mainly consists of replenishing liquid and electrolytes (Christenson, 2013). Antimicrobial therapy should however be considered for infants and those individuals with high-risk medical conditions, such as HIV, sickle cell anemia, and cancer, because they have a high incidence of extraintestinal complications, such as bacteremia, meningitis, and osteomyelitis (Christenson, 2013).

Ampicillin, chloramphenicol and cotrimoxazole were effectively used for the treatment of *Salmonella* infection in the past. However, the organisms have become resistant to these antibiotics due to the irrational use of these antibiotics (Akinyemi *et al.,* 2000). Treatment of *Salmonella* infections is now done with the fluoroquinolones and cephalosporins (Rotimi *et al.,* 2008). However, resistance and treatment failures of flouroquinolones (ciprofloxacin) have been observed and reported in certain areas. In these areas, third-generation cephalosporins are being used to treat *Salmonella* infections. (Capoor *et al.,* 2007).

A single type of bactericidal drug can be used to treat *Salmonella* bacteremia for 10-14 days. However, a combination of a fluoroquinolone and a third –generation cephalosporin may be required for the empirical treatment of life-threatening *Salmonella* infections until the susceptibilities of antimicrobial agents are determined (Hohmann, 2001).

2.8 Anti-microbial resistance

The irrational use of antibiotics in human health as well as in agricultural practices has led to the emergence of resistant microorganisms (Khachatourians, 1998). For instance, it has been reported in the United States that antibiotics are used as growth promoters to feed farm animals (Goldman, 2004). Low levels of antimicrobials are added to feeds in the pork and poultry industry (Khachatourians, 1998). Reports of *Salmonella* being resistant to many common antibiotics used in medical treatment of the disease have been published worldwide. In the U.S. alone, it is estimated that 4,760 deaths occur each due to antibiotic-resistant *Salmonella* (Khachatourians, 1998).

Resistant to the first line antimicrobial drugs including ampicillin, chloramphenicol and cotrimoxazole by 9.7% (408/4200) of *Salmonella* Typhi isolates was observed in a study done in Pakistan, these isolates were thus labeled as Multi drug resistant (MDR) *Salmonella* Typhi (Mahmood *et al.,* 2012).

In Nigeria, a study conducted by Abdullahi *et al.,* (2012), showed that, high proportions of *Salmonella* Typhi and *Salmonella* Paratyphi A were resistant to ampicillin, chloramphenicol and cotrimoxazole. All the isolates were however susceptible to ciprofloxacin and ofloxacin.

In Abidjan, Cote d'Ivoire, a study done by Boni-Cissé *et al.,* (2012), showed that resistant to amoxicillin and amoxicillin-clavulanic acid by *Salmonella* isolates were 74.2% and 58.1% respectively. Resistant to ciprofloxacin was also found to be 14%.

In Ghana, multi drug resistant was observed in 52% (30/58) of *Salmonella* Typhi isolates in a study conducted by Mills-Robertson *et al.,* (2002). Non-typhoidal strains may be complicated by resistant to the common antimicrobial agents (Wilkins *et al.,* 1997).

Resistance to antimicrobial agents is a great challenge to clinicians in the management of infections (Mølbak, 2005).

2.9 *Salmonella* and Extended Spectrum Beta Lactamase

The emergence of bacterial resistance through the production of extended-spectrum beta-lactamase (ESBLs) in recent times, poses a serious antibiotic problem worldwide (Bush, 2008; Canton *et al.,* 2008). The widely use of β-Lactams drugs in treating *Salmonella* infections, has led to the development of resistant to different β-Lactams through the production of β-Lactam enzymes (β-Lactamases) (Bush, 2001). ESBL enzymes are spread easily among bacteria belonging to the family *Enterobacteriaceae*. This enables the transfer of resistance to β-Lactams and other antimicrobial drugs (Kocagoz *et al.,* 2006). Production of ESBLs in *Salmonella* species is not common, as compared with other enteric bacteria (Kocagoz *et al.,* 2006). Different serovers of *Salmonella enterica* have been found to be ESBL producers in many countries. This is a major public health concern, since cephalosporins are the drug of choice in the treatment of *Salmonella* infections in children and in areas where treatment failures with flouroquinolones are common (Hasman *et al.,* 2005, Nashwan *et al.,* 2008).

In a study conducted by Mahmood *et al.,* (2012), in Pakistan, it was observed that of the 408 Salmonella isolates, 3, representing 0.7% were found to be ESBL producing strains. ESBL production was detected in *Salmonella enterica* serover Typhi isolated from a 54 year old Dutch man who had just returned from Philippine (Nashwan *et al.,* 2008). Three (3) *Salmonella* Paratyphi A isolates were also found to be ESBL producers in Nepal (Pokharel *et al.,* 2006).

2.10 Prevention and control of *Salmonella* infections

Salmonella infections are prevented by both public health and personal hygiene measures. These include proper sewage disposal or treatment and provision of water supply that is chlorinated and monitored for contamination by coliform bacteria. Also, stool samples of food handlers should be cultured to identify chronic carriers, so that they can be treated (Crump and Mintz, 2010).

Typhoid fever can also be prevented by vaccination. Two vaccines are licensed for the prevention of typhoid. The live attenuated oral Ty21a vaccine and the parenteral Vi vaccine (Fraser *et al.,* 2007).

CHAPTER THREE

3.0 METHODS

3.1 Study Design

This was a cross sectional study. It was conducted to determine the Prevalence of *Salmonella* infections among food handlers and patients in Jirapa. Stool and blood samples from patients and food handlers were analyzed with standard laboratory procedures.

3.2 Study Site

The study was conducted in Jirapa in the Jirapa District in the Upper West Region of Ghana. Jirapa District has a territorial size of 1,188.6 square kilometers representing 6.4 percent of the regional landmass.

It shares borders with the following districts; Nadowli to the south, Lambussie to the North, Lawra to the west and Sissala West to the East. Jirapa is the district capital, and it is about 62 km away from the regional capital, Wa. The district's strategic location within the region/country and its ready access to neighboring Burkina Faso is a great potential for trade promotion and joint development especially on Sheanuts/butter, mangoes etc to enhance the growth and development of the district. The district has a poor drainage system, as there no major rivers.

The 2010 National Population and Housing census results put the district total population at 88,402 distributed across all ages and different sexes. The total population consists of 53.0 percent females and 47.0 percent for males. With a land size of 1,188.6 Km^2, the District's population density stands at 74.4 persons/km^2. The implication is that the pressure on land and other existing socio-economic facilities is going to be high. The

District is divided into eight (8) sub-districts. These are Jirapa, Douri, Hain, Sabuli, Tuggo, Ullo, and Yagha (Jirapa District Assembly, 2014)

Table 3.1: Breakdown of sub-district population in Jirapa District in 2010

SUB – DISTRICT	POPULATION (2010)
DUORI	7,127
HAIN	10,887
JIRAPA	36,391
SABULI	7,178
TUGGO	8,468
ULLO	9,480
YAGHA	8,871
DISTRICT TOTAL	**88,402**

Source: Jirapa District Health Administration, 2011

3.3 Study population

The study population consists of food handlers who had a direct contact to food preparation and food delivery and agreed to participate in the study.

Also, individuals who came to the hospital with complaints of headache, fever, abdominal pains and diarrhea during the period of study were included. Those who refused consent were excluded.

3.4 Sample Size

The sample size was determined by using a single population formula considering the following assumptions. Za/2=1.96 for the standard scale of 95% level of confidence, Response distribution, P of 50% and level of precision of 5%.

Given the formula $N= [Z^2 (P) (1-P)] / (Error)^2$

Where N= Sample size, Z= 1.96, Error = 5%, P= 50%

A sample size of **381** was representative for Jirapa population of 36,392.

3.5 Criteria for inclusion

1. Food handlers who agreed to participate were included
2. Individuals who come to St. Joseph's Hospital, Jirapa with complaints of enteric fever and gastroenteritis.

3.6 Criteria for exclusion

1. Food handlers and patients who were on antibiotics were excluded
2. Food handlers who declined to participate

3.7 Ethical Consideration

Ethical and Scientific approvals were sought from the KNUST Committee for Human Research, Publications and Ethics (CHRPE) and the St. Joseph's Hospital.

3.8 Enrollment of participants

Consultations were made with the Environmental and Sanitation Officer at Jirapa District Assembly, the headmasters/principals of schools and clinicians at the hospital to help enroll the participants for the study. The objectives of the study were explained to participants and their parents/caretakers. Those who satisfied the inclusion criteria and agreed to participate filled the consent form, and were enrolled for the study.

3.9 Sample collection and handling

Both blood and stool specimens were obtained from patients, whereas only stool specimen was obtained from food handlers.

3.9.1 Collection and analysis of blood

With gloves worn, a tourniquet was applied to the forearm of the participant to enable the veins to be felt. Using 70% ethanol, about 50mm diameter of the venipuncture site was cleansed and allowed to dry. The site for venipuncture was cleansed again using 2% tincture of iodine in a circular action, beginning at the point where the needle will enter the vein and also allowed to air-dry. Using a sterile syringe and needle, blood was drawn into brain heart infusion broth (Liofilchem, Italy). The caps of the culture bottles were swabbed with 70% ethanol and the needle used to withdraw the blood was changed with a new sterile needle, before dispensing into the broth. The blood was immediately mixed with the broth, labeled and incubated at 37°c in aerobic medium for 7 days. The samples were examined daily for signs of bacterial growth such as turbidity and haemolysis. The samples from the broth were subcultured each day for seven days on the following media: Blood agar (Liofilchem, Italy), MacConkey agar (Liofilchem, Italy) and Salmonella-Shigella (SS) agar (Liofilchem, Italy). The plates were incubated aerobically at 37°C for 24hrs. Bacterial growths with colonies of 2-3mm in diameter and pale non-lactose fermenting were presumed to be *Salmonella* and were selected for identification.

3.9.2 Collection and analysis of stool specimen

Participants were counseled on how to obtain stool sample to prevent urine contamination. Sterile containers with wide-necked and screw capped were given to the participants to obtain the stool. Stool samples were obtained by participants themselves

or assisted by their parents/guardians in the case of children. Samples were received at the St. Joseph's hospital's Laboratory, Jirapa, and labeled appropriately. Portions of the specimen were enriched in Selenite broth *(Liofilchem, Italy).* A loop full was inoculated on Macconkay agar *(Liofilchem, Italy)* and Salmonella Shigella (SS) agar *(Liofilchem, Italy)* and incubated for 24 hours at 37 ºC. A subculture of the selenite broth was made on Deoxycholate-citrate agar (DCA) *(Oxoid, UK)* , Macconkay agar *(Liofilchem, Italy)*, and Salmonella Shigella (SS) agar *(Liofilchem, Italy)* the following day and incubated at 37 ºC for 24 hours in an aerobic medium for isolation of *Salmonella* species.

3.9.3 Bacterial Identification

The bacteria were identified by their characteristic appearance on their respective media. Bacterial growths with colourless (non-lactose fermenters) colonies of 2-4mm in diameter and H_2S production or otherwise were selected for gram staining and confirmed by the biochemical and serological methods.

3.9.3.1 Gram stain

Gram stain was done to classify the bacteria as gram negative. A smear of the suspected *Salmonella* colony was made on a clean microscope slide. The smear was allowed to air dry and heat fixed by passing it through a flame three times. The smear was stained with crystal violet stain for 1-2 minutes and washed with water. Lugol's iodine was flooded on the smear for 1 minute and washed with water. It was decolorized with acetone alcohol for some few seconds, washed and finally counter stained with neutral red for 1-2 minutes. The slide was washed, excess water drained off and allowed to air dry. The slide was examined with 100x in oil immersion under a light microscope for gram negative rods, typical of *Salmonella*.

3.9.3.2 Biochemical tests

Presumptive colonies were subcultured on Nutrient agar (Oxoid, UK) and incubated at 37°C for 24 hours, so that a pure culture of the *Salmonella* isolates could be obtained for biochemical identification. In the biochemical test, 2-3 identical colonies were picked by a sterile straight loop and inoculated onto the media. The biochemical tests performed included Triple Sugar Iron (TSI), Urea, Indole, motility and Citrate utilization tests.

3.9.3.2.1 Triple Sugar Iron (TSI) test

2-3 suspected *Salmonella* colonies were picked with a straight loop and carefully inoculated into a tube of TSI agar *(Liofilchem, Italy)* by stabbing the butt first, before .streaking on the surface of the slant, and incubated at 37 ºC overnight. *Salmonella* usually shows an alkaline slant (red) with an acid butt (yellow). H_2S and gas may be produced depending on the *Salmonella* species isolated (Hohmann, 2001).

3.9.3.2.2 Citrate Utilization Test

Suspected *Salmonella* colonies were inoculated into a tube of Simon Citrate agar *(Bioteck, UK)* and incubated at 37 ºC for 24hrs. A change in the colour of the media, from green to blue indicates positive citrate test results, no change in the colour, shows negative citrate test results. Most *Salmonella* serovers are citrate positive (Cheesbrough, 2000).

3.9.3.2.3 Motility Indole Urea (MIU) Tests

Suspected *Salmonella* colonies were stab-inoculated into a tube of MIU agar *(Himedia, India)* and incubated at 37 ºC overnight. Motility was confirmed by observing diffuse growth or turbidity extending away from stab inoculation line. If the growth is seen

along the stab line, then the organism is not motile or motility is negative. Most *Salmonella* species are motile (Cheesbrough, 2000).

A change in colour of the media to pink-red indicates a positive urea test results, while no colour change in the media shows negative urea test result. *Salmonella* species are urea negative (Cheesbrough, 2000).

In the indole test, two drops of Kovac's reagent was added to the MIU agar. A positive indole test results in the formation of a red ring on interface of the media. A negative test leaves no colour change on the interface of the media. *Salmonella* species are indole negative (Cheesbrough, 2000).

3.9.3.3 Serology

Suspected *Salmonella* isolates were further confirmed by serological means using Oxoid *Salmonella* test kit and Becton Dickinson (BD) polyvalent and monovalent antisera. Samples were serotyped with antisera O and H grouping and Vi-sera using Difco *Salmonella* 'O' poly A – I & Vi antiserum, Difco *Salmonella* 'O' group 'B' antiserum, Difco *Salmonella* 'O' group D_1 antiserum, *Salmonella* 'H' poly a – z antiserum and Salmonella Vi antiserum.

3.9.3.3.1 Oxoid *Salmonella* Test Kit

A loop full of suspected *Salmonella* colonies was transferred to a drop of sterile physiological saline on a clean slide and emulsified. One drop of the Oxoid *Salmonella* test kit was added to the suspension and mixed thoroughly. The slide was rotated and the

result was read in two minutes. Positive result shows rapid agglutination in a form of visible clumps. No agglutination within 2 minutes is a negative result.

3.9.3.3.2 *Salmonella* O and Vi slide test

One drop (35 µL) of each Difco *Salmonella* O or Vi (BD, USA) antiserum was dispensed on an agglutination slide labelled 'T' (test slide). One drop of sterile physiological saline was dispensed on a second slide labelled 'N' (negative control). A loopful of suspected *Salmonella* colonies from a nutrient agar plate was transferred to each agglutination slide and mix thoroughly. The slides were rotated for 1 min and read for agglutination. Results were read within 1 min. Positive results show agglutination with clear to slightly hazy background within 1 minute. Negative results show no agglutination within 1 minute.

3.9.3.3.3 *Salmonella* H (poly a – z) Tube Test

A 1:25 dilution was prepared by adding 0.1ml of Difco *Salmonella* H poly a-z antiserum (BD, USA) to 2.4 mL of sterile physiological saline in a test tube to make the working reagent. A suspension of the suspected *Salmonella* isolate was made by transferring about 2-3 loopfull of the *Salmonella* colonies from a nutrient agar plate into 1 mL of sterile physiological saline in a test tube.

0.5 mL of the diluted antiserum was dispensed into a test tube. 0.5 mL of the test isolate suspension was added to the test tube containing the diluted antiserum and mixed. In the negative control, 0.5 mL sterile physiological saline was added to another test tube containing 0.5 mL of the test isolate and mixed. The tubes were incubated in a water bath at 50 ± 2°C for 1 h and read for flocculation. Positive results show flocculation (agglutination) with clear to slightly hazy background. A negative result shows no agglutination.

3.9.4 Sensitivity testing

Kirby-Bauer disk diffusion method was used in testing the antimicrobial susceptibility of all *Salmonella* isolates. This was done under CLSI guidelines. Using a sterile loop, 2-3 *Salmonella* colonies were touched and inoculated in a tube containing peptone water and the turbidity of the inoculum was adjusted to 0.5 McFarland standards. A Mueller-Hinton agar (Liofilchem, Italy) was inoculated by dipping a sterile cotton swab into the inoculum and any excess moisture was expressed by pressing the swab stick against the side of the tube and swabbing completely on the plate. The swabbing process was repeated after the plate was turned to 90 degrees. Using sterile forceps, antibiotic disks were placed on the inoculated agar plate and incubated overnight.

Nine antibiotics were tested. These include ampicillin (AMP) 10 µg, ceftriaxone (CTR) 30 µg, gentamicin (CN) 10 µg, tetracycline (TE) 30 µg, chloramphenicol (CHL) 30 µg, Ciprofloxacin (CPR) 5 µg, cefotaxime (CTX) 30µg, ceftazidime (CAZ) 30µg, and Cotrimoxazole (COT) 25 µg. Finally, the zones of inhibition were measured with a metric rule in millimeters and compared with standard chart. Results were reported as sensitive (S), intermediate (I) and resistant (R).

3.9.5 Testing *Salmonella* isolates for ESBL

The disk diffusion technique was used to test all *Salmonella* isolates for ESBL production. A Mueller-Hinton agar plate was inoculated with *Salmonella* inoculum. Using sterile forceps, an amoxicillin (20ug) plus clavulanic acid (10ug) (Augmentin 30µg) disk was placed in the center of the inoculated plate, ceftazidime (30µg), ceftriaxone (30µg) and cefotaxime (30µg) were then placed around the Augmentin disk and incubated overnight at 37°C. An increase of the zone of inhibition of any one of the

test antibiotics towards the Augmentin disc was regarded as a probable ESBL production.

3.10 Quality Control

All laboratory tests were performed using standard bacteriological procedure and appropriate internal quality controls. The sterility of every batch of prepared media was checked by incubating one of the freshly prepared media plate at 37°C overnight, and observed for contamination.

Some media and reagents were tested with both positive and negative control organisms obtained from Upper West Regional Hospital, Wa, and Komfo Anokye Teaching Hospital (KATH), Kumasi, as indicated in the table below.

Table 3.2: Quality control organisms

Test	**Media/Reagent**	**Positive control organism**	**Source**	**Negative Control**	**Source**
Citrate	Simon Citrate Agar	*Klebsiella pneumonia*	KATH	E. coli	Wa Hospital
ESBL	Mueller Hinton Agar	ESBL producing *Klebsiella Pneumonia*	KATH	Non-ESBL producing *E. coli*	KATH
Urea	Motility Indole Urea Agar	*Proteus vulgaris*	Wa Hospital	E. *coli*	Wa Hospital
Serology	*Salmonella* antisera	*Salmonella spp*	Wa Hospital	E. *coli*	Wa Hospital

3.11 Data Analysis

All Laboratory results and questionnaires were entered in Microsoft Excel. Data analysis was done with SPSS version 15 software. The data were presented in summary tables. Data presented as categorical proportions were compared by the X^2 test while continuous data were compared using the t-test. Significant differences between proportions were set at 0.05.

CHAPTER FOUR

4.0 RESULTS

The study was carried out to determine the prevalence of *Salmonella* infections among food handlers and patients in Jirapa from January to May 2015. Stool specimens were obtained from 170 food handlers and stool and blood specimens were collected from 219 patients. The samples were cultured for *Salmonella*. A total of 38/464 (8.2%) samples were positive to *Salmonella*. All biochemically positive Salmonella isolates showed agglutination with Poly 'O' and a positive reaction to Poly 'H' antisera. Among the 38 *Salmonella* isolates from both food handlers and patients, there were 16 (42.1%) belonging to Serogroup D1 and 3 (7.9%) belonged to serogroup B. 36.8% (14/38) were serotyped and identified as *S*. Typhi. The remaining 24 (63.2%) could not be typed with the antisera available.

4.1 Demographic characteristics of food handlers in Jirapa

The age and sex distribution of food handlers are presented in Table 4.1. The study was conducted on one hundred and seventy (170) asymptomatic food handlers involved in the preparation and serving of various kinds of food including khebab, koose, zonkuon, pito, rice, Tuo zafi, fufu, kenkey and among others. 35.9% of the food handlers were in the age group 20 - 30 years. 136 (80%) of the participants were females, and 34 (20%) were males. 66.5% food handlers had basic education and 12% had no formal education.

4.2 Prevalence of *salmonella* infection among food handlers

Stool samples from 170 participants were tested and seven (7) were infected with *Salmonella*, giving *Salmonella* carriage of 4.1%. Out of the 7 isolates, five (5) were *S.* Typhi representing 71.4% (5/7) and the remaining 2 (28.6) were other non-typhoidal

Salmonella types. Most, (85.7%) of the food handlers infected were females. Only one was a male. *Salmonella* was isolated from three (3) food handlers in school canteen. Most of the food handlers (57.1%) infected were involved in food preparation and serving. All the Salmonellae were isolated from food handlers who lack training in food safety and also lack knowledge about how typhoid fever is transmitted. Two (2) of the infected food handlers do not practice hand hygiene before touching food. The details of these results are presented in table 4.1.

Table 4.1: Frequency of Salmonella infection among food handlers by sex, age, Educational status, category of food handlers, role in food preparation, trained food handler, and hand hygiene, at Jirapa.

PARAMETER		TOTAL NUMBER EXAMINED	*SALMONELLA* POSITIVE
		NUMBER (%)	NUMBER (%)
Age group	<20	8(4.7%)	0
	20 – 29	58(34.1%)	2(3.4%)
	30 – 39	61(35.9%)	2(3.3%)
	40 – 49	29(17.1%)	1(3.4%)
	50 +	14(8.2%)	2(14.3%)
Sex	Male	34(20%)	1(2.9%)
	Female	136(80%)	6(4.4%)
Educational status	None	20(11.8%)	2(10%)
	Basic	105(61.8%)	4(3.8%)
	Secondary	38(22.4%)	1(2.6%)
	Tertiary	7(4.1%)	0
Food category	School canteen	50(29.4%)	3(6.0%)
	Street food	94(55.3%)	2(2.1%)
	Chop bar	11(6.5%)	1(9.1%)
	Restaurant	15(8.8%)	1(6.7%)
Role in food preparation	Preparation	67(34.4%)	3(4.5%)
	Serving	17(10.0%)	0
	Preparation and serving	86(50.6)	4(4.7%)
Training on food safety	Trained	9(5.3%)	0
	Not trained	161(94.7%)	7(4.1%)
Periodic medical screening	YES	164(96.5%)	7(4.1%)
	NO	6(3.5%)	0
Hand washing before touching food	YES	110(64.7%)	5(4.5%)
	NO	60(35.3%)	2(3.3%)
Typhoid knowledge	YES	164(96.5%)	5(3.0%)
	NO	6 (3.5%)	2(33.3%)
Knowledge about how typhoid is transmitted	YES	18(10.6%)	0
	NO	152(89.4%)	7(4.6%)

4.3 Antimicrobial susceptibility pattern of Salmonella isolates from food handlers in Jirapa.

High frequency of resistance for *Salmonella* isolates was observed towards tetracycline (85.7%), chloramphenicol (71.4%) and ampicillin (57.1%). However, all the isolates were sensitive to ciprofloxacin the cephalosporins (ceftriaxone, cefotaxime and ceftazidime). Multidrug resistant was observed in three (3) of the isolates, representing 42.9% (3/7). These were typhoidal isolates (*S*. Typhi). No MDR was detected in the other *Salmonella* strains.

Table 4.2: Socio-demographic characteristics of patients reporting to the St. Joseph's hospital, Jirapa.

Characteristics	Frequency (N)	Percentage (%)
Age Group (N = 219)		
0-5	28	12.8
6-15	6	2.7
16-30	105	47.9
31-50	60	27.4
50+	20	9.1
Sex (N = 219)		
Male	70	31.9
Female	149	68.0
Religion (N = 219)		
Christianity	208	95.0
Islam	11	5.0
Marital Status (N= 219)		
Married	120	54.7
Single	1	0.5
separated	3	1.4
widow	1	0.5
Divorced	120	54.7
Education (N=219)		
None	57	26.0
Basic	91	41.5
Secondary	41	18.7
Tertiary	30	13.7

Occupation (N= 219)		
Unemployed	69	31.5
Public	45	20.5
Self	71	32.4
Student	33	15.1
Other	1	0.5
Eat outside		
Yes	161	73.5
No	58	26.5
Place of eating (N = 161)		
School Canteen	33	20.5
Chop bar	28	17.4
Street food	92	57.1
restaurant	8	5.0
Contact with Animals (N =219)		
Yes	180	82.1
No	39	17.8
Type of Toilet (N = 219)		
Water closet	28	12.8
Pit Latrine	136	62.1
Free Range	38	17.4
Other	17	7.8

4.4 *Salmonella* isolates in relation to the demographic characteristics of patients attending St. Joseph's hospital, Jirapa.

A total of 219 patients participated in the study. 294 stool and blood samples were examined. 31 yielded positive to *Salmonella*, representing 10.4% (31/294). Stool samples yielded more *Salmonella* isolates 74.2% (22/31) than blood samples 25.8% (8/31). The *Salmonella* strains isolated comprise of *S*. Typhi representing 29.1% (9/31) and 70.9% (22/31) were other *Salmonella* species. Of the nine *S*. Typhi isolated, eight (8) were isolated from blood samples and one (1) was obtained from stool samples. All the other *Salmonella* species were isolated from stool samples. *Salmonella* infection was high in age group 16 – 30 years. More females (67.7%) were infected than males (32.3%).

Table 4.3: Salmonella isolates in relation to the Socio-demographic characteristics of patients attending St. Joseph's Hospital, Jirapa

Parameter		*Salmonella* Infection				Total		p- value
		Salmonella spp.		*S.* Typhi		Freq	(%)	
		Freq	(%)	Freq	(%)			
Age group	0-5	4	18.1	0	0	4	12.9	**0.203**
	6-15	2	9.1	0	0	2	6.5	
	16-30	8	36.4	7	77.8	15	48.4	
	31-50	5	22.7	2	22.2	7	22.6	
	50+	3	13.6	0	0	3	9.7	
	Total	**22**	**100**	**9**	**100**	**31**	**100**	
Sex	Male	9	40.9	1	11.1	10	32.3	**0.107**
	Female	13	59.1	8	88.9	21	67.7	
	T0tal	**22**	**100**	**9**	**100**	**31**	**100**	
Sample	blood	0	0	8	88.9	8	25.8	**<0.001**
	Stool	22	95.6	1	11.1	23	74.2	
	Total	**22**	**100**	**9**	**100**	**31**	**100**	

Data are presented as frequencies (freq.) and percentages (%). Data were analyzed using chi-square statistics at 95% confidence intervals. P< 0.05 was considered to be significant.

4.4.1 Antimicrobial susceptibility pattern of salmonella isolates from patients attending St. Joseph's hospital, Jirapa

The antimicrobial susceptibility testing results of all the 31 *Salmonella* isolates illustrated in table 4.3 below. The highest proportion of the Salmonellae were susceptible to ciprofloxacilin, ceftriaxone, ceftazidime and cefotazime, all of the isolates (100%) were sensitive. On the other hand, there were varied proportions resistant to tetracycline with 58.1%, ampicillin 45.2% and to chloramphenicol 48.4%.

Table 4:4: Antimicrobial susceptibility pattern of *Salmonella* isolates from food handlers and patients in Jirapa

DRUG	***SALMONELLA* ISOLATES FROM FOOD HANDLERS (N = 7)**						***SALMONELLA* ISOLATES FROM PATIENTS (N = 31)**					
	***S.* TYPHI (N=5)**			***SALMONELLA* SPP. (N=2)**			***S.* TYPHI (N=9)**			***SALMONELLA* SPP. (N=22)**		
	R	**I**	**S**	**R**	**I**	**S**	**R**	**I**	**S**	**R**	**I**	**S**
TETRACYCLINE	5 (100%)	0	0	1 (50.0%)	0	1 (50.0%)	8 (88.9%)	1 (11.1%)	0	10 (45.5%)	2 (9.2%)	10 (45.5%)
AMPICILINE	3 (60.0%)	2 (40.0%)	0	1 (50.0%)	0	1 (50.0%)	6 (66.7%)	0	3 (33.3%)	8 (36.4%)	2 (9.1%)	12 (54.4%)
CHLORAMPHENICOL	4 (80.0%)	1 (20.0%)	0	1 (50.0%)	1 (50.0%)	0	6 (66.7%)	1 (11.1%)	2 (22.2%)	9 (40.9%)	2 (9.1%)	11 (50.0%)
COTRIMOXAZOLE	2 (40.0%)	0	3 (60.0%)	0	0	2 (100.0%)	2 (22.2%)	1 (11.1%)	6 (66.7%)	4 (18.2%)	4 (18.2%)	14 (63.6%)
GENTAMICIN	3 (60.0%)	1 (20.0%)	1 (20.0%)	0	0	2 (100.0%)	2 (22.2%)	0	7 (77.8%)	3 (13.6%)	2 (9.1%)	17 (77.3%)
CIPROFLOXACILIN	0	0	5 (100.0%)	0	0	2 (100.0%)	0	0	9 (100.0%)	0	0	22 (100.0%)
CEFTRIAXONE	0	0	5 (100.0%)	0	0	2 (100.0%)	0	0	9 (100.0%)	0	0	22 (100.0%)
CEFOTAXIME	0	0	5 (100.0%)	0	0	2 (100.0%)	0	0	9 (100.0%)	0	0	22 (100.0%)
CEFTAZIDIME	0	0	5 (100.0%)	0	0	2 (100%)	0	0	9 (100.0%)	0	0	22 (100.0%)

(R = Resistance, I = Intermediate S = Sensitive)

4.4.2 General characteristics of patients stratified by complaints

83 patients had various complaints. Majority of the infected patients being 28.9% (24/83) reported of abdominal pains. Most of the complaints were in the 16 – 30 age groups. Individuals with *S*. Typhi infection complained of fever, abdominal pains and headache, those with *Salmonella* spp. Infection complained of abdominal pains and diarrhea.

Table 4.5: General characteristics of patients stratified by complaints

		Fever 12 (14.6%)			Headache 14 (16.9%)			Abdominal pain 24 (28.9%)			Diarrhea 16 (19.3%)			Nausea 12 (14.6%)			Chills 5 (6.0%)			Total cases 83 (100%)		
		TS	**TS**	**Total (%)**	**ST**	**SP**	**Total (%)**	**ST**	**SP**	**Total (%)**	**ST**	**SP**	**Total (%)**	**ST**	**SP**	**Total (%)**	**ST**	**SP**	**Total (%)**	**ST**	**SP**	**Total (%)**
Age	0 – 5	0	2	2(16.7%)	0	0	0	0	1	1(4.2%)	0	4	4(25%)	0	0	0	0	0	0	0	7	7(8.4%)
	6 – 15	0	1	1(8.3%)	0	0	0	0	2	2(8.3%)	0	2	2(12.5%)	0	0	0	0	0	0	0	5	5(6.0%)
	16 – 30	7	0	7(58.3%)	6	3	9(64.3%)	5	7	12(50%)	0	4	4(25%)	4	2	6(50%)	4	0	4(80%)	26	16	42(50.6%)
	31 – 50	0	0	0	0	2	2(14.3%)	0	4	4(16.7%)	0	4	4(25%)	0	3	3(25%)	0	0	0	0	13	13(15.7%)
	51+	2	0	2(16.7%)	2	1	3(21.4%)	2	3	5(20.8%)	0	2	2(12.5%)	1	2	3(25%)	1	0	1(20%)	8	8	16(19.3%)
	Total	**9**	**3**	**12(100%)**	**8**	**6**	**14(100%)**	**7**	**17**	**24(100%)**	**0**	**16**	**16(100%)**	**5**	**7**	**12(100%)**	**5**	**0**	**5(100%)**	**34**	**49**	**83(100%)**
Sex	Male	1	2	3(25%)	1	4	5(35.7%)	1	7	8(33.3%)	0	7	7(43.8%)	1	3	4(33.3%)	0	0	0	4	23	27(32.5%)
	Female	8	1	9(75%)	7	2	9(64.3%)	6	10	16(66.7%)	0	9	9(56.3%)	4	4	8(66.7%)	5	0	5(100%)	30	26	56(67.5%)
	Total	**9**	**3**	**12(100%)**	**8**	**6**	**14(100%)**	**7**	**17**	**24(100%)**	**0**	**16**	**16(100%)**	**5**	**7**	**12(100%)**	**5**	**0**	**5(100%)**	**34**	**49**	**83(100%)**
Sample type	Blood	8	0	8(66.7%)	7	0	7(50%)	6	0	6(25%)	0	0	0	4	0	4(33.3%)	4	0	4(80%)	29	0	29(34.9%)
	Stool	1	3	4(33.3%)	1	6	7(50%)	1	17	18(75%)	0	16	16(100%)	1	7	8(66.7%)	1	0	1(20%)	5	49	54(54%)
	Total	**9**	**3**	**12(100%)**	**8**	**6**	**14(100%)**	**7**	**17**	**24(100%)**	**0**	**16**	**16(100%)**	**5**	**7**	**12**	**5**	**0**	**5(100%)**	**34**	**49**	**83(100%)**

Keys: ST = *Salmonella* Typhi, SP = *Salmonella* species.

CHAPTER FIVE

5.0 DISCUSSION

Salmonella infection is a global public health problem, often associated with unhygienic food processing and preparations, unsafe water supplies and inadequate sanitary conditions (Addo *et al.*, 2007).

Typhoid fever is among the widespread diseases affecting the population of Jirapa and has been rated seventh (7th) among these common infections (Annual report, 2014). The prevalence and the source of spread in Jirapa are not known. Food handlers have been reported by Mensah *et al.,* (1997) and Feglo *et al.,* (2004) in Accra and Kumasi respectively in Ghana, to contribute to the spread of *Salmonella* infections. This study was undertaken to determine the prevalence of *Salmonella* infections among food handlers and patients attending St. Joseph's hospital, Jirapa and also detect the antimicrobial susceptibility patterns of the *Salmonella* isolates obtained. The study was conducted among patients and food handlers in the Jirapa community.

5.1 Food Handlers

The study established a 4.1% prevalence of *salmonella* carriage among food handlers in Jirapa. This is consistent with similar studies done in Ghana and other Developing countries. In Kumasi, Ghana, among 258 asymptomatic food handlers screened, 6(2.3%) proved positive for *Salmonella* carriage (Feglo *et al.,* 2004). In Addis Ababa, out of the 423 food handlers screened, 13 (3.4%) were also positive for *Salmonella* (Legesse *et al.,* 2014).

The *Salmonella* prevalence among food handlers obtained by this study was however lower than the 7% reported in Jarkarta, Indonesia, the 13.6% reported in Addis Ababa, Ethiopia and the 18% reported in Nigeria (Yolland *et al.,* 2004, Okoye *et al.,* 2005, Addis *et al.,* 2011).

The study indicated that, 4 representing 57.1% out of the total seven (7) *Salmonella* isolates from food handlers were found in the age group 20 – 39 years. This is similar to a study done by Garedew-Kifelew *et al.,* (2014), who found out that all the *Salmonella* positive food handlers were in the age groups 18 – 38 years.

Additionally, in this study, it was observed that more females 6(4.4%) had *Salmonella* infection out of the 136 females screened than males 1(2.9%) out of the 34 males screened. A higher prevalence of *Salmonella* carriage in females than males was also reported by Getnet, (2011), who noted that all the asymptomatic *Salmonella* food handlers were females. This could be due to the fact that more females are involved in food business than males.

Of the seven (7) *Salmonella* species isolated from food handlers at Jirapa, five (5) were *S*. Typhi (2.9%) and two (2) were non-typhoidal *Salmonella* species (1.2%). The high prevalence of *S*. Typhi carriage among food handlers as indicated in the current study is in agreement with studies done in Bahir Dar Town in Northwest Ethiopia where 1.6% food handlers were found to be infected with *S*. Typhi and in Karachi, Pakistan where 3.3% of food handlers were infected with *S*. Typhi (Bayeh *et al.,* 2010, Siddiqui *et al.,* 2015).

The current study also noted a high prevalence of 42.9% (3/7) of *Salmonella* infections among food handlers from school canteens. This is of much concern to school children, since children are more susceptible to *Salmonella* infections and the disease tends to be more severe in them than adults (Christenson, 2013). Majority (96.5%) of the food handlers in Jirapa do periodic medical screening. Lack of periodic medical screening did not have any association with *Salmonella* infections among the food handlers. However, *Salmonella* is not part of the infectious organisms being screened in Jirapa.

Lack of training on food safety and knowledge about how typhoid is transmitted were however associated with Salmonella infections. All the food handlers infected did not have any training on food safety and lacked knowledge about how typhoid fever is transmitted. These food handlers could be potential risk to food safety, since they may have little or no understanding on the risk of microbial or chemical contamination of food and how to avoid them.

The result of the antimicrobial susceptibility test of this study indicated that, *Salmonella* isolates from the food handlers showed a high resistance to tetracycline with a level of 85.7%, chloramphenicol (71.4%) and ampicillin (57.1%). This is similar to findings from Ethiopia by Garedew-Kifelew (2014), who reported that 54.8% of *Salmonella* isolates were resistant to ampicillin. In the USA, Makinata *et al.,* (2010) also reported that 92.9% of isolates were resistant to tetracycline.

Multidrug resistance (resistance to tetracycline, chloramphenicol and ampicillin) was detected in three (3) out of the seven (7) isolates, representing 42.9%. This is similar to the report by Qaiser *et al.,* (2011), who observed that 30.5% of typhoidal isolates were resistant to all the three first line antibiotics. This is a great public health concern, since these food handlers can spread these multidrug resistant strains.

Interestingly, all the *Salmonella* isolates obtained from food handlers were susceptible to ciprofloxacin and the cephalosporins tested (ceftriaxone, cefotaxime and ceftaxidime). This compares favorably with similar findings in other developing countries. Getnet *et al.,* (2011) in Addis Ababa, Ethiopia reported that *Salmonella* isolates were highly susceptible to ceftriaxone and fluoroquinolones. In Nigeria, Smith *et al.,* (2009) also noted that all *Salmonella* isolates were susceptible to ciprofloxacin. In Kenya, Yegon and Yegon (2012) observed that all the eight (8) *Salmonella* isolates from asymptomatic food handlers in Westland Nairobi, were susceptible to ciprofloxacin. However, it contrasts what Surinder et al; (2008) reported. They observed 7.1%, 7.9% and 5.0% resistance of *S*. Typhi to ciprofloxacin, ceftriaxone and cefotaxime respectively.

5.2 Patients

The present study had a *Salmonella* prevalence of 10.4% among patients attending St. Joseph's hospital, Jirapa. This is similar to what Addis *et al.,* (2011) reported at Ethiopia, where a prevalence of 13.6% *Salmonella* was reported. The prevalence rate obtained in this study is however lower than the 36.0% reported in Thailand (Padungtod and Kaneene, 2006).

Of the thirty one (31) *Salmonella* species isolated from patients, 9 (29.0%) were identified as *Salmonella* Typhi and 22 (71.0%) were other *Salmonella* species. These *Salmonella* strains could not be typed, because, the antisera for serotyping them was not available. There were eight (8) Salmonellae isolated from blood. This represents 7.1% (8/112) prevalence of isolation from blood. All the *Salmonella* isolates from blood culture were *Salmonella* enterica serover Typhi. Similar finding was reported by

Akinyemi *et al.,* (2007) in Lagos, but contrast that of Labi *et al.,* (2014) in Accra who reported a higher prevalence of non-typoidal bacteremia (63.5%) than typhoidal bacteremia (36.5%) among patients in Korle-Bu Teaching Hospital, Ghana. The high prevalence of isolation of *S*. Typhi from blood samples recorded in this study may be attributed to the virulent nature of *Salmonella* Typhi, which enables it to invade the bloodstream.

Out of the 182 stool samples cultured, twenty three (23) yielded *Salmonella* representing 12.6% (23/182). This is consistent with the 13.6% reported by Addis *et al.,* (2011) in Ethiopia. 22 out of the total 23 Salmonellae isolated from stool samples were other *Salmonella* species. This represents 95.6% (22/23). One (1) out of the 23 was *S*. Typhi, which also represents 4.3% (1/23). The high recovery of *Salmonella* species in stool samples may be attributed to the fact that these strains lack the ability to invade the bloodstream under normal circumstances, but may cause bacteremia in immune compromised individuals.

A high prevalence of *Salmonella* infections of 48.4% (15/31) was recorded in the age group 16 – 30 years. This does not agree with the work of Abdullahi *et al.,* (2012) in Kano, Nigeria who reported a high prevalence rate of *Salmonella* infections among children.

Moreover, *Salmonella* infection was higher in females 67.7% (21/31) than in males 32.3 % (10/31). This is similar to the work of Umeh and Agbulu (2010) in Nigeria, who reported of 58.0% prevalence of *Salmonella* Typhi in females. Majority (73.5%) of the patients patronize foods and drinks outside their homes including street foods (57.1%), “chop bars” (17.4%), school canteens (20.5%) and restaurants (5.0%). Also, Most of the

patients (82.1%) have contact with animals. These are important risk factors for the acquisition of *Salmonella* infections.

The commonest complaints presented by patients in this study were abdominal pains, headache and diarrhea. The current study unveiled that, out of the 31 patients infected, 77% (24/31) complained of abdominal pains. 45.2% (14/31) complaints were recorded for both headache and diarrhea. In the absence of other diseases, these complaints can be indicative of *Salmonella* infections.

Treatment of *Salmonella* infections with the appropriate antimicrobials is very essential in the management of the infection. However, reports of increase in antimicrobial resistance are common worldwide. Hence the need to determine the susceptibility pattern of antimicrobials before treatment.

The result of antimicrobial susceptibility test on *Salmonella* isolates obtained from patients in this study indicated that a high antimicrobial resistant pattern was observed in tetracycline (58.1%), chloramphenicol (48.1%) and ampicillin (45.2%). Labi *et al.,* (2014) observed that over 70% of both nontyphoidal and typhoidal Salmonella isolates were resistant to tetracycline, ampicillin and chloramphenicol in Ghana. High resistances of Salmonella isolates to these antimicrobial drugs have been reported in Ethiopia, India and USA (Garedew-Kifelew *et al.,* 2014, Surinder *et al.,* 2008, Makinata *et al.,* 2010). This high resistance of *Salmonella* isolates towards antimicrobials including tetracycline, ampicillin and chloramphenicol as observed in this study could be due to the ready availability of these antimicrobials drugs in the study area, leading to their misuse. Nonetheless, high proportions of *Salmonella* isolates were susceptible to gentamicin (77.4%) and cotrimoxazole (64.5%) in this study.

Five (16.1%) out of the total 31 *Salmonella* isolates were classified as MDR based on their resistance to three common antimicrobial agents including ampicillin, chloramphenicol and cotrimoxazole. These inexpensive drugs which were successfully used to treat *Salmonella* infections previously are now showing increasing resistance towards *Salmonella* strains, especially *S*. Typhi (Mills-Robertson *et al.,* 2002). The 16.1% of MDR *Salmonella* strains recorded by the current study is similar to the 10.0% reported in Karachi (Sidiqui *et al.,* 2015), but lower than the 50% reported by Marks *et al.,* (2010) in Ghana.

In this study, all the *Salmonella* isolates were susceptible to ciprofloxacin, ceftriaxone, ceftazidime and cefotazime. High susceptibility of *Salmonella* isolates to ciprofloxacin was recorded in studies done by Mark *et al.,* (2010) and Grob *et al.,* (2011). Another study in Ghana by Namboodiri *et al.,* (2011) also indicated that most *Salmonella* species were susceptible to ciprofloxacin.

In Ghana, ciprofloxacin is the recommended antimicrobial drug for the treatment of *Salmonella* infections, especially multi-drug resistant typhoidal strains, replacing chloramphenicol (Mandal, 2004, Ministry of Health Ghana, 2010). However, there have been few reports of emergence of resistance to ciprofloxacin, this is said to be potential problem in Africa (Graham, 2010). Treatment failures are being reported due to it indiscriminate use. A decrease in susceptibility from 89% to 81% (1997 – 2001) of *Salmonella* Typhi towards ciprofloxacin was reported by Gautam *et al.,* (2001). Similar report has also been made in the United Kingdom (Thredfall *et al.,* 2001).

This study also recorded that all the *Salmonella* isolates were susceptible to the cephalosporins (ceftriaxone, cefotaxime and ceftazidime) tested. This is consistent with with the results recorded by Garedew-Kifelew *et al.,* (2014), who observed that all the isolates obtained were susceptible to ceftriaxone in Ethiopia. High susceptibility of *Salmonella* isolates to cephalosporins has also been reported by Gautam *et al.,* (2002) and Nath *et al.,* (2003).

The high susceptibility pattern exhibited by *Salmonella* isolates towards the ciprofloxacin and the cephalosporins as indicated by this study can be attributed to the fact that, these antimicrobial drugs (especially the cephalosporins) are expensive and are not readily available to the people in the study area, thus preventing their irrational use.

5.3 ESBL Production

The use of β-Lactams drugs in treating *Salmonella* infections, has led to the development of resistant to different β-Lactams through the production of β-Lactam enzymes (β-Lactamases) (Bush, 2001). Different serovers of *Salmonella enterica* have been found to be ESBL producers in many countries. This is a major public health concern, since cephalosporins are the drug of choice in the treatment of *Salmonella* infections in children and in areas where treatment failures with flouroquinolones are common (Hasman *et al.,* 2005, Nashwan *et al.,* 2008).

In this study, no *Salmonella* strain was found to produce extended spectrum beta lactamase. This might be due to the fact that beta lactam drugs such as cephalosporins are not commonly used in treating Salmonella infections in Jirapa. Also these drugs are relatively expensive and are not easily affordable by the local community, thereby

limiting their abuse and reducing resistance. The results obtained in this study is consistent with the findings from Cote d'Ivoire by Boni-Cisse *et al.,* (2012) who reported of no ESBL producing *Salmonella* species in their study, but contrast the report from Pakistan by Mahmood *et al.,* (2012) who observed three (3) *Salmonella* isolates produced ESBL. ESBL production was detected in *Salmonella enterica* serover Typhi isolated from a 54 year old Dutch man who had just returned from Philippine (Nashwan *et al.,* 2008). Three (3) *Salmonella* Paratyphi A isolates were also found to be ESBL producers in Nepal (Pokharel *et al.,* 2006).

5.4 Conclusion

In this study, 170 stool samples obtained from food handlers in Jirapa and 294 stool and blood samples from patients attending the St. Joseph's hospital at Jirapa were screened for *Salmonella* infection. The study indicated that the prevalence of *Salmonella* infections among food handlers and patients was 4.1% and 10.4% respectively.

Abdominal pains, headache and diarrhea were the common complaints presented by patients in this study. These complaints may thus be indicative of Salmonella infections when other diseases are ruled out.

All the *Salmonella* isolates obtained in this study were susceptible to ciprofloxacin, ceftriaxone, ceftaxidime and cefotaxime. These antimicrobials drugs can thus be used for the empiric treatment of *Salmonella* infections in Jirapa. Most of the *Salmonella* strains were resistant to some common antimicrobial drugs including ampicillin, tetracycline and chloramphenicol. No ESBL-producing *Salmonella* strain was found in this study.

It can be concluded from the present study that, 4.1% of food handlers in Jirapa are reservoirs of Salmonellae. These food handlers constitute a potential source for the

transmission of the *Salmonella* pathogen, and may suggest one of the reasons for the high prevalence of *Salmonella* infections in Jirapa.

5.4 Recommendations

1. Food handlers should be screened for *Salmonella* annually, so that those who are found to be infected could be treated and monitored until they are cleared of the disease.
2. Food handlers should be trained on basic principles of food safety and handling. Their training should include essential information on safe food handling and source of microbial contamination.
3. Blood culture is recommended in the diagnoses of *S*. Typhi infections
4. Controlled and judicial use of antimicrobial drugs to help prevent the emergence of resistance towards ciprofloxacin, ceftriaxone, cefotaxime and ceftaxidime and also ESBL-producing *Salmonella* strains is recommended.

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APPENDICES

APPENDIX 1: MEDIA USED FOR THE ISOLATION OF SALMONELLA

Below is a list of media used for the isolation of Salmonella in the laboratory.

1. Blood agar
2. Brain Heart Infusion broth
3. Simons Citrate Agar
4. Kovacs reagent
5. MacConkey Agar
6. Nutrient Agar
7. Peptone Water
8. Salmonella-Shigella Agar
9. Deoxycholate Citrate Agar
10. Selenite F Broth
11. Triple Sugar Iron
12. Motility Indole Urea (MIU) Agar

THE COMPOSITION, PREPARATION AND MODE OF ACTION OF MEDIA

BRAIN HEART INFUSION BROTH (Liofilchem, Italy)

Composition (typical g/L)

Brain-Heart Infusion Solids (Porcine) 17.5g

Peptone 10.0g

Glucose 2.0g

Sodium Chloride 5.0g

Disodium Hydrogen Phosphate 2.5g

pH 7.4+/- 0.2

Method of preparation – Weigh 37g of the medium into 1000ml of distilled water, mix and heat gently to dissolve completely. Dispense into sterile blood culture bottles and sterilize by autoclaving for 15minutes at 121°C

Mode of action - This is an enriched medium for the cultivation of fastidious bacteria, yeast and moulds.

MACCONKEY AGAR (Liofilchem, Italy)

Media Composition

Pancreatic digest of gelatin 17.00g

Peptones (meat and casein) 3.0g

Lactose monohydrate 10.0g

Bile salts 1.5g

Sodium chloride 5.00g

Neutral red 0.03g

Agar 15.00g

pH 7.1+/- 0.2 at 25 ^{o}C

Method of preparation – Suspend 51.5g in 1000ml distilled water and heat to dissolve the medium completely. Sterilize by autoclaving at 121 ^{o}C for 15 minutes. Cool to 45-50°C and pour into sterile Petri plates.

Mode of action –it serves as a differential media the media differentiates lactose fermenting and non- lactose fermenting organisms. Lactose fermenters produce acids which upon acting on the bile salt present in the media take up the neutral red colour as seen in its colonies.

Non-lactose fermenters however give of an alkaline reaction which does not absorb the neutral red colour. This result in their pale/colourless colonies. The presence of bile salt mixture in the media also inhibits gram positive organisms.

MUELLER HINTON AGAR (LIOFILCHEM, ITALY)

Media Composition

Beef extracts 2.0g

Casamino acids technical 17.5g

Starch 1.5g

Agar 15.0g

pH 7.3 +/- 0.1 at 25 ^{o}C

Method of Preparation - Weigh and dissolve 36 grams of the powder into 1000ml of distilled water. Sterilize at 121°C for 15mins.

Mode of action - This medium is recommended for the disc diffusion method of antimicrobial susceptibility testing of bacteria.

NUTRIENT AGAR (Oxoid, UK)

Composition (typical g/L)

Peptone 5.078g

Beef Extract 3.0g

Sodium chloride 8.0g

Agar No. 2 12.0

pH 7.3 +/- 0.2

Method of preparation – Weigh 28g of powder into 1 litre of Distilled water. Heat to dissolve completely and sterilize by autoclaving for 15 minutes at 121°C, mix well and pour into sterile plates.

Mode of action - A basic culture media usually for the cultivation of non-fastidious organisms and sub-culture of organisms for purity growths. It can also be used for the preparation of enriched media (Blood and Chocolate agar), which can be used for the growth of Neisseria and Haemophilus species...

SALMONELLA – SHIGELLA AGAR (LIOFILCHEM, ITALY)

Composition

Meat extract-5.0g

Yeast extract 5.0g

Peptone 5.5g

Lactose 10.0g

Sodium citrate 1.0g

Sodium thiosulphate 8.5g

Ferric Ammonium Citrate 1.5g

Brilliant green 0.00033g

Neutral red 0.025g

Agar 14.0g

pH 7.0 +/- 0.2 at 25oC

Method of preparation - Weigh and suspend 52.0 grams in 1 litre of distilled water. Take to the boil until completely dissolved. Do not autoclave.

Mode of action - This is a selective as well as differential medium used in the isolation of Salmonella and Shigella species. This medium inhibits the growth of gram positives organism and coliforms, due to the presence of bile salts and brilliant green. Colonies of lactose fermenters are red whiles that of non-fermenters are colourless. Organisms that produce hydrogen sulphide show black centres on this medium due to the presence of ferric citrate.

DEOXYCHOLATE CITRATE AGAR (Oxoid, UK)

Media Composition (Gram/Litre)

Peptone 5.0g

Lab-Lemco powder 5.0g

Lactose 10.0g

Sodim Citrate 5.0g

Sodium thiosulphate 5.0g

Ferric citrate 1.0g

Sodium deoxcholate 2.5g

Neutral red 0.025g

Agar 15.0g

pH7.0

Mode of preparation: suspend 48.5g in 1 litre of distilled water. Heat to dissolve completely. Mix well and pour into plates immediately. Do not autoclave

Mode of action: DCA is a selective medium for the isolation of *Salmonella* and Shigella species. Colonies of lactose fermenters are red whiles that of non-fermenters are colourless. Organisms that produce hydrogen sulphide show black centres on this medium due to the presence of ferric citrate.

SELENITE F BROTH (Liofilchem, Italy)

Composition

Tryptone 5.0g

Lactose 4.0g

Sodium Phosphate 10.0g

pH 7.0 +/- 0.2 at 25 ^{o}C

Method of preparation-Weigh 23.0grams of the powder into 1L of distilled water.Heat to dissolve completely. Do not autoclave. Dispense aseptically into sterile bijoux bottles.

Mode of action- This is an enrichment medium used for the overnight growth of faecal material. It promotes the growth of Salmonella and Shigella over the commensals and thus helps in their selective isolation.

STERILE SALINE (PHYSIOLOGICAL SALINE)

Composition

NaCl 8.5g

Distilled water 1 litre

Method of preparation - Dissolve 8.5g NaCL in distilled water. Autoclaved for 15mins at 121°C.Cool to room temperature.

Mode of action - Builds a Neutral environment maintaining the pH and morphology of the organism.

APPENDIX II: BIOCHEMICAL TEST REAGENTS

Triple Sugar Iron Agar (Liofilchem, Italy)

Media Composition (Formula in g/l)

Peptospecial 20.0g

Glucose 1.0g

Lactose 10.0g

Sucrose 10.0g

Sodium chloride 5.0g

Sodium thiosulphate 0.3g

Ferrous sulphate 0.2g

Phenol red 0.025g

Agar 12.0g

pH 7.3 +/- 0.2 at 25 ^{o}C

Method of preparation – Suspend 64.5g in one litre of distilled water and heat to dissolve the medium completely. Dispense into test tubes and sterilize by autoclaving at121 ^{o}C for 15 minutes. Allow to cool in a slanted position such that deep butts are formed.

Mode of action - This is a medium for the differentiation of gram negative enteric bacteria on the basis of carbohydrate fermentation and the production of hydrogen sulphide. Growth of an organism on the TSI slant indicates the type of sugar fermented. Acid production turns the phenol red indicator yellow. Alkaline reaction turns the indicator pinkish-red. Production of hydrogen sulphide is indicated by the formation of a black colour as hydrogen sulphide combines with ferrous ammonium sulphate. Cracks in the medium usually at the butt indicate gas production.

SIMMONS CITRATE AGAR (BIOTEC, UK)

This is a medium used in the differentiation of Enterobacteriaceae.

Media Composition (Formula in g/l)

Magnesium sulphate 0.2g

Ammonium dihydrogen phosphate 1.0g

Dipotassium phosphate 1.0g

Sodium citrate 2.0g

Sodium chloride 5.0g

Bromothymol blue 0.08g

Agar Agar 15.0g

pH 6.9 +/- 0.2

Mode of preparation – Weigh 24g of powder and add to 1 litre of distilled water. Heat to dissolve. Dispense into tubes or bottles then sterilize by autoclaving at 121 ^{o}C for 15 minutes. Allow to set as slopes.

Mode of action - This is based on the organisms ability to utilize citrate as a sole of carbon and monoammonium phosphate as the sole source of nitrogen. Utilization of the citrate causes a change in the pH of the medium resulting in the bromothymol blue colour from its characteristic green colour.

MOTILITY INDOLE UREA MEDIUM BASE (Himedia, India)

Media Composition (Gms / Litre)

Casein enzymic hydrolysate 10.000g

Dextrose 1.000g

Sodium chloride 5.000g

Phenol red 0.010g

Agar 2.000g

Final pH (at 25°C) 6.8±0.2

Mode of preparation: Suspend 18 grams in 950 ml distilled water. Heat to boiling to dissolve the medium completely. Dispense in 95 ml amounts into flasks and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to about 50-55°C and add aseptically 5 ml sterile 40% Urea solution (FD048) per 95 ml basal medium. Mix well and dispense into sterile test tubes. Allow to cool in an upright position.

MIU Medium Base is formulated to detect motility, urease and indole production in single tube. Casein enzymic hydrolysate provides amino acids and other nitrogenous substances. Sodium chloride maintains osmotic equilibrium. Dextrose is fermentable carbohydrate. Phenol red is the pH indicator which turns pink- red in alkaline conditions. The test cultures are stab-inoculated.

Motility and urease reactions are read before testing Indole production. Motile organisms show either diffused growth or turbidity extending away from stab inoculation line while nonmotile organisms grow along the stabline. Organisms that utilize urea, produce

ammonia which makes the medium alkaline, showing pink-red colour by change in the phenol red indicator (1). Indole is produced from tryptophan present in casein enzymic hydrolysate. The indole produced combines with the aldehyde present in the Kovac's reagent to form a red complex.

KOVAC'S REAGENT

Composition

4-Dimthylamino-benzaldehyde 10grams

Iso-amyl alcohol 150ml

Conc. Hydrochloric acid 50ml

Weigh and dissolve 10grams of 4-Dimthylamino-benzaldehyde in 150mls of Iso-amyl alcohol. Add 50mls Conc. Hydrochloric acid after dissolving. Store away from sunlight.

Kovac's reagent is used in detecting the indole compound

APPENDIX III: GRAM STAIN AND REAGENTS

Principle

A smear after heat fixing is stained with crystal violet for 1-2 minutes, followed by the use of a mordant (iodine), decolorizing with acetone alcohol and use of a counter stain (neutral red). The air dried fixed smear of bacteria, when stained with crystal violet picks up the stain and retains its when the mordant is applied hence giving it a purple colour. The mordant iodine enhances the union between the dye and the internal content of the organism. On the other hand acetone alcohol may cause the bacteria to lose the initial dye (crystal violet) and pick up the counter satin- neutral red. This is designated as gram negative.

Preparation of the crystal violet Stain

Crystal violet 20 g

Ammonium 9 g

Ethanol or methanol, absolute 95 ml

Distilled water 1 Litre

Weigh the crystal violet on a piece of clean paper. Transfer to a brown bottle. Add the absolute ethanol and mix until the dye is completely dissolved. Weigh the ammonium oxalate and dissolve in 200 ml of distilled water. Add to the stain. Make up to the 1 litre with distilled water, and mix well.

Preparation of Lugols iodine

Potassium iodide 20 g

Iodine 10 g

Distilled water 1 Litre

Weigh the potassium iodide, and transfer to a brown bottle. Add a quantity of the volume of water, and mix until the potassium iodide is completely dissolved. Weigh the iodine, and add it to the potassium iodide solution. Mix until the iodine is dissolved. Make up to 1 litre with distilled water, and mix well. Label the bottle, and store in a dark place at room temperature.

Preparation of Acetone alcohol

Acetone 500 ml

Ethanol or methanol, absolute475 ml

Distilled water25 ml

Mix the distilled water with the absolute ethanol (ethyl alcohol) .Transfer the solution to a screw-cap bottle. Measure the acetone, and add it to the alcohol solution. Mix well.

Preparation of Neutral red

Neutral red1 g

Distilled water 1 Litre

Weigh the neutral red on a piece of clean paper, and transfer it to a reagent bottle. Add the volume of water, and mix until the dye is completely dissolved. Label the bottle and store at room temperature.

APPENDIX IV: STERILIZATION

Sterilization of glass ware

Allglassware were sterilized by dry heat in a hot air oven, a temperature of 160^oC held for 60 minutes was used, timed from when the items in the oven have reached this temperature.

Glassware was left in the oven to cool to room temperature before use.

Sterilization of metals (loop, wire and forceps)

Decontamination and sterilization was done by flaming until the mentioned metals were red hot with a Bunsen burner flame and allowed to cool before use.

Media storage

Culture media plates were packed in cellophane bags and stored at $2–8^oC$. Media in screw-cap tubes and bottles were also stored in the refrigerator at $2–8\ ^oC$.

Disposal of waste

Blood

Blood in brain heart infusion broth are autoclaved at $121\ ^oC$ for 15mins.this is allowed to cool before carefully pouring the content of the bottles into a waste bucket. After all culture bottles have been emptied, the waste was poured directly into the water closet, avoiding splashes. The water closet was carefully and thoroughly rinsed with water.

Stool

Disposable containers were used for the collection of stool samples. Disposal of the specimens was by incineration at the St. Joseph's Hospital incinerator.

Sharps (needles)

These were discarded into a puncture resistant sharp bin and incinerated at the St. Joseph's Hospital incinerator.

Culture

All cultures were disposed by incineration after the cultures were autoclaved at 121°C for 15 minutes.

APPENDIX V: QUESTIONNAIRE

SALMONELLA CARRIAGE AMONG FOOD HANDLERS AND PATIENTS ATTENDING ST. JOSEPH'S HOSPITAL, JIRAPA

QUESTIONNAIRE FOR FOOD HANDLERS

Study No						ID
Date of interview (00/00/00)						DATE
Time			.			TIME
Age (years)						AGE
Marital status	Married	Single	divorced	widow	separated	MARITAL
Residence						RESIDENCE
occupation	unemployed	Self employed	Public	Other		OCCUP
Education	Basic	Secondary	Tertiary	None		EDU
Religion	Christianity	Islam	Traditional	Other		RELI
What category of food service are you	School Canteen	Public canteen	Chop bars	Street food vendor		FOOD CATEGORY
Are you trained in food safety?	Yes	No				SAFETY
How long have you been a food vendor?						VENDOR
What type of food (s) do you prepare						FOOD TYPE
What role do you play in the food preparation						ROLE
Do you undertake periodic medical screening?						MEDIC

If yes, when was the last time you did a medical screening?						LAST MEDIC
What type of toilet do you use	Water closet	Pit latrine	'free range'		Other	TOILET
Do you wash your hands with soap and water After visiting the toilet			Yes		No	WASH
Do you have pet(s) in your house			Yes		No	PET
If yes, what type of pet(s) do you have	Dog		Cat		Other	PET TYPE
Do you rear farm animal(s)			Yes		No	ANIMAL
What type of farm animal(s) do you rear	Pigs	Cattle	poultry		Other	ANIMAL TYPE
Do you know about the disease called typhoid fever?				Yes	No	TYPHOID
Do you know how typhoid fever is transmitted?				Yes	No	TRANS
Have you had typhoid before?				Yes	No	BTYPHOID
Do you have the following today?						
Fever	Yes			No		FEVER
Headache	Yes			No		HEADACHE
Abdominal pains	Yes			No		APAINS
Diarrhea	Yes			No		DIARR
Constipation	Yes			No		CONSTI
Loss of appetite	Yes			No		APPETITE
Are you on antibiotic drugs	Yes			No		ADRUGS

SALMONELLA CARRIAGE AMONG FOOD HANDLERS AND PATIENTS ATTENDING ST. JOSEPH'S HOSPITAL, JIRAPA

QUESTIONNAIRE FOR PATIENTS

Study No						ID
Date of interview (00/00/00)						DATE
Time			.			TIME
Age (years)						AGE
Marital status	Married	Single	divorced	widow	separated	MARITAL
Residence						RESIDENCE
occupation	unemployed	Self employed	Public	Other		OCCUP
Education	Basic	Secondary	Tertiary	None		EDU
Religion	Christianity	Islam	Traditional	Other		RELI
Where do you usually eat apart from your house?	Canteen	Chop bar	Street food	Other		EAT
Where is it located?						LOC
Do you have pet(s) in your house	Yes		No			PET
If yes, what type of pet(s) do you have	Dog	Cat	Turtle	Other		PET T
Do you rear animals?	Yes		No			ANIR
If yes What type of animal do you rear	Pigs	Cattle	Poultry	Other		ANIP
What type of toilet do you use	Water closet	Pit latrines	Free range	Other		TTOILET

Do you have the following today?			
Fever	Yes	No	FEVER
Headache	Yes	No	HEADACHE
Abdominal pains	Yes	No	APAINS
Diarrhea	Yes	No	DIARR
Nausea	Yes	No	NAUSEA
Chills	Yes	No	CHILLS
Are you on antibiotic drugs	Yes	No	ADRUGS