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DEPARTMENT OF CLINICAL MICROBIOLOGY

PREVALENCE OF*SALMONELLA* INFECTIONS IN PATIENTS ATTENDING ST. DOMINIC HOSPITAL, (AKWATIA)-EASTERN REGION

By

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DECLARATION

I hereby declare that this submission is my own work towards the MPhil 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 husband Mr. Adzakpah Godwin and daughter Miss Nadine Ewoenam Adzakpah.

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I do thank the Almighty God for this great height achieved. I would like to also convey my sincerest gratitude to my project supervisor, Dr. Patrick Feglo who helped in shaping this project. I do appreciate his time slot for me in his busy schedule. This project would not have been possible without the help of Mr. James Owusu, Mr. Samuel Nartey and Mr. Isaac Sarsah all of the laboratory Department of the St. Dominic Hospital and also Hamza Yakubu of Health Information Department of St. Dominic Hospital. I do thank Mr. Mark Ayerteh of Aqua Vitens Rand, Mr. Stephen Ofori of the Koforidua Regional Hospital and especially Mr. Lartey of the Komfo Anokye Teaching Hospital for their immense support.

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ABSTRACT

The St. Dominic hospital in Akwatia reports high *Salmonella* infection with the source of these infections unknown. The prevailing *Salmonellae* species and their antimicrobial susceptibility pattern are not known. It is also not known if the *Salmonellae* there produce Extended Spectrum Beta Lactamase (ESBL). This study therefore investigated the prevalence of *Salmonella serotypes* isolated from both clinical and water samples in Akwatia. Four hundred and sixty four (464) samples comprising 270 Stool and 194 Blood samples were collected from patients attending the St. Dominic Hospital in Akwatia. One hundred and eighty eight (188) water samples were also cultured for *Salmonella*. Prevalence of *Salmonella* was 11.6% (54/464) for the clinical samples tested and 2.7% (5/188) for the water samples. Out of the 54 clinical isolates, 31.4% (16/54) were typhoidal *Salmonellae* while 68.6% (38/54) were non-typhoidal *Salmonellae*. *Salmonella* Typhi recording 20.4% (11/54) was the most frequently encountered typhoidal isolate while *S.* Enterica, 29.6% (16/54) followed by *Salmonella* Typhimurium 24.1% (13/54) were predominant among the non typhoidal isolates. Abdominal pain and fever were the most common patient complaints. The water samples tested had bacterial count of 2.56 $x10^3$-1.2 x 10^{13} per millilitre. *Salmonella* were isolated from 9.4% (5/188) of the water samples collected. The *Salmonella* isolates had high proportions resistant to ampicillin 69.5% (41/59), piperacillin 69.5% (41/59) and Trimethoprim- Sulfamethoxaxole 76.3% (45/59). All the *Salmonella* isolates were, however, susceptible to cefoxitin (100%), cefotaxime (100%), cefepime (100%), Imipenem (100%), meropenem (100%) and amikacin (100%). No isolate was found to be resistant to norfloxacin and ciprofloxacin. Varying resistance patterns were observed with the other antibiotics. No strain was found to produce ESBL. *Salmonella* infection can be acquired from the well water. The use of cephalosporins, quinolones, carbapenems and amikacin are recommended as the drug of choice for both typhoidal and non-typhoidal Salmonellosis.

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LIST OF ABBREVIATIONS

ABNC	-	Active but non-culturable
AFLP	-	Amplified fragment length polymorphism
AST	-	Antimicrobial Susceptibility Tests
CDC	-	Centre for Disease Control
CSN	-	Central Nervous System
ESBLs	-	Extended-spectrum beta-lactamase
GWCL	-	Ghana Water Company Limited
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
USA	-	United States of America
VBNC	-	Viable but non-culturable
WHO	-	World Health Organization

CHAPTER ONE

1.0 INTRODUCTION

Salmonellae are of immense medical importance as intracellular pathogens that cause a variety of diseases (Haraga *et al.*, 2008). *Salmonellae* are commonly found in food-borne outbreaks (Levine *et al*., 2001). They have been recognized as pathogens of both humans and animals for over a century (Alam *et al*., 2005) with their reservoir being the digestive tract of vertebrates and many animals such as chickens, cattle, swine, fish and reptiles (Weill, 2008), as well as wild birds (Tizard, 2004). Some *Salmonella* serovars are host-specific. *Salmonella* Typhi and *Salmonella* Paratyphi *A, B* and *C* are associated with humans (Luxemburger and Dutta, 2005; Whitaker *et al*., 2009) with *Salmonella* Pullorum being associated with chickens whereas *Salmonella* Abortusovis is found in sheep (Pui *et al.*, 2011). Some *Salmonella* serotypes affect wildlife (Chambers and Hulse, 2006) , while others have a broad host range (Pui *et al.*, 2011).

Salmonella are classified into two major groups. These are the invasive and the non-invasive *Salmonella* (Samal and Sahu, 1991). Invasive *Salmonella* cause enteric fevers and are also called typhoidal *Salmonellae* (Samal and Sahu, 1991). Typhoidal *Salmonellae* include *Salmonella* Typhi and Paratyphi. The non–invasive *Salmonella* are termed non-typhoidal *Salmonellae* (NTS) and are made up of *Salmonella* species which usually cause gastroenteritis (Samal and Sahu, 1991). This includes serotypes such as *Salmonella* Typhimurium and *Salmonella* Enteritidis. The different serotypes of *Salmonella* can co-infect an individual or cause infections separately (Samal and Sahu, 1991).

Typhoidal and non-typhoidal *Salmonella* infections are major public health problems in developing countries. The problems, according to many findings, are associated with unhygienic processing and preparation of foods, substandard water supply, inadequate sanitary measures and the emergence of multidrug resistance in some *Salmonella* strains (Bryan, 1988; Addo *et al.*, 2007; Chau *et al.*, 2007; Setti *et al.*, 2009).

Transmission of *Salmonella* is by faecal-oral route, through the consumption of contaminated water or food (WHO, 1996). Consumption of about 10^5 *Salmonella* per gram of food (Jay *et al.*, 2003) and10^4 organisms per litre of water (Burrows and Renner, 1999) can cause infection when ingested. After ingestion, *Salmonella* evades the stomach acidity because they are intracellular. Upon reaching the small intestines *Salmonella* are phagocytosized by macrophages in the intestinal mucosa. However, possession of a Vi antigen help prolong the life of the bacteria, preventing it from being phagocytosed. The bacteria after 10 to 14 days of incubation, invade other sites of the body and this leads to illness (Giannella, 2002). *Salmonella* infections are characterized by fever, weakness, anaemia, body weight loss, abdominal pain, vomiting, and diarrhoea (Samal and Sahu, 1991) or constipation (WHO, 1996) and sometimes maculopapular rash on the skin (Miller and Pegues, 2000).

1.1 EPIDEMIOLOGY

Globally, *Salmonella* cause an estimated 16 million cases of illness each year, resulting in over 600,000 deaths per annum (WHO, 1996), increasing to about 22,000,000 symptomatic infections and 220,000 fatalities worldwide annually (Crump *et al.*, 2004). The annual prevalence in the United States in 2007, was 14.9 per 100,000 populations

(CDC, 2007). Studies in England and Wales estimated 220 human Salmonellosis per 100,000 populations (Wheeler *et al.*, 1999) while in the Netherlands, an estimated 340 cases of Salmonellosis per 100,000 populations was recorded (de Wit *et al.*, 2001a; de Wit *et al.*, 2001b; van Pelt *et al.*, 2003). High prevalence is reported in the African continent but moderate in Mediterranean north Africa including Morocco, Algeria, Tunisia, Libya and Egypt (Ghenghesh *et al.*, 2009). Case fatality rates of *Salmonella* infection in developing countries range between 12% and 32% as compared to less than 2% in industrialized countries (Carmeli and Shapiro, 1993).

1.2 PREVALENCE OF *SALMONELLA*

The highest incidence of *Salmonella* cases worldwide occurs in south East Asian countries, recording 274 cases per 100,000 persons (Brooks *et al*., 2005). In Africa, a population-based incidence of *Salmonella* causing typhoid infections in Egypt was 59 cases per 100,000 persons per year (Uba *et al.*, 2007). A high prevalence of *Salmonella* is also noted in Latin America with 50 cases per 100,000 persons (Brooks *et al*., 2005). The prevalence of *Salmonella* infection in many parts of sub-Saharan Africa is largely unknown. This is attributed to the lack of diagnostic laboratories and fatal *Salmonella* may be attributed to malaria (Evans *et al.*, 2004; Reddy *et al*., 2010).

Clinical and laboratory data coalesced over 16 years (1975 – 1990) from four African countries (Ghana, Zambia, Tanzania, and Kenya) showed that Ghana has a national incidence of one in a thousand, the highest among the countries surveyed (Petit and Wamola, 1994). *Salmonella* infections in Ghana rank among the leading 20 causes of outpatient illness, accounting for 0.92% of hospital admissions (Sory, 2009). Marks *et al*.,

(2010) reported 37 (12.4%) isolates (2.5% of the 1,456 hospitalized children) positive for *Salmonella enterica* serovar Typhi in a study in the Ashanti Region of Ghana (Marks *et al.*, 2010).

1.3 *SALMONELLA* INFECTIONS IN AKWATIA

Salmonella infections are commonly diagnosed at the St. Dominic Hospital in Akwatia. Recent laboratory data indicated that all age groups and gender are affected by the disease as shown in Tables 1.1 and Table 1.2. From 2005 through to 2011 typhoid fever was diagnosed at high levels. There was also high case fatalities recorded for typhoid fever throughout the periods as shown in Table 1.3

Table 1.1: Five year trend of typhoid cases seen at OPD of St. Dominic hospital

Typhoid fever (TYPHOID)	**AGE-GROUP**											
	<1	**1-4**	**5-9**	**10-14**	**15-17**	**18-19**	**20-34**	**35-49**	**50-59**	**60-69**	**70+**	**TOTAL**
2005	11	51	61	36	56	50	151	102	89	49	0	**656**
2006	0	12	37	40	27	23	94	86	43	23	17	**402**
2007	7	38	43	24	48	40	88	79	72	51	27	**517**
2008	1	9	30	28	13	4	95	74	28	17	25	**324**
2009	1	28	88	62	47	138	243	217	82	39	55	**1000**
2010	8	118	142	112	35	41	210	188	65	35	48	**1002**
2011	8	116	164	150	57	37	215	164	67	35	41	**1054**

(Collected from laboratory records books at St. Dominic Hospital, Akwatia)

Table 1.2: Gender distribution of Typhoid cases at St. Dominic hospital, Akwatia

Typhoid fever (TYPHOID)	**MALE**		**FEMALE**		**TOTAL**	
	<5yrs	**>5yrs**	**<5yrs**	**>5yrs**	**<5yrs**	**>5yrs**
2005	35	240	27	354	62	594
2006	6	154	6	236	12	390
2007	14	107	31	365	45	472
2008	1	125	9	189	10	314
2009	18	173	11	798	29	971
2010	62	349	64	527	126	876
2011	64	346	60	584	124	930

(Collected from laboratory records books at St. Dominic Hospital, Akwatia)

Table 1.3: Typhoid case fatality rate (%) from 2005 to 2011 at the St. Dominic's Hospital, Akwatia

Year	**Admissions**	**No. Of deaths**	**Typhoid Case Fatality Rate**
2005	22	1	4.6%
2006	125	0	0.0%
2007	41	1	2.4%
2008	155	4	2.6%
2009	161	2	1.2%
2010	168	2	1.2%
2011	259	1	0.4%

(Collected from laboratory records books at St. Dominic Hospital, Akwatia)

1.4 PROBLEM STATEMENT

Typhoid and paratyphoid infection are common in the Akwatia district affecting both males and females of all ages. The disease may lead to complications and deaths in the district. So far, the species of *Salmonella* causing the infections and the disease burden are not known. Above all the sources of spread of the *Salmonella* in the community are also not known.

1.5 JUSTIFICATION

Human *Salmonella* carriers are a source of human *Salmonella* spread (Alam *et al.*, 2005). They do this through the contamination of food and water they handle. This is because the disease is spread by the faecal-oral route (Feglo *et al.*, 2004). *Salmonella* infections are not only food-borne but also waterborne especially where wastewater is directly discharged into surface water such as lakes, ponds, and rivers contaminating them (Ohno *et al.*, 1997). Infected persons presenting with disease and asymptomatic carriers alike, shed the bacteria in their stool. Through the disposal of human excreta into water bodies, *Salmonella* finds its way into rivers and streams. Rain water also washes human excreta in the environment (from indiscriminate defaecation) into these rivers, streams and low-lying wells. Water from these rivers, streams and wells is used for cleaning and as drinking water, making it one of the most important sources of *Salmonella* infection. Sepage from manholes into wells built in close proximity can also contaminate these wells with *Salmonella* if present, thus causing infections to inhabitants who drink or use them for food preparation. *Salmonella* infections continue to be diagnosed, resulting in

very high statistical figures being generated at the St. Dominic Hospital in Akwatia, thus prompting this investigation. Monitoring of typhoidal and non-typhoidal *Salmonella* infection is important to establish the prevailing serotypes in a community. This study will also establish the emergence and prevalence of antimicrobial resistance as well as detect extended – spectrum beta-lactamase producers. The acquisition of this epidemiological information will aid in effecting change in the treatment patterns of *Salmonella* infections in the near future.

1.6 AIM

To determine if potable water contributes to the source of *Salmonella* infection and the prevalence of the species of *Salmonella* infecting patients reporting to the St. Dominic Hospital, Akwatia, and among the water samples in Akwatia

1.6.1 Objective

1. To determine the prevalence of *Salmonella* species in stool and blood samples of patients attending St. Dominic Hospital.
2. To determine the prevalence of *Salmonella* species in potable water samples in the community.
3. To determine the antimicrobial sensitivity pattern and ESBL-production of *Salmonella* isolates obtained.
4. To determine if there is an association among the antibiotic sensitivity pattern and ESBL-production and demographic characteristics of patients and environmental samples.

CHAPTER TWO

LITERATURE REVIEW

2.1 THE ORGANISM *SALMONELLA*

2.1.1 History

Salmonellae have been the cause of enteric diseases for over a century. D. E. Salmon and T. Smith isolated *Salmonella* Choleraesuis from pigs diagnosed with hog cholera in 1880. The genus was named in honour of D. E. Salmon in 1890 (Ziprin, 1994).

2.1.2 The genus Salmonella

Salmonella is a Gram-negative bacterium (Davos, 2005). In 2005, *Salmonella enterica* was approved as the type species of the genus *Salmonella*. The genus also includes the species *Salmonella bongori* (Su and Chiu, 2007). *Salmonella subterranean*, was recognized in 2005 as a strain isolated from subsurface sediments contaminated with nitrate and having a low-pH (Tindall *et al.*, 2005; Su and Chiu, 2007).

2.1.3 Nomenclature and Classification

Salmonella species are named in accordance with the Kaufmann-White typing system. The Kaufmann-White typing system classifies *Salmonella* strains into serovars on the basis of the extensive diversity of antigens possessed by the bacteria (Giannella, 2002; Grimont and Weill, 2007). These are the O, H and Vi antigens (Giannella, 2002; Grimont and Weill, 2007). The Kaufmann-White typing system further divides *Salmonella enterica* into six subspecies. These are *Salmonella enterica* subspecies *Enterica*, *Salmonella enterica* subspecies *Salamae*, *Salmonella enterica* subspecies *Arizonae*, *Salmonella* enterica subspecies *Diarizonae*, *Salmonella* enterica subspecies *Houtenae* and

Salmonella enterica subspecies *Indica* (Brenner *et al.*, 2000). The first letter of the serovars name is a capital letter and the name is not italicized to indicate that they are not separate species. *Salmonella Typhi* and *Salmonella Paratyphi* then becomes *Salmonella enterica subsp. Enterica (I) serovars* Typhi *and* Paratyphi (Chengappa *et al.*, 1993). The use of the genus and serotype name alone, for example, *Salmonella* Typhi, is accepted (Brenner *et al.*, 2000). The subspecies of species *Salmonella e*nterica can be differentiated through biochemical means and through genomic relatedness (Brenner *et al.*, 2000). The serotype of *Salmonella Bongori* retained the symbol V. *Salmonella Bongori* has only one subspecies known as subspecies V(Chengappa *et al.*, 1993).The Kaufmann-White typing system has over the years classified over 2500 distinct types of *Salmonella* (Brenner *et al.*, 2000).

2.1.4 Description of Salmonella species

Salmonella are short plump shaped rods (Freeman, 1985) 2-5 micrometres long and 0.8-1.5 micrometres wide (Lightfoot, 2004). The organisms are facultative anaerobes, as they have both respiratory and fermentative metabolism. With the exception of *Salmonella* Typhi, *Salmonella* are non-capsulate (Cheesbrough, 2000); non –spore forming and non-lactose fermenting (Varnam and Evans, 1991). Most *Salmonella* species are motile by flagella (Giannella, 2002). Most of the serovars produce hydrogen sulphide in an optimal growth temperature of 35 to 37^{o}C (Hohmann, 2001).

2.1.4.1 Antigenic composition of Salmonella bacteria

Salmonella have three antigens: O antigen, H antigen, and Vi antigen (Giannella, 2002). The O antigen is located in the cell wall of the bacterium, on the surface of the outer membrane. The O antigen is also referred to as the somatic antigen (Giannella, 2002). H antigen is a flagella antigen. The H antigen can be damaged by heat (Giannella, 2002). The Vi antigen is a superficial antigen, which lies over the O antigen on the surface of the bacterium (Giannella, 2002). The presence of the Vi antigens makes the bacteria more virulent than others without it. Some *Salmonella* species with the Vi antigen are *Salmonella* Typhi, *Salmonella* Paratyphi C and *Salmonella* Dublin (Giannella, 2002). Over 40 secreted virulence factors have been identified in *Salmonella* (Haraga *et al.*, 2008; McGhie *et al.*, 2009). The virulence plasmid of *Salmonella* is important for bacterial multiplication in the reticuloendothelial system of warm-blooded vertebrates (Guiney *et al.*, 1994).

2.2 PATHOGENESIS OF *SALMONELLA* INFECTIONS

2.2.1 Infective Dose

Salmonella infection begins from the transmission of the *Salmonella* bacterium to a susceptible host. This is usually through the consumption of contaminated food or water (Wannissorn, 2001). About 10^4 bacterial cells per gram or per litre of water are needed to cause infection (Raffatellu *et al.*, 2006). However, low gastric acidity, which is common in elderly persons and among individuals who use antacids, may reduce the infective dose to 10^3 cells (Raffatellu *et al.*, 2006). Vaccination against S*almonella* can increase the infective dose of an individual to 10^9cells (Raffatellu *et al.*, 2006). The inoculum required

for infection varies from strain to strain and physiological wellbeing of the host (Wannissorn, 2001).

2.2.2 Incubation period

Non-typhoidal *Salmonella* infections are usually acute presenting with symptoms within 18-48 hours after ingestion of the bacterium. The duration of infection varies but generally may last from 2-5 days (Pui *et al.*, 2011). Typhoidal infections present with a mild onset and usually long incubation periods. These generally range from 7-14 days for typhoid fever and for paratyphoid fever 1-10 days (Pui *et al.*, 2011).

2.2.3 Pathology of Salmonella infections

The ingested *Salmonella* species are engulfed by phagocytic cells. The bacteria are transported into the ileum and colon where they invade the intestinal epithelium (Parry *et al.*, 2002; Raffatellu *et al.*, 2006). Multiplication of the bacteria, however, occurs in the intestinal epithelium and lymphoid follicles (Parry *et al.*, 2002; Raffatellu *et al.*, 2006). Macrophages and intestinal epithelial cells then draw T cells and neutrophils and interleukin 8 (IL-8), causing inflammation which suppress the infection (Parry *et al.*, 2002; Raffatellu *et al.*, 2006).

Some *Salmonella* species have specialized fimbriae that enable them adhere to the epithelium over the Peyers patches (clusters of lymphoid tissue) in the ileum (Parry *et al.*, 2002). Others have a Vi capsular antigen that masks pathogen-associated molecular patterns (PAMPs) and thus avoid neutrophils-based inflammation (Parry *et al.*, 2002).

The bacteria then cause their host macrophages to attract more macrophages (Raffatellu *et al.*, 2006). *Salmonella* invades the macrophages taking up the macrophages' cellular activity for its own reproduction. Large numbers of *Salmonella* are then released. To spread systematically, the bacteria travel by the systemic circulation and lymph to the reticuloendothelial tissues of the liver, spleen, bone marrow, and lymph nodes (Parry *et al.*, 2002). *Salmonella*, however, continues to multiply until an adequate density is reached after which, the bacteria breaks out into the bloodstream to invade other parts of the body including the gall bladder (Parry *et al.*, 2002). The organism re-enters the gastrointestinal tract reinfecting the Peyers patches. The bacteria are then shed in the stool (Parry *et al.*, 2002).

2.3 CLINICAL FORMS OF *SALMONELLA* INFECTION IN HUMANS

Salmonella has four disease patterns: gastroenteritis, enteric fever (Typhoid and Paratyphoid Fever), bacteraemia/septicaemia with or without intestinal infection, and a carrier state (Pui *et al.*, 2011). Gastroenteritis is a *Salmonella* infection caused by at least 150 *Salmonella* serotypes. *Salmonella* Enteritidis is the most common serotype. The major symptom is diarrhoea. Sometimes bloody diarrhoea, fever and abdominal pain can also be observed. After recovery, the *Salmonella* bacteria can be found in stool samples for up to 12 weeks (Pui *et al.*, 2011).

Typhoid fever and paratyphoid fever are jointly termed ‘enteric fevers’, and present with similar symptoms and febrile systemic illness (Levine *et al.*, 2001; Parry, 2004;Luxemburger and Dutta, 2005; Whitaker *et al.*, 2009). These infections are also termed systemic *Salmonella* infections (Levine *et al.*, 2001; Parry, 2004). Severity of

symptoms depends on the causative agent. Enteric fever caused by *Salmonella* Typhi is more deadly than that of paratyphoid fever. Symptoms for typhoid fever include prolonged fever, diarrhoea and abdominal pain. *Salmonella* infections may damage internal organs such as the liver, spleen, respiratory and neurological system (Pui *et al.*, 2011). Intestinal perforation or haemorrhages are observed in 0.5% to 1% of cases, especially in non-treated or inappropriately-treated cases (WHO, 1996). Arthritis, septicaemia (fatal in hosts with impaired defences as in the aged and immune-suppressed) (Dhanoa and Fatt, 2009) and urinary tract infections are also caused by typhoidal *Salmonella* (Jones *et al.*, 2008). Enteric fever is endemic in less developed countries where poor sanitation, poor food hygiene and reduced access to treated water facilitate the spread of the causative organism (Crump and Mintz, 2010).

Not all serotypes of this bacteria cause enteric fever. Some *Salmonellae* cause non-typhoidal Salmonellosis (Chengappa *et al.*, 1993). *Salmonella* species causing non-typhoidal diseases are common among enteric bacterial pathogens (Li *et al.*, 2005). Non-typhoidal *Salmonella* causes infections which are often self-limiting, but they can cause severe infections such as bacteraemia, meningitis and urinary tract infections (Parry, 2003; Jones *et al.*, 2008). Salmonellosis presents with diarrhoea, headache, abdominal cramps, fever, nausea and vomiting (Pui *et al.*, 2011).

Common symptoms associated with *Salmonella* bacteraemia include chills, high fever and anorexia. The bacterium may localize in any organ in the body and produce focal lesions resulting in complications including meningitis, pneumonia, endocarditis, vascular infections, cholecystitis, hepatic and splenic abscesses, urinary tract infections,

pneumonia or empyema, meningitis, septic arthritis, osteomyelitis and CNS infections (Hohmann, 2001; Percival *et al.*, 2004). In pregnancy salmonellosis may result in abortion and foetal death, transplacental infection of the foetus (especially in the infection of *Salmonella* Typhi) and maternal death (Carroll and Williams, 2008). Untreated typhoid infections can develop into chronic carrier state. These chronic carriers can spread Salmonella infections (Pui *et al.*, 2011).

A chronic carrier state exists for both typhoidal and non-typhoidal salmonellosis. About 3% of typhoidal infections and 0.1% of non-typhoidal infections become chronic carriers (Giannella, 2002). This condition may last from weeks to years. A chronic carrier state can be found in both humans and animals (Giannella, 2002).

2.4 TRANSMISSION OF *SALMONELLA* SPECIES

2.4.1 Routes or Vehicles of Human Infection

The transmission of *Salmonella* species takes the oral-faecal route, by means of ingesting contaminated food and water (Abulreesh *et al.*, 2007). Risk factors for salmonellosis include reduced gastric acidity, recent use or abuse of antibiotics, extremes of age, immunosuppressive conditions (Crum-Cianflone, 2008) and intestinal schistosomiasis (Gendrel *et al.*, 1994).

Almost any type of food product could serve as a source for infection (Crum-Cianflone, 2008). Powdered infant formula was implicated in two consecutive large outbreaks of *Salmonella enterica* serotype Agona among infants in France (Brouard *et al.*, 2007). *Salmonella* Typhimurium, believed to be carried in pork, has made more than 1000 people ill in Denmark from 1980 to 2000 (Ethelberg *et al.*, 2008). *Salmonella*

Typhimurium present in municipal tap water affected over 400 people in Colorado in the United States (Berg, 2008).

Person-to-person spread of *Salmonellae* is the main mode of infection in outbreaks in day care centres and hospitals and other institutions, particularly where unhygienic conditions persist (Swanson *et al.*, 2007). Sexual transmission of *Salmonella* infection through oral or anal intercourse is probable (Luxemburger and Dutta, 2005). The sexual transmission of typhoid fever among nine homosexual men who had relations with one asymptomatic *Salmonella* Typhi carrier in 2000 was noted by Reller *et al.*, (2003).

Direct contact with infected animals also serve as a source for *Salmonella* infections (Tauxe, 1991). A study of 28 cases of *Salmonella* Typhimurium pointed pet rodents as a source of human *Salmonella* infection (Swanson *et al.*, 2007).

2.5 PREVALENCE AND CASES OF *SALMONELLA*

2.5.1 Non – typhoidal cases

Non-typhoidal *Salmonellae* have been recognized as a leading cause of bacterial enteritis in the UK and worldwide (Pui *et al.*, 2011). *Salmonella* infections in Japan are often associated with boxed lunches, school lunch, and cakes and were all said to have been caused by *S.* Enteritidis (Denny *et al.*, 2008).

In the United States the prevalence of *Salmonella* infections has been stable since 2004 (Voetsch *et al.*, 2004). The annual burden of non-typhoidal *Salmonella* infection in the United States is considered to be 520 cases per 100,000 people compared with 13.4

laboratory-confirmed cases per 100,000 people per year. This indicates an estimate of 38.6 cases of non-typhoidal *Salmonella* infection (Voetsch *et al.*, 2004).

Africa, Asia and Latin America reports of *Salmonella* Typhimurium in 10–13% of outbreaks making it the most common isolate in these areas (Denny *et al.*, 2008). NTS were the most common isolate from blood culture in Ghanaian children with persistent fever (Commey *et al.*, 1994).

2.5.2 Typhoidal cases

Salmonella Paratyphi A is said to account for a growing proportion of enteric fever in the United States of America (Gupta *et al.*, 2008). From 2005 to 2006, 149 paratyphoid fever cases were confirmed in the United States. Most of the patients had recently travelled to South Asia (Gupta *et al.*, 2008). The prevalence of paratyphoid fevers had increased in the past few decades in some parts of South and Southeast Asia as well (Gupta *et al.*, 2008). Paratyphoid fevers account for 50% of *Salmonella* isolates obtained from blood cultures among patients with enteric fever (Ochiai *et al.*, 2005; Woods *et al.*, 2006).

Typhoid fever is endemic in Asia, Africa, Latin America, the Caribbean and Oceania but 80% of cases come from Bangledesh, China, India, Indonesia, Laos, Nepal, Pakistan or Vietnam due to poor sanitary conditions (Chau *et al.*, 2007). Typhoid fever is usually associated with travel to developing countries (currently recording 72% of approximately 400 cases per year) (Linam and Gerber, 2007).

Salmonella Typhi, most virulent of the *Salmonella* species, is associated with waterborne outbreaks in developing countries, with one in Nepal in 2002 affecting nearly 6000 people (Lewis *et al.*, 2005). Outbreaks of typhoid fever are frequently reported from sub-

Saharan Africa, often with a sizable number of patients presenting with intestinal perforations (Muyembe-Tamfum *et al.*, 2009).

A total of 151 bacterial isolates recovered from 442 septicaemic children in the University College Hospital., Ibadan, yielded a prevalence of 12.6% and 3.9% for *Salmonella* Typhi and *Salmonella* Paratyphi respectively (Ogunleye *et al.*, 2005).

A study in Ghana identified 212 bacterial isolates from blood cultures out of which *Salmonella enterica* was 100 (69.0%). *Salmonella enterica serovar* Typhi accounted for 59 (40.7%) (Uwe Groß *et al.*, 2011). Another study in Ghana in 2009, resulted in 48 (48.5%) pathogenic bacteria out of 99 blood cultures (Uwe Groß *et al.*, 2011). In that study, *Salmonella enterica* was found in 50% (24/48) of all pathogen-positive blood cultures with *Salmonella enterica serovar* Typhi remaining the most prevalent species (Uwe Groß *et al.*, 2011). Prevalence of chronic typhoidal *Salmonella* carriers among food vendors in Kumasi, Ghana was 2.3% (six out of 258). These six were equally split among non-typhoidal *Salmonella* and typhoidal *Salmonella* (Feglo *et al.*, 2004).

2.5.3 Age and gender-specific prevalence of Salmonella

The prevalence of non-typhoidal *Salmonella* is usually highest in infants and young children (Olsen *et al.*, 2001). Among infants and young children in the United States, the prevalence is usually higher in boys than in girls (Olsen *et al.*, 2001). *Salmonella* Typhi infection is also noted in preschool children or children 5–19 years old in endemic areas (Olsen *et al.*, 2001). Ogunleye *et al.*, (2005), showed that *Salmonella* septicaemia was high (56%) among children of age 5-11 years followed by 1-5years and below group (36%) in Ibadan, Nigeria.

The prevalence of *Salmonella* in women in the United States of America was estimated as 10.2 per 100 000 and that of men as 8.8 per 100 000 from 1987 to 1997 (Olsen *et al.*, 2001). The prevalence in males was found to be higher than that of females among the elderly. However, a higher prevalence is observed among middle-aged women than their male counterparts in the United states (Olsen *et al.*, 2001).

The biological or epidemiological basis for the age- and gender-specific prevalence of salmonellosis is not clear (Mølbak, 2005). The true prevalence among very young children and infants may be higher (Dutta *et al.*, 2001). Clinical presentations in these age groups may be atypical and thus a *Salmonella* infection may be misdiagnosed (Dutta *et al.*, 2001). Adults according to Olsen *et al.* (2001) have immunity usually from previous exposures.

2.6 *SALMONELLAE* AND THE ENVIRONMENT

Pathogenic microorganisms and their presence in the environment are attributed to several factors including contamination through water, soil, food processing equipments, food contact surfaces and most importantly food handlers (Shamsuddeen and Ameh, 2008; Kawo and Abdulmumin, 2009).

2.6.1 Salmonellae in the aquatic environments

Bacterial contamination of surface water has over the years been a water quality issue because of their ability to transmit disease (Miller and Pegues, 2000). Although salmonellosis traditionally was thought of as an animal-originated food-borne disease (Clark *et al.*, 1996), recent outbreaks resulting from water confirm the ability of water to transmit the disease to humans (Mohle-Boetani *et al.*, 2002). *Salmonella* species have

been found in sewage, freshwater, marine coastal water, groundwater (Baudart *et al.*, 2000) and natural water (Martinez-Urtaza *et al.*, 2004) suggesting that *Salmonella* infection can be acquired from aquatic sources (Baudart *et al.*, 2000; Olsen *et al.*, 2001). The species of *Salmonella* in aquatic environments could depend on sources of contamination (Setti *et al.*, 2009). Survival of the different *Salmonella* serotypes can be affected by the climatic conditions and the multiplicity and dynamics of *Salmonella* serotypes in aquatic environments (Simental and Martinez-Urtaza, 2008; Haley *et al.*, 2009).

Salmonella is also viable for a longer period of time than many enteric bacteria in freshwaters (Baudart *et al.*, 2000). This suggests that water may be a stable environment for these bacteria, thus heightening the threat of *Salmonella* infections from water sources (Baudart *et al.*, 2000). Water is therefore being increasingly investigated as a potentially significant reservoir for *Salmonella* transmission and spread (Schutze *et al.*, 1999).

Achieving the World Health Organization's Millennium Development Goal number seven – to 'halve the proportion of people without secure access to safe drinking water and adequate sanitation by 2015' (Clasen *et al.*, 2007) seems slow, but would make major impact toward the control of the global burden of enteric fever (Lynch *et al.*, 2009; Whitaker *et al.*, 2009).

In Ghana, the majority of consumers of water get water supply from sources other than from the Ghana Water Company Limited (GWCL). The danger associated with water obtained from sources other than the GWCL is not well established (Obiri *et al.*, 2003).

The most reliable but expensive source of drinking water is bottled water. It is of good bacteriological quality but only accessible to the rich in society (Obiri *et al.*, 2003).

A study by Addo *et al*., 2009 in Accra, Ghana, found Five (16.7 %) out of the total of 30 samples being excellent, 5 (16.7%) being satisfactory, 9 (30%) were suspicious and 11 (36.7%) were unsatisfactory using the MPN values. The quality of the water samples was assessed based on the World Health Organization (WHO) classification system for drinking water.

Although there is scarcity of documented data on the prevalence of water-borne diseases involving the consumption of "pure" water, it has been widely observed that with its arrival, the numbers of cases of salmonellosis have significantly increased in recent years (Addo *et al.*, 2009).

2.7 SEASONAL TRENDS OF SALMONELLOSIS

In Italy the isolation of *Salmonella* species from polluted rivers was easily done during summer than autumn (Pianetti *et al.*, 1998) as was found in the Salmon River during the warm seasons (spring and summer) in British Columbia (Jokinen *et al.*, 2010). In Spain, however, the isolation of *Salmonella* serovars was higher in colder months (October-December) than in warmer months (July-September) (Martinez-Urtaza *et al.*, 2004).

Seasonal trends of non - typhoidal salmonellosis (Enteritidis) in humans in East Asia and (Typhimurium) in India was found to be highest in colder months (November-December) (Ekdahl *et al.*, 2005) while the highest peak of non – typhoidal *Salmonellae* infection was recorded during the summer (June-August) in Korea (Cho *et al.*, 2008).

A seasonal trend in the frequency of *Salmonella* infections in Ghana was reported by Marbell et al in 1974. The infections were said to peak in July coinciding with the peak of rainfall seasons in the country.

2.8 MORTALITY/MORBIDITY OF *SALMONELLA* INFECTION

Salmonellosis is sometimes fatal. About 15,000 cases in the U.S. require hospitalization every year and over 400 deaths occur (Voetsch *et al.*, 2004). Bacteraemia worsens the prognosis (Hohmann, 2001). In the United States, in the pre-antibiotic times recorded 9%-13% case fatality (Crump *et al.*, 2008). About 80% of deaths caused by *Salmonella* infection are found in Africa (Onyango *et al.*, 2009).

2.9 LABORATORY DIAGNOSIS OF *SALMONELLA* INFECTION

The early stage of *Salmonella* infection is often difficult to diagnose. Definitive diagnosis of enteric fever depends on the isolation of *Salmonella* from blood, stool, urine, bone marrow, bile or other body fluids (Geddes, 1974).

Conventional culture method for the isolation of *Salmonella* is widely used. This involves pre-enrichment, selective enrichment and selective plating followed by biochemical or serological confirmatory tests of suspected colonies (Varnam and Evans, 1991).

Blood cultures are the standard diagnostic method. Blood cultures requires a large volume of blood to be cultured (15 ml in adults). Blood cultures are positive in 60 to 80 percent of patients with typhoid (Gilman, 1975; Vallenas *et al.*, 1985; Hoffman *et al.*,

1986; Wain *et al.*, 2001). Culture of bone marrow is a more sensitive diagnostic tool for *Salmonella* infections (Gilman, 1975; Vallenas *et al.*, 1985; Hoffman *et al.*, 1986; Wain *et al.*, 2001). The result is positive in 80 to 95 percent of patients with typhoid. Patients who have been taking antibiotics for several days, irrespective of the duration of illness do show positive test results (Gilman, 1975; Vallenas *et al.*, 1985; Hoffman *et al.*, 1986; Wain *et al.*, 2001).

Cultures have also been made from the buffy coat of blood (Rubin *et al.*, 1990), streptokinase-treated blood clots, (Hoffman *et al.*, 1986) and skin snips of rose spots (Gilman *et al.*, 1975). The sensitivity of stool culture depends on the amount of faeces cultured, and the positivity of stool cultures increases with the duration of the illness (Gilman, 1975; Vallenas *et al.*, 1985; Hoffman *et al.*, 1986; Wain *et al.*, 2001). Stool cultures are positive in 30 percent of patients with acute typhoid fever (Gilman, 1975; Vallenas *et al.*, 1985; Hoffman *et al.*, 1986; Wain *et al.*, 2001).

Laboratory typing methods, both phenotypic and molecular methods for the differentiating of the *Salmonella* strains and for tracing the routes of *Salmonella* dissemination are available. This is needed due to the limitation of biochemical test analysis in performing such functions (Riley, 2004).

Phenotypic methods include serotyping (Popoff *et al.*, 1993; Davos, 2005) and Phage typing (Hald *et al.*, 2007). Molecular methods are based on genotypic information and includes Pulsed-field gel electrophoresis (PFGE) (Hu *et al.*, 2002), Polymerase chain reaction (PCR) such as Multiplex PCR (Maurer., 2006), Real time PCR (Higuchi *et al.*, 1993) and Isothermal PCR (Jung *et al.*, 2010). Others are plasmid profiling, ribotyping,

insertion sequence (IS) typing, RAPD (randomly amplified polymorphic DNA), AFLP (amplified fragment length polymorphism) and MLST (multilocus sequence typing) (Bender *et al.*, 2001; Pavlov *et al.*, 2004).

2.9.1 Culture and isolation of Salmonella species

2.9.1.1 Samples useful for the isolation of Salmonella

Blood, urine and faeces /rectal swab are suitable specimen for the isolation of *Salmonella* species (Murray *et al.*, 1999). Food and water may be sent for culture in the event of suspected outbreaks. Aspirates and body fluids from sterile sites may also be used for the culture and isolation of *Salmonella* species if infection is suspected to be disseminated (Murray *et al.*, 1999).

2.9.1.2 Transportation and storage

Samples may be transported to the laboratory immediately. Samples can be stored at 4° C if transportation will take longer than 2 hours. Samples can equally be stored at 4° C for up to 24 hours before culture (Murray *et al.*, 1999). Rectal swabs in modified Stuart's transport medium, can be stored at room temperature for 24 hours (Murray *et al.*, 1999).

2.9.1.3 Pre-enrichment

To reduce the chances of obtaining false negative results, a non-selective pre-enrichment of faeces, food and water sample is performed (Gracias and McKillip, 2004). Pre-enrichment medium (usually non-selective) such as alkaline peptone water is used. (Gracias and McKillip, 2004).

2.9.1.4 Culture media

Many selective agar media are available for *Salmonella* isolation. These include MacConkey agar, Hektoen enteric agar or Xylose Lysine Deoxycholate (XLD) agar, Bismuth sulphite agar, Mannitol Lysine Crystal Violet Brilliant Green agar, Deoxycholate-citrate agar and *Salmonella-Shigella* agar (Hohmann, 2001).

Newer and more-selective chromogenic agars based on biochemical characteristics of *Salmonella* are available. The media may contain inhibitors in order to stop or delay the growth of non-target organisms (Manafi, 2000). Such biochemical characteristics include beta-galactosidase activity (Perry and Freydiere, 2007; Schonenbrucher *et al.*, 2008). In the absence of beta-galactosidase activity, C8-esterase activity, catabolism of glucuronate, glycerol and propylene glycol, hydrolysis of X-5-Gal, and H_2S production are also available and useful (Perry and Freydiere, 2007; Schonenbrucher *et al.*, 2008). Some of these media however, have not yet made their way into routine clinical use (Perry and Freydiere, 2007; Schonenbrucher *et al.*, 2008).

After overnight (18-24 hours) incubation, *Salmonella* colonies on MacConkey Agar (MAC) produce colourless (lactose-negative) colonies, 2-4 mm in diameter. *Salmonella* typically produce clear colonies with distinct black centres (hydrogen sulphide: H_2S) on Hektoen Enteric agar (Hohmann, 2001). Colonies of *Salmonella* serovar Typhi are clear with pinpoint black centres and colonies of *Salmonella* ser. Paratyphi *A* are clear. Paratyphi *A* does not produce H_2S on Xylose Lysine Deoxycholate Agar (XLD). A

Salmonella colony has a slightly transparent zone of reddish colour and a black centre on a Brilliant Green Agar plate (Hohmann, 2001).

2.9.1.4.1 Non-culturable Salmonella species

The viable but nonculturable (VBNC) state of enteric bacteria means the bacteria continue its basal metabolic activities yet do not grow on artificial media (Oliver, 2010). This state is to improve the chances of survival of enteric bacteria against environmental factors affecting their survival (Barer and Harwood, 1999). Factors for the possible VBNC state in *Salmonella* may include exposure to elevated temperatures, U. V. irradiation, salinity, exposure to antibiotics, chlorination (Oliver, 2005) and a decrease in the source of nutrient (Roszak *et al.*, 1984).

Resuscitation of VBNC *Salmonella* cells may be possible (Dhiaf *et al.*, 2010), but the cells may lose their virulence or ability to cause infection (Caro *et al.*, 1999). *Salmonella* Typhimurium in VBNC forms and active but nonculturable (ABNC) were unable to infect laboratory animals (Smith *et al.*, 2002).

2.9.2 *Isolation of Salmonella from water*

Tests for coliform bacteria are the most important routine microbiological examinations carried out on drinking water (Barrow and Feltham, 1993). These tests provide a sensitive means for detecting faecal contamination of water. They are also for assessing water quality, the effectiveness of water treatment and for monitoring of the quality water (Barrow and Feltham, 1993).

One of the oldest methods of water quality assessment is called the multiple tube method, also known as the most probable number technique (Barrow and Feltham, 1993). Bacteria

are cultured in a liquid pre-enrichment medium. This is followed by confirmation tests for acid production. In this test, measured volumes of sample, or dilution of sample, are added to a series of tubes or bottles containing liquid differential medium. The number of positive bottles are counted and used to determine the most probable number of organisms in the sample using appropriate probability tables. Confirmation of organism in the positive tubes is by sub-culturing the content of the tubes on appropriate media (Barrow and Feltham, 1993).

An Adenosine Triphosphate Test is a rapid method measuring active microorganisms in water through detection of Adenosine Triphosphate, or ATP (Barrow and Feltham, 1993). Pour plates is another method used when looking for bacterial species that grow poorly in air (Barrow and Feltham, 1993). The total number of colonies found is referred to as the Total Viable Count (TVC) and measured in Cfu/ml or colony forming units per millilitre (Barrow and Feltham, 1993).

The plate count method has the bacteria growing on the surface of an artificial nutrient so that the colony becomes visible to the eye and can be counted (Barrow and Feltham, 1993). A serial dilution of the original sample is done to achieve average counts between 30 and 300 colonies of bacterium. Less than 30 colonies makes the interpretation statistically unsound whilst greater than 300 colonies often results in overlapping colonies and imprecision in the count. It, however, requires the culture of several dilutions (Barrow and Feltham, 1993).

Membrane filtration or concentration method requires that the water samples to be filtered through membrane filters and these filters are themselves laid on nutrient medium within sealed plates (Barrow and Feltham, 1993). Membranes have a millimetre grid printed on them and can be reliably used to count the number of colonies under a binocular microscope (Barrow and Feltham, 1993).

2.9.3 *Biochemical differentiation of Salmonellae*

The characteristics of the *Salmonella* bacterium used for biochemical differentiation and confirmation of the different species are the fermentation of D-glucose with the production of acid and usually gas (Le Minor, 1984). Other carbohydrates usually fermented are L-arabinose, maltose, D-mannitol, D-mannose, L-rhamnose, D-sorbitol (except ssp VI), trehalose, D-xylose and dulcitol (Le Minor, 1984).

Commonly used biochemical tests used to differentiate Salmonella from other members of the family Enterobacteriaceae are listed in Table 2.1. The biochemical differentiation of the species and subspecies are enlisted in Table 2.2.

Table 2.1: Biochemical differentiation of *Salmonella* from other Enterobacteria

	Salmonella	*Shiegella*	*Citrobacter*	*Edwardsiella*
β-galactosidase	-[2]	+ / -	+	-
Arginine dihydrolase	+ / -	-	+ / -	-
Lysine decarboxylase	+	-	-	+
Ornthine decarboxylase	+	-	+ / -	+
Simmon's citrate	+	-	+	-
H_2S production	+	-	- / +	+
Acid from				
Lactose	-[2]	-[4]	+ / -	-
Dulcitol	+ / -	-	- / +	-
Melibiose	+	- / +	-	-
Sorbitol	+	- / +	+	-
Xylose	+	-	+	-
Motility	+	-	+	+

[1] Reactions for *Salmonella* are based on those of ubiquitous serovars of importance in food poisoning. Reactions of *Salmonella* Typhi and other host adapted serovars may differ.

[2] *S. enterica* subsp. *Arizonae* and strains of some other serovars are positive.

[3] *Shigella sonnei* is positive.

[4] Delayed fermentation is a feature of some strains of *Shigella sonnei*

Source: Varnam and Evans (1991)

Table 2.2: Biochemical differentiation of *Salmonella* Species and Sub-Species

Species	*S. enterica*						*S. bongori*
Subspecies	*enterica*	*salamae*	*arizonae*	*diarizonae*	*houtenae*	*indica*	
Characters							
Dulcitol	+	+	-	-	-	**d**	+
ONPG (2 h)	-	-	+	+	-	**d**	+
Malonate	-	+	+	+	-	-	-
Gelatinase	-	+	+	+	+	+	-
Sorbitol	+	+	+	+	+	-	+
Culture with KCN	-	-	-	-	+	-	+
L(+)-tartrate(a)	+	-	-	-	-	-	-
Galacturonate	-	+	-	+	+	+	+
ɣ-glutamytransferase	+ **(*)**	+	-	+	+	+	+
β-glucuronidase	**d**	**d**	-	+	-	**d**	-
Mucate	+	+	+	- **(70%)**	-	+	+
Salicine	-	-	-	-	+	-	-
Lactose	-	-	- **(75%)**	+ **(75%)**	-	**d**	-
Lysis by phage O1	+	+	-	+	-	+	**d**
Usual habitat	Warm-blooded animals		Cold-blooded animals and environment				

(a) = *d*-tartrate.
(*) - Typhimurium d, Dublin -.
+ = 90% or more positive reactions.
- = 90% or more negative reactions.
d = different reactions given by different serovars

Source: Popoff and Le Minor (1997)

2.9.4 Rapid methods for diagnosing Salmonella

Advances in immunology, molecular biology, biotechnology and engineering have given rise to new age of technologies for the isolation of *Salmonella* (Cox and Fleet, 1998). These technologies are fast, automated, cost effective and reliable (Feng, 1992). They involve the use of miniature biochemical tests, new media formulations, DNA probe and antibody dependent assays (Feng, 1992).

Some of the new technologies include, Polyclonal enzyme immunoassay screening method, motility enrichment on modified semi-solid Rappaport-Vassiliadis (MSRV) medium, Fluorogenic and colorimetric monoclonal immunoassay (Q-Trol) screening method, Biochemical identification Kit method (for example API 20E) and the Biochemical Systems identification (Vitek GNI) screening method (Andrews *et al.*, 1998).

The Vitek 2 Technology is one of such rapid methods developed by Biomérieux (France). The Vitek 2 compact system is an automated analyser for the identification of bacteria, yeast and for the susceptibility testing of medically important bacteria. It entails the analysis, data management programmes, an automated results output system and a quality control set up which validates the Vitek 2 compact system. It also makes use of specifically produced reagent cards. A required test suspension is manually prepared and paired with a gram specific test card such as the Vitek 2 gram negative identification cards (Pincus, 2006).

There is pressure differences generated by means of a vacuum pump that causes the suspension to be forced into the wells of the test cards trough transfer tubes. The test card undergoes incubation and reading cycles' at an average temperature of 35.5^{o}C (Pincus, 2006).

The Vitek 2 compact performs identification and susceptibility analysis by continuously monitoring the growth and activity of organisms inoculated into its test card micro wells. The light emitting diodes of the optical framework uses visible light to measure the growth of inoculated organism after an initial reading is performed. The measurement of

growth of the organism is repeated every 15 minutes by how much light is prevented from passing through the micro wells. This is captured on a silicon photo detector. The results are automatically transmitted to the computer interface of the laboratory information system (Pincus, 2006).

2.9.4.1 *Identification of Salmonella Species using Vitek 2 Compact*

The Vitek 2 Gram-Negative identification card (GN) is used for the identification of clinically-significant fermenting and non-fermenting gram- negative bacilli. The GN card is based on standardized biochemical methods and newly developed substrates measuring carbon source utilization, enzymatic activities and resistance (Pincus, 2006). Identification of an organism is based on the characteristics of the data and knowledge about the organism and resulting reactions being analysed. The software compares the test set of reactions to the expected set of reactions of the organism or organism group. A quantitative percent probability value is calculated and relates to how well the observed reactions compares to the typical reactions of each organism as seen in the table below. This forms part of the identification process (Pincus, 2006).

There are 47 biochemical tests and one negative control well results appear as +,-, or ? (Pincus, 2006). In this test, a sterile swab stick is used to transfer a sufficient number of morphologically similar colonies of the isolates into a 3.0ml of sterile saline (aqueous 0.45% NaCl) of pH 4.5, preferably in a clear plastic test tube. The homogenous organism suspension with a density equivalent to McFarland No.0.50 using a calibrated VITEK^{R} 2 DensiCHEK ^{TM} and less than 30minutes old is placed into the cassette together with the GN card. The user manual instructions are followed to initiate the test.

Table 2.3: Identification Card Qualifying Messages

ID MESSAGE CONFIDENCE LEVEL	CHOICES	PERCENTAGE PROBABILTY	COMMENTS
EXCELLENT	1	96-99	
VERY GOOD	1	93-95	
GOOD	1	89-92	
ACCEPTABLE	1	85-88	
LOW DISCRIMINATION	2 TO 3	SUM OF CHOICES = 100;AFTER RESOLUTION	2 TO 3 TAXA EXHIBIT SAME BIOPATTERN
INCONCLUSIVE/ UNIDENTIFIED ORG	>3 OR 0	N/A	>3 EXHIBIT SAME PATTERN, VERY ATYPICAL BIOPATTERN

Source: (bioMérieux VITEK, 2004)

2.10 Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing is necessary for the administration of appropriate treatment to patients (Atlas, 1995). Many methods exist and include the disk diffusion, broth dilution and agar dilution susceptibility tests (Atlas, 1995).

The disk diffusion method is performed on solid culture medium inoculated with bacteria. Disks impregnated with a specific antimicrobial agent of known concentration are also used. The antibiotics in the disks diffuse into the medium creating a concentration gradient that could be measured in a clear zone around the antibiotic disk (Atlas, 1995). This zone indicates the inhibition of growth of the organism and establishes the organism as either sensitive or resistant to the antibiotics used (Finegold *et al.*, 1978).

Advancements in the performance of the antimicrobial susceptibility tests are also available (Feng, 1992) and include the use of Biochemical identification Kit method (for example API 20E) and the Biochemical Systems identification (Vitek GNI) screening method (Andrews *et al.*, 1998).

2.10.1 Antimicrobial Susceptibility Testing using the Vitek 2 Compact

A Vitek 2 Compact Automated analyzer involves a set of miniaturized version of the double dilution technique of Minimum Inhibitory Concentration (MIC) determination using the micro dilution method. The Antimicrobial Susceptibility Tests (AST) card is of 64 micro wells, one of which is the control well. The control well contains only microbiological culture media. The remaining wells contain culture media impregnated with premeasured quantities of antimicrobials.

The organism suspension to be tested must be diluted to a standardized concentration in 0.45% saline before being used to rehydrate the antimicrobial medium within the test card. The card is then filled, covered, and placed in the instrument reader manually (as with VITEK 2 Compact). The instrument monitors the growth of each well in the card over a defined period of time (up to 18 hours for bacteria). At the completion of the incubation cycle, MIC values (or test results) are determined for each antimicrobial contained on the card.

2.11 ANTIBIOTICS FOR THE TREATMENT OF *SALMONELLA*

Salmonella infection was formerly treated effectively with ampicillin, cotrimoxazole and other common antibiotics but due to the abuse and over use of these antibiotics, the organisms have become resistant to these (Akinyemi *et al.*, 2000). Antibiotics of choice

for the treatment of *Salmonella* infections now are the cephalosporins and fluoroquinolones (Rotimi *et al*., 2008). *Salmonella* bacteraemia is treated with a single bactericidal drug for 10-14 days (Hohmann, 2001). Life-threatening *Salmonella* infections may be treated with both a third-generation cephalosporin and a fluoroquinolones until the susceptibilities of antimicrobial agents are known (Hohmann, 2001). Recently, third-generation cephalosporins have been used in regions with high fluoroquinolones resistance (Capoor *et al*., 2007). Kundu *et al.* (2006) recommended cefixime as a second-line agent and azithromycin for empirical treatment of uncomplicated typhoid fever. Complicated typhoid fever however, attracts the use of ceftriaxone. Aztreonam and imipenem are second-line antibiotics for complicated cases. Involvement of the Central Nervous System (CNS) requires high-dose ceftriaxone as the recommended drug of choice for effective penetration of the blood-brain barrier (Hohmann, 2001). Early treatment of *Salmonella* infections with appropriate antibiotics reduces the effect of the disease and improves the chances of survival of the infected persons (Lynch *et al.*, 2009).

Out of 4200 *Salmonella* Typhi isolates, 408 (9.7%) were resistant to all the first-line anti-typhoid drugs (chloramphenicol, ampicillin and trimethoprim-sulfamethoxaxole) and were labelled as Multi drug resistant (MDR) *Salmonella* Typhi in a study in Pakistan (Mahmood *et al.*, 2012).

Antibiogram of isolates in a study by Nagshetty et al., (2010) in India revealed that all the isolates of *Salmonella* Typhi were sensitive to Imipenem. Ampicillin (29.47%) and chloramphenicol (28.42%) were among the antibiotics with high resistance. In this same

study, Multi drug resistant (MDR) isolates were mainly resistant to three antibiotics: ampicillin, chloramphenicol and cotrimoxazole. Among the multidrug resistant isolates were 28 ampicillin-resistant *Salmonella* Typhi isolates and 27 chloramphenicol- resistant *Salmonella* Typhi.

In a study by Abdullahi et al., (2012) in Funtua, Nigeria *Salmonella* Typhi and *Salmonella* Paratyphi A isolates obtained were all resistant to ampicillin; (93.8%) and 66.7% were resistant to chloramphenicol, while proportions of *S.* Typhi and *S.* Paratyphi A, resistant to cotrimoxazole were 37.5% and 66.7%, respectively. No resistance of the strains to fluoroquinolones (ciprofloxacin and ofloxacin) was observed. Resistance to ampicillin by *Salmonella* Enteritidis and *Salmonella* Typhimurium (non-typhoidal *Salmonellae*) were 57.1% and 100% respectively. Non-typhoidal *Salmonellae* were not observed to show any resistance against these fluoroquinolones.

A study on the antimicrobial susceptibility pattern of *Salmonella* isolate by Ogunleye et al., (2005) in Ibadan, Nigeria showed 100% sensitivity of *Salmonella* Typhi and *Salmonella* Paratyphi to ceftriaxone with varied sensitivity/resistance to gentamicin, ofloxacin, ceftazidime, Augmentin, pefloxacin, chloramphenicol, amoxicillin, cotrimoxazole.

Boni-Cissé et al., (2012), in Abidjan, Cote d'Ivoire, found the resistance patterns of *Salmonella* isolates to be 74.2% to amoxicillin and 58.1% to amoxicillin-clavulanic acid. Five (5) or 8.1% of strains were resistant to cefotaxime. Most of the strains were resistant to chloramphenicol, tetracycline and cotrimoxazole. Multi Drug Resistance was estimated to be 30%. Further, the resistance to ciprofloxacin was 14%. The proportion of

Salmonella Typhi resistant to both amoxicillin and cotrimoxazole was 66.6% while resistance to chloramphenicol was 50%. The distribution of multi drug resistant strains was as follows: 33.3% for *Salmonella* Typhi; 34.1% for non-typhoidal *Salmonella*; 28.6% for *Salmonella* Typhimurium; 30.8% for *Salmonella* Enteritidis.

Setti et al., (2009) in Agadir, Morocco found out that about 50% of the *Salmonella* isolates obtained in their study showed resistance to at least one antibiotic. Overall, 22 of the isolates were resistant to ampicillin (38.6%), 2 isolates were resistant to sulphonamide compound (3.5%), and 1 was resistant to tetracycline (1.7%). A study by Mermin et al., (1999) in Tajikistan found 29 *Salmonella* Typhi isolates out of which 27 (93%) were resistant to many antibiotics including ampicillin, tetracycline, and trimethoprim-sulfamethoxaxole but these 27 (93%) were susceptible to amoxicillin-clavulanic acid, ceftriaxone, ciprofloxacin and gentamicin. One isolate (3%) was resistant to Sulfamethoxaxole and streptomycin only, and 1 isolate (3%) was sensitive to all agents (Mermin *et al.*, 1999).

Oubrim et al (2012) worked on 144 samples in Settat and Soualem, Morocco and found a total of 42 strains of *Salmonella*. Six different serovars of *Salmonella* species were identified. *Salmonella* Typhimurium accounted for 57.14% of the isolates. *Salmonella* Hadar was identified as 21.42% of the isolates, 7.14% were *Salmonella* Senftenberg and 4.76% were found to be *Salmonella* Newport, *Salmonella* Give and *Salmonella* Anatum respectively. Only serotypes *Salmonella* Typhimurium, *Salmonella* Hadar and *Salmonella* Senftenberg showed resistance to two or more antibiotics. None of the isolates from the other serovars (*Salmonella* Give, *Salmonella* Newport and *Salmonella*

Anatum) were resistant to any of the 14 antibiotics tested. All *Salmonella* isolates were susceptible to amikacin, Cephalothin, ceftazidime, cefotaxime, gentamicin, Imipenem, Sulphonamides and ciprofloxacin (Oubrim *et al.*, 2012).

A study in Ghana indicated that 30 (52%) out of 58 confirmed isolates of *Salmonella* Typhi were multi drug resistant (Mills-Robertson *et al.*, 2002). They also found 10 of the 58 isolates to be resistant to the three first line antibiotics: ampicillin, chloramphenicol and cotrimoxazole (Mills-Robertson *et al.*, 2002).

The resistance to well-known and trusted antimicrobial agents is recognized as one of the greatest challenges that physicians face in the management of infections (Mølbak, 2005). Antimicrobial resistance in disease-causing bacteria, especially in enteric bacteria is a major public health issue attributable to the overuse of antibiotics in food-producing animals, mass treatment and long-term administration of antimicrobial growth promoters, excessive use of antibiotics in treatment "selective pressure" and transferable resistance genes, in response to environmental conditions (Mølbak, 2005; D'Aoust and Maurer, 2007).

Antibiotic-resistant bacteria are not found in human isolates only but also in strains isolated from various aquatic environments (Cernat *et al.*, 2002; Shehabi *et al.*, 2006). These isolates have been detected in the faeces of diseased and apparently healthy livestock and poultry (Ahmed *et al.*, 2009), food (dairy products, meat, poultry products) (Dallal *et al.*, 2010), free-living wild animals and birds (Abulreesh, 2011), domesticated animals (Ebani *et al.*, 2005), natural waters (fresh and marine) (Harakeh *et al.*, 2006),

sewage effluents and sludge (Espigares *et al.*, 2006) and from diarrhoeal patients (Graziani *et al.*, 2008) worldwide.

2.12 *SALMONELLA* AND EXTENDED SPECTRUM BETA LACTAMASE

Bacterial resistance arising through the production of extended-spectrum beta-lactamase (ESBLs) have been recognized as a worldwide therapeutic problem (Bush, 2008; Canton *et al.*, 2008). Enzymes are easily transmitted among members of enterobacteriaceae, thus helping in the spread of resistance not only to β lactams but also to other commonly used antibiotics such as quinolones and aminoglycosides (Kocagoz *et al.*, 2006). ESBL-producing *Salmonella* isolates are less common than other Enterobacteria (Kocagoz *et al.*, 2006).

Mahmood *et al.*, (2012) found ESBL production in suspected isolates in Pakistan showing reduced susceptibility to ceftriaxone, ceftazidime or cefotaxime and were tested for ESBL production. Only three isolates (one in each year from 2006-2008) out of 408 (0.7%) were found to be ESBL producers. No incidence of ESBL production was observed in 2009. Boni-Cissé *et al.*, (2012) also reports no strain producing an extended spectrum beta lactamase (ESBL) in Cote d'Ivoire.

2.13 PREVENTION AND CONTROL OF *SALMONELLA* SPECIES

2.13.1 Vaccine measures

The use of typhoid vaccines to reduce the susceptibility of hosts to infection is another way of controlling typhoid infections. There are 2 vaccines available: The Ty21a vaccine is a live, attenuated, oral vaccine containing the *S.* Typhi strain Ty21a. Ty21a is available

as enteric capsules. In the United States, it is licensed for use in children 6 years of age (Crump and Mintz, 2010).

The parenteral Vi vaccine is based on the *S.*Typhi Vi antigen. It is a vaccine is licensed in the United States for children aged 2 years. The vaccine has been found most effective in young children with proven protection of unvaccinated neighbors. A limitation in the use of the Vi vaccine is that, it is unlikely to provide protection against paratyphoid fever since the *Salmonellae* species involved lack the Vi antigen. There are currently no licensed vaccines against *S.* Paratyphi (Crump and Mintz, 2010).

However, recently developed Vi conjugate vaccine has been shown to have a greater than 90% protective efficacy in children of 2-5 years in Vietnam over a period of at least 27 months post immunization (Bhan *et al.*, 2005). The Vi-rEPA, includes Vi antigen bound to a non-toxic recombinant protein. The vaccine is currently being evaluated (Crump and Mintz, 2010).

Recommendations of mass vaccination in endemic areas, travellers, antimicrobial resistant areas along with creation of public awareness, improvement of sanitation and planned water with improvement of personal hygiene have been suggested (Parry, 2004; Bhan *et al.*, 2005).

2.13.2 Non-vaccine measures

Enteric fever prevention involves improved sanitation, ensuring the safety of food and water supplies and the identification and treatment of chronic carriers of *S.* Typhi (Crump and Mintz, 2010).

Salmonellae contaminated water and food are major vehicles for transmission of typhoid fever (WHO, 1996). Enteric fever which was endemic in Western Europe and North America decreased with the introduction of treatment of municipal water, pasteurization of dairy products, and the exclusion of human feces from food production (Clasen *et al.*, 2007). Extending the benefits of improved sanitation and the availability of safe water and food to low and middle-income countries may achieve similar results (Clasen *et al.*, 2007).

Many a research suggests that improving water quality can significantly reduce diarrhea. Although this proposition has not been tested with enteric fever, it is likely that interventions that reduce diarrheal diseases transmitted through contaminated water, food, and poor hygiene would have similar effects on enteric fever (Crump and Mintz, 2010).

Identification and treatment of *S.* Typhi carriers, particularly those involved with food production is an important strategy for the control of typhoid fever (Feglo *et al.*, 2004). This approach may be useful in low-prevalence settings. Although carriers can be identified by serial culture of stool specimens, this approach is labor intensive (Crump and Mintz, 2010).

CHAPTER THREE

METHODOLOGY

3.1 THE STUDY SITE

This study was conducted at Akwatia in the Kwaebibirem District of the Eastern Region of Ghana. The Kwaebibirem District, with a land size of 1230 km^2, is located on the South-Western portions of the Eastern region. The District lies in the west semi-equatorial climatic zone with bi-modal rainfall around 1120.3 mm that supports plant growth. The average temperature ranges between about 26.5^oC in July and 37^oC in January (Annual Report, 2011).

The district is bounded to the north by Atiwa District, to the North-West by Birim North District, to the East by East Akim Municipality, to the South-East by West Akim Municipality, to the South-West by Birim Central Municipality and to the West by Akyimansa District. The district has many rivers namely Birim, Kadepon, Pram, Subinso, Emo and Abaam (Annual Report, 2011).

According to the 2010 census as released by the Ghana Statistical Service, the district has a population of 192,562 (a population density of 154/km^2) residing in the 159 communities (320 settlements) including notable towns like Kade, Akwatia, Okumaning, Boadua and Asuom. Other localities include Takorase, Akim Wenchi, Pramkese, Nkwantanang, Kusi, Abaam, Apinamang, Topremang, Otumi, Takyiman, Kwae, Adankrono, Tweapease, Abodom and Number 4. It is estimated that the population is growing at 2.1 percent per annum (Annual Report, 2011).

Table 3.1: Breakdown of sub-district population in 2010

Sub- district	**Population**
KADE	30,695
OKUMANING	11,201
AKWATIA	26,960
APINAMANG	24,162
PRAMKESE	24,865
ABAAM	10,679
ASUOM	27,257
TAKORASE	21,233
OTUMI	15,511
TOTAL	**192,562**

Source: *(Annual Report, 2011)*

Agriculture is the predominant occupation in the district. Crops like cocoa, oil palm, citrus, maize and rice are cultivated here. Other crops cultivated are coffee and rubber as well as food crops like cocoyam, cassava and plantain. Sugarcane and vegetables are also cultivated. Agriculture in the district is on subsistence level, and very few farmers engage in plantation-farming (Annual Report, 2011).

Akwatia in the Kwaebibirem District of the Eastern Region of Ghana is about 11 km from Kade, the district capital and 96km from Koforidua, the Regional Capital. The inhabitants usually are farmers. Others engage in small-scale mining and petty-trading. There are also artisans, government and company workers and hawkers (Annual Report, 2011).

Sources of water for both drinking and domestic purposes of the people of Akwatia include rivers, stand pipes, sachet water ("pure" water) and predominantly well water. The inhabitants engage in indiscriminate defecation at Akwatia. It is also common to see

over-flowing rubbish bins and racked-up gutters with liquid waste running along rain-made gulleys (Annual Report, 2011).

Small-scale mining operations are common in the district. This has brought along with it environmental degradation and water pollution as well as silting of rivers in the district. During heavy rains, these rivers flood into homes and farmlands. These flood waters collect debris from farm lands and sewage from homes and deposit them into the aquatic environments. Water from these sources may be used by the inhabitants for domestic chores and for drinking.

3.2 APPROVAL FOR THE STUDY

The study was approved by the ethical committee of KNUST and the St. Dominic Hospital.

3.3 INCLUSION CRITERIA

- Patients attending St. Dominic hospital presenting with abdominal pain and fever.

3.4 EXCLUSION CRITERIA

- Patients attending the St. Dominic hospital several times for the same condition were not included in the study.
- Patients refusing to participate in the study were excluded.
- Patients already on antibiotics were not included in the study.

3.5 SAMPLES FOR THE STUDY

3.5.1 Patients Sample

Out-patients and In- patients reporting with fever, abdominal pains and diarrhoea who were referred to the laboratory for blood or stool culture or both were selected. Patients who had intestinal perforations and had their blood and/or stool samples sent to the laboratory for culture were also selected. These patients were identified by the documented complaints registered in their patient folders.

The study population was made up of patients of St. Dominic. In all 464 patients Samples were collected. Patient samples comprised 194 blood samples and 270 Stool samples both for bacteriological culture.

3.5.2 Water Samples

188 water samples were tested in the study. Samples of potable water comprised communal wells, home-owned wells, standpipes, ponds, rivers, and sachet water.

3.6 COLLECTION OF WATER SAMPLES

Water samples were collected in 50ml sterile glass bottles from selected water sites (ponds, well, river water and stand pipes) in the study area. The bottles were autoclaved at 121^{o}C for 15minutes before use. They were then wrapped in brown paper and secured with an autoclave tape. The receptacles were dried at 160^{o}C for 120 minutes in hot air oven. The water receptacles were allowed to cool to room temperature before being used to collect the water samples. These receptacles were packed into an ice chest with no ice in it and sent to the sites for sample collection.

To collect well water, fixed drawing vessel for fetching from the wells were used. It was lowered into the well while holding on to its attached rope. The drawing vessel gets filled with water under its weight and holding on to the rope it was pulled out of the well. Some of the water in the drawing vessel was poured directly into the sterile receptacle and the receptacle capped tightly. The sample was labelled, placed in an ice chest with ice and transported to the laboratory for analysis.

One edge of the sachet water bag was sterilised with 70% ethanol and cut open with a sterile pair of scissors. The pair of scissors was wiped with sterile gauze containing 70% ethanol. Some of the water was poured directly into the sterile glass receptacles and capped tightly. The tap water was fetched directly into the sterile receptacles. River and pond water were fetched while holding on to the cord attached to the neck of the sterile receptacles. The receptacles get filled with water under its weight. The receptacle is drawn out when filled and tightly capped. The samples were labelled, placed in an ice chest with ice and transported to the laboratory for analysis.

3.7 ANALYSIS OF WATER SAMPLE

3.7.1 Determination of total viable count

For quantitative analysis, water samples were serially diluted. To prepare serial dilutions, 5 test tubes were labelled 1-5 and arranged in a test tube rack. Each of the test tubes was filled with nine millilitres of buffered peptone water.

Using an automatic pipette, 1ml of the water sample was added to the test tube labelled 1. After manually shaking the tube labelled 1 to mix its content; 1ml of it was taken and

added to the tube labelled 2. Tube 2 was also shaken manually and 1ml of its content taken and added to the tube labelled 3, and so on until the last tube was serially diluted to obtain a dilution of 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} respectively. 0.1ml of each tubes' content was transferred into sterile plate count agar (PCA) using automatic pipette. A sterile glass rod was used to spread the inoculum on the agar surface. This was immediately incubated at 37^{o}C overnight.

3.7.2 Incubation of culture plates

All cultures plates were inverted and incubated at 37^{o}C under aerobic conditions overnight after which the culture plates were observed for bacterial growth.

3.7.3 Bacterial Count

The culture plates with bacterial growth were examined. Plates that had between 30-300 colonies growing on them were selected for the determination of colony forming units per millilitre. Bacterial counts were made using a scientific colony counter (Stuart Scientific Colony Counter, United Kingdom). The number of CFU/ml was calculated by multiplying the number of colonies counted by the dilution factor. Bacterial counts were expressed as log of colony forming unit per millilitre of water using formula: $(C|V) \times DF$

Where:

C is the number of colonies counted

V is the volume (0.1ml) of water dispensed onto each of the dried plate count agar medium.

DF is the dilution factor.

All discrete colonies were counted and expressed as colony forming units /millilitre (Cfu/ml of water samples).

3.7.4 Determination of Salmonella in water samples

The concentration method was used for the determination of *Salmonella* in the water samples (Dufour *et al.*, 2003). The method was chosen to help improve the chances of obtaining the *Salmonella* organisms from the volume of water samples collected. The rest of the water samples (after the dilution- 49ml) were filtered through a sterile Whatman filter paper and sterile glass funnel. After the filtration, the Whatman filter paper was picked with a sterile forceps and placed into 40ml of 5% Selenite F broth. The bottle was shaken to remove the bacteria off the filter paper into the broth media and incubated at 37^{o}C overnight. After incubation the broth medium was sub-cultured onto *Salmonella-Shigella* agar (Liofilchem, Italy) and incubated at 37^{o}C overnight.

Bacterial colonies that grew were picked and sub-cultured on Nutrient agar (Oxoid, England) to obtain pure culture. Pure isolates prepared on Nutrient agar were used for biochemical tests to identify the *Salmonella*. If *Salmonella* was not identified the test ended and culture plates were discarded.

3.8 COLLECTION AND ANALYSIS OF BLOOD SAMPLES

To collect blood, the site for venepuncture was disinfected with 70% ethanol. A tourniquet was applied to the upper arm and venous blood was drawn into brain heart infusion broth. Sterile syringes were used in drawing the blood samples. The caps of the culture bottles were disinfected with 70% ethanol. A fresh sterile needle was used to

puncture the cap of the culture bottles to dispense the blood into the brain heart infusion broth. The blood was gently mixed with the broth.

Blood culture samples were labelled and incubated aerobically at 37^{o}c for 7 days but were examined daily for signs of bacterial growth. Subcultures were made on Blood agar (Liofilchem, Italy) and MacConkey agar (Liofilchem, Italy) every other day for seven days. The subcultures on MacConkey agar (Liofilchem, Italy) and Blood agar (Liofilchem, Italy) were incubated aerobically at 37^{o}C. Bacterial growths with colonies of 2-3mm in diameter and pale non-lactose fermenting colonies of 2-4mm in diameter were suspected as *Salmonella* but required full identification.

3.9 COLLECTION AND ANALYSIS OF STOOL SAMPLES

Stool samples were received from patients of the St. Dominic hospital for culture. Patients were counselled on the collection and handling of the stool specimen to avoid contaminating of the samples especially with urine. All stool specimens were collected by patients themselves or by their guardians in the case of children. The specimens were collected into sterile, clean wide-necked receptacles, labelled with patient's details. The specimens were collected in the morning when the patient first woke up from sleep. The samples were transported to the laboratory for culture and were given appropriate laboratory numbers. Portions of the stool samples were inoculated into 5% Selenite F broth. A loop full was cultured on *Salmonella-Shigella* agar. The plates were incubated aerobically at 37^{o}C for 18-24 hours. A subculture of the Selenite F broth was made onto *Salmonella-Shigella* agar the following day and was incubated aerobically at 37^{o}C.

3.10 BACTERIAL IDENTIFICATION

Bacterial identification was done using the colonial morphology of the bacteria on the culture media, H_2S production or otherwise (where applicable), preparation of smear from discrete colonies for gram stain which was then followed by biochemical tests (Indole, Triple Sugar Iron, citrate and urea).

3.10.1 Gram staining technique

Gram stain was performed to classify the bacteria as gram negative. A drop of distilled water was placed on a slide. A colony of bacteria suspected to be *Salmonellae* was emulsified in the distilled water to make a smear on the slide. The smear was allowed to dry and fixed by passing the slide over a Bunsen flame. Crystal violet stain, Lugol's iodine, Acetone and Neutral red were used to stain the slide. The slide was drained of excess water and air dried. The slide was examined with 100 xs in oil immersion under a light microscope for gram negative rods, characteristic of *Salmonella* (Reagent details can be found in Appendix I).

3.10.2 Biochemical testing

Suspected *Salmonella* colonies were sub-cultured on Nutrient agar. This was to obtain a pure culture of the *Salmonella* isolates for biochemical tests. In the biochemical tests, a straight wire was sterilized with a flame of a Bunsen burner and allowed to cool. The sterile wire was used to pick up bacterial isolates by touching the top of 2-3 identical colonies and inoculating them in the test reagent. The biochemical tests performed included Indole test, Triple sugar iron agar test, Citrate utilization test, Urea test and motility test.

3.10.2.1 Indole test

This was done by inoculating 2-3 colonies of *Salmonella* into sterile peptone water (Liofilchem, Italy) with a sterile straight wire and incubated at 37^{o}C overnight. A few drops of Kovacs' reagent were then added to the culture using a Pasteur pipette. A red layer settled on the top of the culture indicating a positive test. If no red layer formed, then the test was considered negative. Colonies of E.coli were used as a positive control and colonies of Pseudomonas aeruginosa used as negative control.

3.10.2.2 Triple sugar iron agar (Liofilchem, Italy)

A sterile straight wire was used to pick up 2-3 colonies of suspected *Salmonella.* This was carefully inoculated into a tube of triple sugar iron agar avoiding the walls of the tubes. The butt of the media was first inoculated and a continuing streak made on the surface of the slant after the straight wire was withdrawn from the butt of the media. The test tube was tightly capped and incubated at 37^{o}C overnight. *Salmonella* usually shows an alkaline slant with an acid butt. Gas production is expressed with some strains (Le Minor, 1984). H_2S production to varying degrees may also be expressed depending on the species of *Salmonella* isolated (Hohmann, 2001). The different reactions observed with *Salmonella* species can be found in Appendix 1. *Proteus vulgaris* was used as positive control organisms and *Pseudomonas aeruginosa* as negative control.

3.10.2.3 Citrate test (Biotec, United Kingdom)

This was done by inoculating 2-3 colonies of suspected *Salmonella* colonies into sterile citrate agar with a sterile straight wire and incubated at 37^{o}C overnight. A positive citrate test results in a blue colour at the slant while a negative test, shows no colour change of

the agar, retaining its characteristic green colour. Klebsiella pneumoniae was used as a positive control while E.coli was for negative control. (See Appendix I)

3.10.2.4 Urease test (Liofilchem, Italy)

This was done by inoculating 2-3 colonies of suspected *Salmonella* into sterile urea agar with a sterile straight wire and incubated at 37^{o}C overnight. A positive test results in a pink-red coloration and alkalinity of the media. A negative test leaves the media yellow–orange in colour. Proteus vulgaris was used for positive control and E.coli used as negative control.

The Indole test together with the urease test helps differentiate between *Salmonella* and Proteus species, *Salmonella* are characteristically negative to both Indole and urease test (Cheesbrough, 2000).

3.10.3 Motility test

This test was done to show if the organism was motile. *Salmonella* are usually motile by means of flagella. In this test, a sterile straight wire was used to pick up 2-3 colonies of the organism. The isolate was inoculated into peptone water and incubated for about 8 hours. A drop of this culture was placed on a microscope slide and a cover slip placed over. The set –up was observed under the microscope and observed with x40 objective lens for motility.

3.11 APPLICATION OF VITEK SYSTEM FOR THE IDENTIFICATION OF *SALMONELLA*

In this test, 3.0 ml of sterile saline of concentration 0.45 and pH of 4.5 was aseptically transferred into a clear plastic test tube. A sterile swab was used to transfer 2-3 colonies of the *Salmonella* bacterium into the saline. This was manually mixed to prepare a homogenous solution. The density of this suspension was adjusted to 0.5 McFarland standard with a VITEK 2 Densi CHEK^{TM}. The suspension was placed in the VITEK 2 Gram-Negative identification card (GN). Appropriate information such as the specimen identification number was keyed into the computer user interface. The VITEK 2 Gram-Negative identification cards (GN) were loaded into the analyzer and the test initiated.

The Vitek 2 compact performs identification and susceptibility analysis by continuously monitoring the growth and activity of organisms inoculated into its test card micro wells. The light emitting diodes of the optical framework uses visible light to measure the growth of inoculated organism after an initial reading is performed. The measurement of growth of the organism is repeated every 15minutes by how much light is prevented from passing through the micro wells. This is captured on a silicon photo detector. The results are automatically transmitted to the computer interface of the laboratory information system.

3.12 APPLICATION OF VITEK SYSTEM FOR ANTIMICROBIAL SUSCEPTIBILITY OF ISOLATES

In this test, 2-3 colonies of *Salmonella* were picked from a pure plate (Nutrient Agar) and emulsified in 3 ml of sterile saline. This was the initial dilution. The initial dilution was adjusted to a density of 0.5 McFarland standards with a Vitek 2 DensiCHEK. A second

dilution was made by pipetting 145ul of the initial suspension into another 3.0ml saline. This suspension was placed in the test cassette together with a VITEK 2 Antimicrobial Susceptibility Tests (AST) card and test initiated. The light emitting diodes of the optical framework uses visible light to measure the growth of inoculated organism after an initial reading is performed. The measurement of growth of the organism is repeated every 15minutes by how much light is prevented from passing through the micro wells. This is captured on a silicon photo detector. The results are automatically transmitted to the computer interface of the laboratory information system. Antimicrobials included ampicillin, amoxicillin-clavulanic, piperacillin, piperacillin-tazobactam, cefazolin, cefoxitin, cefotaxime, ceftazidime, ceftriaxone, cefuroxime, cefepime, imipenem, meropenem, amikacin, gentamicin, ciprofloxacin, norfloxacin, tetracycline, nitrofurantoin and trimethoprim-sulfamethoxaxole.

3.13 TESTING *SALMONELLA* ISOLATES FOR ESBL

All *Salmonella* strains were tested for ESBL production. This was performed on Mueller-Hinton agar by the disc diffusion technique (Rodriues *et al.*, 2004). The ESBLs were detected by first screening the isolates and then confirming them for ESBL production.

3.13.1 Screening for ESBL producers

In this test, three colonies were inoculated into a tube containing peptone water. The turbidity of the inoculum was adjusted to 0.5 McFarland standards. A sterile cotton swab was dipped into the inoculum to seed the Muller-Hinton agar. The swab was pressed firmly on the inside wall of the tube above the fluid level and rotated to remove excess inoculum from the swab. The surface of the Mueller-Hinton Agar plate was inoculated by

running the swab on the surface of the Agar. Sterile forceps was used to place the antibiotic discs onto the Mueller-Hinton Agar surface. A disc of amoxicillin (20ug) plus clavulanic acid (10ug) (Augmentin 30µg) was placed in the centre of the inoculated agar plate. Antibiotic disc of ceftazidime (30µg), ceftriaxone (30µg) and cefotaxime (30µg) were placed around the Augmentin disc with a sterile forceps. They were the test antibiotics. The discs were placed 20mm apart from each other. This plate was covered and incubated overnight at 37°C.

After overnight incubation the zones of inhibition were measured with vernier calliper in millimetres (mm). Enhancement of the zone of inhibition of any one of the test antibiotics towards the Augmentin disc was regarded as a probable ESBL production. This was done by visual observation. ESBL-producing Klebsiella pneumoniae was used as a positive control while non-ESBL producing E. coli served as a negative control.

3.13.2. Phenotypic confirmatory test for ESBL producers

A Muller Hinton agar was inoculated with a suspension of *Salmonella* isolate in 0.85% sodium chloride. The suspension was made to attain a turbidity of 0.5 McFarland before it was used. An antibiotic disc each of cefpodoxime (10µg) and amoxicillin plus clavulanic acid (Augmentin, 30µg) were placed on the surface of the inoculated medium with a sterile forceps. The antibiotics were placed 20mm apart from each other. This plate was incubated at 37^{o}C overnight. An enhanced zone of inhibition of the cefpodoxime disc towards the Augmentin (30µg) disc was considered as positive and noted as confirmed ESBL-producing organisms (Babu *et al.*, 2010).

3.14 STORAGE OF ISOLATES

Colonies from a pure culture *Salmonella* isolates were also stored in 50% glycerol broth in duplicates. This was done by inoculating 2-3colonies of *Salmonella* into sterile 50% glycerol broth with a sterile straight wire. These were stored in the freezer at -80^{o}C.

3.15 QUALITY CONTROL

Every batch of the media prepared was checked for sterility after 24 hours of preparation. This was done by incubating a freshly prepared media plate in an incubator at 37^{o}C overnight. The media was observed for the signs of bacterial contamination including colour change and gas production in media.

Pure plates of both positive and negative control organisms were prepared from already stored isolates. ESBL-producing Klebsiella pneumoniae was obtained from the Okomfo Anokye Teaching Hospital and used as a positive control in testing for ESBLs and a non-ESBL producing E.Coli used as a negative control. E.coli, Proteus vulgaris, Pseudomonas aeruginosa were obtained from the St. Dominic hospital laboratory.

Two of each batch of media prepared was used for quality control: One for positive control and the other for negative control. Control organisms were handled in an equal manner as was done for isolates obtained in the study in test procedures previously described in this chapter. These are summarised in Table 3.2

Table 3.2: Quality control organisms and media

TEST	MEDIA	POSITIVE CONTROL	NEGATIVE CONTROL
Indole test	Peptone water	*E. coli*	*Klebsiella pneumoniae*
H_2S Production	Triple sugar iron	*Proteus vulgaris*	*P. aeruginosa*
Citrate utilization	Citrate	*Klebsiella pneumoniae*	*E. coli*
Urease production	Urea	*Proteus vulgaris*	*E. coli*
ESBL Production	Mueller Hinton	ESBL producing *Klebsiella pneumoniae*	Non- ESBL producing *E. coli*

3.16 DATA PROCESSING AND ANALYSIS

Data generated from the various activities were presented in summary in tables in Microsoft Excel spreadsheets and analysed by GraphPad Prism version 5 (San Diego California, USA, www.graphpad.com).

The general characteristics of the study population were stratified by *Salmonella* infection. Demographic characteristics, source of sample type (in/out- patient), antibiotic susceptibility and ESBL phenotype were stratified by *Salmonella* isolates. Variables encountered in the study were mainly categorical or continuous. Continuous data were compared to each other using paired t-test, while categorical variables were compared to each other using chi – square analysis. Pearson chi – square test (P-value < 0.05) was used to determine the level of significance of the *Salmonella* isolates among the gender, age and sample types.

CHAPTER FOUR

RESULTS

4.1 DEMOGRAPHIC CHARACTERISTICS OF PATIENTS AND THE PREVALENCE OF *SALMONELLA* INFECTION

The study was conducted to determine the prevalence of *Salmonellae* in patients attending St. Dominic hospital. There were 464 samples of both blood and stool tested. Fifty four (54) *Salmonella* strains were isolated. This represents 11.6% (54/464). Typhoidal *Salmonellae* represented 29.6% (16/54) and comprise 20.4% (11/54) *Salmonella* Typhi and 9.3% (5/54) *Salmonella* Paratyphi B. Non-typhoidal *Salmonellae* represented 70.4% (38/54) of the total isolates. *Salmonella* Enterica had the highest prevalence among the non-typhoidal *Salmonellae* strains with 29.6% (16/54). It was followed by *Salmonella* Typhimurium with 24.1% (13/54) and *Salmonella* Enteritidis with 14.8% (8/54) (Table 4.1). *Salmonella* species isolated was distributed among all age groups. The age group 6-15 were most affected followed by age group 16-30 years. More females, 55.6% (30/54) were infected than males, 44.4% (24/54). Stool samples yielded more *Salmonella* isolates 72.2% (39/54) than blood cultures 27.8% (15/54). All *Salmonella* Typhi isolates were from blood cultures only but *Salmonella* Paratyphi B isolates were obtained from both blood and stool samples. All other *Salmonella* isolates were obtained from stool cultures only. Two different *Salmonella* strains were isolated from 3 patients each. There was mixed infections of *Salmonella* Paratyphi B with *Salmonella* Typhimurium, *Salmonella* Paratyphi B with *Salmonella* Diarizonae, *Salmonella* Enterica with *Salmonella* Enteritidis (Table 4.1).

Table 4.1: Demographic characteristics of the study population stratified by *Salmonella* species infecting patients attending St. Dominic Hospital, Akwatia

Isolates / Parameter	Typhoidal *Salmonellae*		Non-Typhoidal *Salmonellae*				Total 54 (100%)	P-value
	S.Typhi 11 (20.4%)	*S*. Paratyphi B 5 (9.3%)	*S*.Typhimurium 13 (24.1%)	*S*.Enterica 16 (29.6%)	*S*.Enteritidis 8 (14.8%)	*S*.Diarizonae 1 (1.9%)		
	Freq.	Freq.	Freq.	Freq.	Freq.	Freq.	Freq. (%)	
Age(years)								0.912
0-5	1	0	1	1	2	0	5 (9.3%)	
6-15	7	2	6	6	1	1	23 (42.6%)	
16-30	1	2	4	5	4	0	16 (29.6%)	
31-50	1	1	1	3	1	0	7 (13.0%)	
51+	1	0	1	1	0	0	3 (5.6%)	
Sex								0.592
Male	5	1	5	9	3	1	24 (44.4%)	
Female	6	4	8	7	5	0	30 (55.6%)	
Sample type								< 0.0001
Blood	11	4	0	0	0	0	15 (27.8%)	
Stool	0	1	13	16	8	1	39 (72.2%)	
Mixed Infection		1^{A}, 1^{B}	1^{B}	1^{C}	1^{C}	1^{A}		

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. ***Note****: S.* Typhi = *Salmonella* Typhi*; S.* Paratyphi B= *Salmonella* Paratyphi B*; S.* Enterica= *Salmonella* Enterica*; S.* Typhimurium = *Salmonella* Typhimurium*; S.* Enteritidis = *Salmonella* Enteritidis*; S.* Diarizonae = *Salmonella* Diarizonae*.* 1^{A}*=mixed infection of S.*Paratyphi B+*S.*Diarizonae, 1^{B}*=mixed infection of S.*Paratyphi B +*S.*Typhimurium *and* 1^{C}*=mixed infection of S.*Enterica+*S.*Enteritidis*.*

4.2 BACTERIAL CONTAMINANTS OF WATER SOURCES

The water samples tested in this study gave viable counts. The total viable count from well water was the highest giving a mean count of 1.64 cfu/ml. It was followed by various sachet water which gave a mean variable count of 1.19 cfu/ml. The lowest mean count was from river water samples of 1.72×10^{-1} cfu/ml. Other results are presented in Table 4.2. Various bacterial types were isolated from the water samples. Among them were *Salmonella* Typhi (1 isolate) *and Salmonella* Typhimurium (4 isolates). Other bacteria (lactose-fermenting gas-formers) were detected indicating faecal contamination of the water samples.

Table 4.2: Distribution of bacterial isolates from various water samples

Water sources (No. tested)	No. of times each sampled	Viable counts (cfu/ml)		Organism Isolated		Other Bacteria (mean count) cfu/ml
		Range	Mean	*S.* Typhi n=1	*S.* Typhimurium n=4	
Well water (51)	2	3.2×10^{-2} - 2.56×10^{1}	1.64	1	4	1.75
River (3)	4	1.21×10^{-13} - 5.1×10^{-1}	1.72×10^{-1}	0	0	1.72×10^{-1}
Pond (2)	2	1.06×10^{-1} - 1.81×10^{-1}	1.39×10^{-1}	0	0	1.39×10^{-1}
Tap (5)	2	4.4×10^{-2} – 9.0×10^{-2}	6.13×10^{-2}	0	0	6.13×10^{-2}
Stand pipe (3)	2	<30 cfu/ml	-	0	0	-
Sachet water (54)		3.7×10^{-2} - 9.5	1.19	0	0	1.19
Brand 1	9	9.5×10^{-2} - 9.5	2.31	0	0	2.31
Brand 2	9	9.7×10^{-2} - 8.6×10^{-1}	4.8×10^{-1}	0	0	4.8×10^{-1}
Brand 3	9	1.21 - 1.36	1.29	0	0	1.29
Brand 4	9	9.9×10^{-2} - 9.9×10^{-1}	6.87×10^{-1}	0	0	6.87×10^{-1}
Brand 5	9	8.3×10^{-1} - 2.09	1.46	0	0	1.46
Brand 6	9	3.7×10^{-2} - 2.2	9.06×10^{-1}	0	0	9.06×10^{-1}

4.3 ANTIMICROBIAL TEST RESULTS OF *SALMONELLA* SPECIES ISOLATED

All isolates exhibited high resistance ranging from 69.5% (41/59) to 100% (59/59) against ampicillin, piperacillin and trimethoprim-sulfamethoxaxole. No isolate of *Salmonella* Paratyphi B and *Salmonella* Diarizonae (1 isolate) were susceptible to ampicillin, piperacillin and trimethoprim-sulfamethoxaxole. Resistance to ampicillin was at an MIC of >=32ug/ml. Resistance to piperacillin and trimethoprim-sulfamethoxaxole were at >=128ug/ml and >=320ug/ml, respectively. Multidrug resistance was observed among 41 out of the total 59 *Salmonella* isolates, with high proportions of resistance to ampicillin, piperacillin, and trimethoprim-sulfamethoxaxole.

All (59) of the *Salmonella* isolates were susceptible to quinolones (ciprofloxacin and norfloxacin) tested. Fifty six (56) isolates showed susceptibility to ciprofloxacin at an MIC of <=0.25 ug/ml and 3 isolates, susceptible at an MIC of 0.5 ug/ml. Meanwhile, 46 isolates were susceptible to norfloxacin at an MIC of 0.5ug/ml with 13 isolates susceptible at MIC of 2 ug/ml.

There were varied results to the cephalosporins. All isolates were susceptible to cefepime at an MIC of <=1 ug/ml. A total of 53 isolates were susceptible to cefotaxime at an MIC of <=1 ug/ml while 6 of the isolates were susceptible at 2 ug/ml. Susceptibility to cefoxitin was at MIC of <=4 ug/ml. However, 1 isolate was susceptible to cefoxitin at 4 ug/ml and 3 isolates susceptible at 8 ug/ml.

There were also varied results with the Aminoglycosides (amikacin and Gentamycin) tested. Thirty one (31) isolates were susceptible to amikacin at MIC of <=2 ug/ml and 27 isolates susceptible to amikacin at MIC of 4 ug/ml. Only one isolate was susceptible to amikacin at 8 ug/ml. There were 51 isolates susceptible to Gentamycin at <=1 ug/ml whiles 8 isolates were resistant with an MIC of >=16 ug/ml. All *Salmonella* strains isolated were susceptible to both meropenem and Imipenem with MIC of <=1 ug/ml (Table 4.3).

Table 4.3: Minimum inhibitory concentrations (MICs) for *Salmonella* isolates

Antibiotics (generic names)	Sensitive (NO)	Intermediate (NO)	Resistant (NO)
ampicillin	<=2 (16)	16 (2)	>=32 (41)
piperacillin	<=4 (16)	64 (2)	>=128 (41)
piperacillin/tazobactam	<=4 (43) 8 (4)	32 (5)	>=128 (7)
amoxicillin/clavulanic acid	<=2 (18) 4 (9) 8 (29)	16 (3)	-
cefazolin	<=4 (30) 8 (19)	16 (9)	>=64 (1)
cefoxitin	<=4 (55) 4 (1) 8 (3)	-	-
cefotaxime	<=1 (53) 2 (6)	-	-
ceftazidime	<=1 (58)	-	>=32 (1)
ceftriaxone	<=1 (43) <=2 (5)	16 (3)	>=64 (8)
cefuroxime	<=1 (47)	16 (6)	>=32 (6)
cefepime	<=1 (59)	-	-
imipenem	<=1 (59)	-	-
meropenem	<=1 (59)	-	-
amikacin	<=2 (31) 4 (27) 8 (1)	-	-
gentamycin	<=1 (51)	-	>=16 (8)
ciprofloxacin	<=0.25 (56) 0.5 (3)	-	-
norfloxacin	<=0.5 (46) 2 (13)	-	-
tetracycline	<=1 (41) 4 (2)	-	>=16 (16)
nitofurantoin	<=16 (13) 32 (26)	64 (14)	128 (3) 256 (2) <=512 (1)
trimethoprim-sulfamethoxaxole	<=20 (14)	-	>=320 (45)

4.4 GENERAL CHARACTERISTICS OF PATIENTS STRATIFIED BY TYPE OF COMPLAINTS

A total of 149 patient complaints were reported by patients from whom *Salmonella* were isolated. Abdominal pain was the commonest of these complaints representing 24.5% (36/149) of the total complaints reported. Other complaints included fever 23.8% (35/149) and headache 12.9% (19/149). The rest were nausea and chills recording 12.2% (18/149) each. Less occurring complaints are indicated in Table 4.4.

Most of the complaints came from patients within the age group of 6-15 years. Female patients recorded the highest complaints 61.1% (91/149) compared to 38.9% (58/149) for males. *Salmonella* Enterica was the most common isolate associated with the complaints of abdominal pain. *Salmonella* Typhimurium 28.6% (10/35) and *Salmonella* Typhi 25.7% (9/35) were the most common isolates associated with complaints of fever (Table 4.4).

Table 4.4: General characteristics of subjects stratified by type of complaints

	Abdominal pain 36 (24.5%)			Fever 35 (23.8%)			Watery stool 16 (10.9%)			Nausea 18 (12.2%)			Headache 19 (12.9%)			Chills 18 (12.2%)			Weakness 7 (4.8%)			Total Cases 149 (100%)		
	TS	NTS	TOTAL	TS	NTS	TOTAL	TS	NTS	TOTAL	TS	NTS	TOTAL	TS	NTS	TOTAL	TS	NTS	TOTAL	TS	NTS	TOTAL	TS	NTS	TOTAL
Age(years)																								
0-5	1	4	5 (13.9%)	0	2	2 (5.7%)	0	2	2 (12.5%)	0	3	3 (16.7%)	0	0	0 (0.0%)	0	0	0 (0.0%)	0	0	0 (0.0%)	1	11	12 (8.2%)
6-15	7	9	16 (44.4%)	9	11	20 (57.1%)	4	6	10 (62.5%)	3	5	8 (44.4%)	1	7	8 (42.1%	4	3	7 (38.9%)	1	1	2 (28.6%)	29	42	71 (47.7%)
16-30	2	7	9 (25.0%)	2	5	7 (20.0%)	0	2	2 (12.5%)	2	2	4 (22.2%)	2	4	6 (31.6%)	2	3	5 (27.8%)	1	1	2 (28.6%)	11	24	35 (23.5%)
31-50	1	4	5 (13.9%)	0	4	4 (11.4%)	0	1	1 (6.3%)	0	3	3 (16.7%)	1	2	3 (15.8%)	1	2	3 (16.7%)	0	1	1 (14.3%)	3	17	20 (13.4%)
51+	0	1	1 (2.8%)	1	1	2 (5.7%)	1	0	1 (6.3%)	0	0	0 (0.0%)	1	1	2 (10.5%)	1	2	3 (16.7%)	1	1	2 (28.6%)	5	6	11 (7.4%)
Total	**11**	**25**	**36 (100%)**	**12**	**23**	**35 (100%)**	**5**	**11**	**16 (100%)**	**5**	**13**	**18 (100%)**	**5**	**14**	19 (100%)	**8**	**10**	**18 (100%)**	**3**	**4**	**7 (100%)**	**49**	**100**	**149 (100%)**
Sex																								
Male	4	9	13 (36.1%)	5	11	16 (45.7%)	1	5	6 (37.5%)	1	5	6 (33.3%)	0	5	5 (26.3%)	3	6	9 (50.0%)	1	2	3 (42.9%)	15	43	58 (38.9%)
Female	7	16	23 (63.9%)	7	12	19 (54.3%)	4	6	10 (62.5%)	4	8	12 (66.7%)	5	9	14 (73.7%)	5	4	9 (50.0%)	2	2	4 (57.1%)	34	57	91 (61.1%)
Total	**11**	**25**	**36 (100%)**	**12**	**23**	**35 (100%)**	**5**	**11**	**16 (100%)**	**5**	**13**	**18 (100%)**	**5**	**14**	**19 (100%)**	**8**	**10**	**18 (100%)**	**3**	**4**	**7 (100%)**	**49**	**100**	**149 (100%)**
Culture																								
Positive blood	11	0	11 (30.6%)	12	0	12 (33.3%)	5	0	5 (29.4%)	5	0	5 (27.8%)	5	0	5 (25.0%)	8	0	8 (42.1%)	3	0	3 (37.5%)	49	0	49 (31.8%)
Positive stool	0	25	25 (69.4%)	0	24	24 (66.7%)	0	12	12 (70.6%)	0	13	13 (72.2%)	0	15	15 (75.0%)	0	11	11 (57.9%)	0	5	5 (62.5%)	0	105	105 (68.2%)
Total	**11**	**25**	**36 (100%)**	**12**	**24**	**36 (100%)**	**5**	**12**	**17 (100%)**	**5**	**13**	**18 (100%)**	**5**	**15**	**20 (100%)**	**8**	**11**	**19 (100%)**	**3**	**5**	**8 (100%)**	**49**	**105**	**154 (100%)**
Isolates																								
*S.*Typhi	7	0	7 (19.4%)	9	0	9 (25.7%)	4	0	4 (25.0%)	3	0	3 (16.7%)	4	0	4 (21.1%)	6	0	6 (33.3%)	3	0	3 (42.9%)	36	0	36 (24.2%)
*S.*Paratyphi B	4	0	4 (11.1%)	3	0	3 (8.6%)	1	0	1 (6.3%)	2	0	2 (11.1%)	1	0	1 (5.3%)	2	0	2 (11.1%)	0	0	0 (0.0%)	13	0	13 (8.7%)
*S.*Enterica	0	12	12 (33.3%)	0	8	8 (22.9%)	0	7	7 (43.8%)	0	7	7 (38.9%)	0	6	6 (31.6%)	0	5	5 (27.8%)	0	1	1 (14.3%)	0	46	46 (30.9%)
*S.*Enteritidis	0	4	4 (11.1%)	0	5	5 (14.3%)	0	1	1 (6.3%)	0	3	3 (16.7%)	0	3	3 (15.8%)	0	1	1 (5.6%)	0	0	0 (0.0%)	0	17	17 (11.4%)
*S.*Typhimurium	0	9	9 (25.0%)	0	10	10 (28.6%)	0	3	3 (18.8%)	0	3	3 (16.7%)	0	5	5 (26.3%)	0	4	4 (22.2%)	0	3	3 (42.9%)	0	37	37 (24.8%)
Total	**11**	**25**	**36 (100%)**	**12**	**23**	**35 (100%)**	**5**	**11**	**16 (100%)**	**5**	**13**	**18 (100%)**	**5**	**14**	**19 (100%)**	**8**	**10**	**18 (100%)**	**3**	**4**	**7 (100%)**	**49**	**100**	**149 (100%)**

Data are presented as frequencies and percentages; ***Keys: TS****= Typhoidal Salmonellosis;* ***NTS****= Non-Typhoidal Salmonellosis;* ***n****=Number of cases; Figures in parentheses indicate percentages*

4.5 DISTRIBUTION OF ISOLATES

The results indicate that Akwatia recorded the highest number of isolates with 27.8% (15/54). This was followed by Asamankese with 14.9% (7/54) and Kade accounting 7.4% (4/54). Among the 15 isolates originating from Akwatia were 5 isolates of *S.* Enterica, 3 each of *S.* Enteritidis and *S.* Typhimurium and 2 each of *S.* Paratyphi B and *S.*Typhi. This shows an even spread of the various isolates in the community (Table 4.5).

Table 4.5: Organisms and locality

LOCALITY	*S.*Diarizonae	*S.*Enterica	*S.*Enteritidis	*S.*Paratyphi B	*S.*Typhi	*S.*Typhimurium	Total	%
AKWATIA		5	3	2	2	3	15	27.8%
ASAMANKESE		3	3		1	1	7	14.8%
KADE		3				1	4	7.4%
BOADUA		1	1			1	3	5.6%
ABAAM					1	1	2	3.7%
AKWATIA MANSO					1	1	2	3.7%
OKUMANING		1			1		2	3.7%
PRAMKESE	1			1			2	3.7%
ABENASO NKWANTA		1					1	1.85%
ABOMOSU			1				1	1.85%
ADWAFOAKWA					1		1	1.85%
AFOSU					1		1	1.85%
AKROSO		1					1	1.85%
AKYEM TWEAPEASE				1			1	1.85%
AKYINSO					1		1	1.85%
AMANFROM					1		1	1.85%
AMANTEM NKWANTA						1	1	1.85%
AMONOM				1			1	1.85%
APINAMANG						1	1	1.85%
BREKUMANSO ZONGO						1	1	1.85%
DWENASE		1					1	1.85%
OSENASE					1		1	1.85%
STAFF VILLAGE						1	1	1.85%
TOPREMANG						1	1	1.85%
TOTAL	**1**	**16**	**8**	**5**	**12**	**13**	**59**	**100%**

Data is presented as frequencies and percentages

CHAPTER FIVE

5.0 DISCUSSION

This study set out to establish the prevalence of *Salmonella* among patients attending St. Dominic Hospital and that in potable water samples in Akwatia, Ghana. There were 54 *Salmonella* isolates from patients, which consisted of 27.8 % (15/54) from blood samples and 72.2 % (39/54) from stool samples. Five *Salmonella* (isolates were also isolated from water in this study. This gave a prevalence of 11.6% (54/464) and 2.7 % (5/188) among the patients and water samples, respectively. The patients involved in the study aged 1day to 79yrs. There were a total of 267 males with 24 positive cases. The total number of females was females 197 with 30 positive cases of *Salmonella* infections.

5.1 PREVALENCE OF *SALMONELLA* SPECIES AMONG PATIENTS

This study recorded more non-typhoidal salmonellosis as compared with typhoidal salmonellosis. Threlfall and Ward, (2001), reported decrease in cases of typhoidal salmonellosis in developed countries and attributed their results to adequate sanitary measures. However, non-typhoidal salmonellosis was more common and was said to be associated with food contaminated by *Salmonellae*. A similar pattern as found in the current study was also seen in Cote d'Ivoire (Boni-Cissé *et al.*, 2012) where non-typhoidal isolates accounted for 76.1% of isolates whiles typhoidal *Salmonella* accounted for 23.9%. The differences in the pattern of *Salmonella* infection found in this study and that found in developed countries may be as a result of unavailability of potable drinking water as findings of this study established.

There were more *Salmonella* Typhi isolates than *Salmonella* Paratyphi B among typhoidal patients. These were commonly isolated in blood than in stool samples. This finding conformed to the report of Akinyemi *et al.,* (2007) who reported higher frequency of *Salmonella* Typhi in Lagos. In a study in Ghana, Uwe Groß *et al.*, (2011) found *Salmonella* Typhi from blood culture to be 59 (40.7%). Peletiri *et al*., (2012) reported 90 (3.2%) positive cases out of the 2,818 blood cultures, out of which 68 (75.6%) were *Salmonella* Typhi and 16 (17.8%) were *Salmonella* Paratyphi B.

The low percentage of blood culture positive cases in this study may be due to prior antimicrobial therapy before reporting to the hospital. The predominance of the typhoidal isolates in the blood cultures in this current study may be attributed to their virulent nature which enables them to cross intestinal barriers and infect the blood (Boyd *et al*., 1993).

This study also showed that non-typhoidal *Salmonellae* were more commonly isolated from stool than typhoidal *Salmonellae*. These *Salmonella* species only colonize the intestinal tract and do not have the ability to cross the intestinal barrier and infect the blood. Non-typhoidal *Salmonellae* also persist in fecal cultures for several weeks leading to chronic carrier states as stated by Pui *et al.,* (2011) and may account for its high recovery in stool samples.

Salmonella Enterica was the most predominant serovars among the patients. The prevalence of *Salmonella* Enterica 29.6% (16/54) from patients was higher than that of *Salmonella* Typhimurium 24.1% (13/54) and *Salmonella* Enteritidis 14.8% (8/54).

Therefore, *Salmonella* Enterica was more frequently isolated in patients than any other non-typhoidal *Salmonella* species.

This finding is not consistent with that of others who found *Salmonella enterica serovars* Typhimurium and Enteritidis to be the most common causes of human Salmonellosis in some African Countries (Kariuki, 2008; Fashea *et al.*, 2010). Studies carried out in Europe and America also do agree that *Salmonella* Enteritidis is the first causative agent of bacteraemia and gastroenteritis (Araque, 2009; Betancor *et al.*, 2009). No invasive non-typhoidal *Salmonellae* were found in this study. The patients involved in this current study, however, were not immunocompromised as in the study of Gordon *et al*, (2008) and Reddy *et al.*, (2010) who reported that invasive NTS disease were common in African adults with advanced immunosuppression. The difference in the prevalence of isolates may be due to the type of *Salmonella* species localized here in the study area as some serotypes may be prevalent in certain areas than others (Bradford, 2001).

Three cases of co-infection (mixed infection) of both typhoid and paratyphoid serotypes were recorded in the current study and agree with the report of Umeh and Agbulu (2010) and that mixed infection with multiple *Salmonella* serotypes is not common. The number of mixed infections in their study was, however, very high with 230 (15.6%) out of 1479 patients showing a mixture of both typhoid and paratyphoid serotypes.

5.2 AGE DISTRIBUTION OF PATIENTS INFECTED WITH *SALMONELLA*

Out of the 54 *Salmonella* isolates from patients in this study, 28 out of the total 54 patients' isolates, representing 51.9% were found in the age group 6-15years. The high

isolation of *Salmonella* in this age group of 6 to 15 years also is consistent with the work of Feasey *et al.,* (2010) and Abdullahi *et al.*,(2012) who reported children of school-going age and young adults were more affected with *Salmonella.* Most probably, the undeveloped immune system of this age group makes them vulnerable to enteric pathogens (Ja'afar *et al*., 2013).

5.3 GENDER DISTRIBUTION OF *SALMONELLAE* ISOLATES

Of the total cases of 54 *Salmonella* isolates from patients, 55.6% (30/54) were from females and 44.4% (24/54) from males in this present study**.** A high prevalence in females than males was observed in a study by Umeh and Agbulu (2010) who found a 58.0% prevalence of *Salmonella* Typhi in females. They also recorded a higher prevalence of *S.* Paratyphi in females than in males between the ages of 11-20 years. Females are more infected with *Salmonella* in this area probably because they tend to do more domestic chores involving water such as fetching water from various water sources, cooking, washing of clothes and cleaning of homes compared to their male counterparts due to cultural reasons. From the results of our study some of this water sources may be contaminated with *Salmonella*.

5.4 TYPES OF COMPLAINTS OF PATIENTS

Presentation of *Salmonella* infection can be confusing and easily misleading since there are the symptoms usually presented are common to most tropical infections. The current study recoded abdominal pain in 24.5% (36/149) patients and 23.8% (35/149) patients presented with fever. These symptoms were singly presented in more than 50% of the patients from whom *Salmonella* were isolated in this study. Truusaluk *et al*.,(2008)

reported that *Salmonella species* from blood samples increased with the duration of fever. The duration of fever and its corresponding *Salmonella* yield was however not considered in this study. Common symptoms in a study by Tayyab *et al.*,(2010) were fever (100% patients), vomiting (94% patients), abdominal distention (100% patients) and tenderness (100% patients). The disparity here may be because the study of Tayyab *et al.*,(2010), was centered on patients with typhoid ileal perforations only with patients showing severe symptoms as recorded in their study. The results of this study show that, out of the total 51 patients infected with *Salmonella*, 70.6% (36/54) and 68.6% (35/54) complained of abdominal pain and fever respectively. Thus, these complaints can be suggestive of a *Salmonella* infection especially when other disease conditions have been ruled out.

5.5 PREVALENCE OF *SALMONELLA* IN WATER SAMPLES

Waterborne diseases are often unreported or under reported in developing countries because of the lack of systematic studies. Baudart et al., (2000) isolated 544 strains of *Salmonella* from water samples collected from a Mediterranean coastal watershed, out of which, more than 40 different *Salmonella* serovars were identified. Another study in Georgia identified *Salmonellae* in 79.2% of water samples tested with 57 *Salmonella* positive samples out of a total 72 water samples (Haley et al., 2009). These studies including this current one which found *Salmonella* and lactose-fermenting gas-formers in the water samples tested provide evidence that the water system can be polluted by various *Salmonella* serovars.

This study, however, recovered very few serovars compared to that of Baudart et al., (2000) and Haley *et al.*,(2009) and may probably be due to to different survival rates of different *Salmonellae* serotypes of the organism in surface waters (Haley *et al.*, 2009; Setti *et al.*, 2009) or probably the *Salmonella* bacteria were present in the water samples tested but were in a viable but nonculturable state (Oliver, 2010).

Pathogenic bacteria that cause waterborne outbreaks include *Escherichia coli*, *Salmonella*, and *Shigella* (Szewzyk *et al.*, 2000). Some of these pathogenic organisms were isolated from water samples in this study, indicative of faecal contamination.

In this study *Salmonella* Enteritidis was not found as the predominant isolate of *Salmonella* in contrast to its predominance reported globally (Galanis *et al.*, 2006). Out of the 5 *Salmonella* isolates found in the water samples 80% (4/5) were *Salmonella* Typhimurium strains and 20% (1/5) *Salmonella* Typhi. *Salmonella* Typhimurium and *Salmonella* Typhi were among the most common isolates obtained from humans.

The diversity of *Salmonella* serovars in water among the different studies and this current one may also be as a result of the difference of host species that act as reservoirs of the organism and excrete their *Salmonella* serovars, which find their way into the water. This may also account for this difference in serovars prevalent in the water samples in the different study areas.

Salmonellae serotypes often found in aquatic environments are normally not the same as that isolated from humans (Setti *et al.*, 2009). The *Salmonella* serovars found in this study from well water samples were commonly associated with human clinical disease. It

is likely that contaminated environment from both humans and animals, more likely human activity, such as indiscriminate defecation contaminate drinking water when fecal matter are washed into the water sources during the rainy seasons and hence a source of infection to the people who drink from them.

A study in Nigeria indicated varied bacteriological concentration in a quality assessment results of the water samples in their study area (Owolabi, 2012). Another study, also in Nigeria found that all wells in the areas where majority of their research work was carried out had high bacterial pollution with results ranging from 100 to 800 col/100ml (Olabisi *et al.*, 2008). The high bacterial contamination observed in well water samples in this study was no different. This can be attributed to the site (low lying) where the wells were located and settlement pattern. There were pit latrines and septic soak-aways located too close to the wells in most households. It is suspected that seepage of these latrines into the wells might also contribute to the presence of faecal bacteria as seen our study.

Various sachet water samples tested had high counts of lactose-fermenting gas-forming bacteria indicative of fecal contamination. Previous studies in other parts of the country reported similar bacterial load indicative of poor water quality as in the study of Addo et al., (2009). In Nigeria, Edema *et al.*,(2011) found 87% of the sachet-packed water samples contaminated with *Salmonella* and/or *Escherichia coli.* A majority of the population may acquire water-borne diseases because many depend on sachet-packed water for drinking. This is because it is relatively cheap together with the assumed safety associated with it and the belief that it is better than pipe-borne water hence its popular acronym “pure water” (Addo *et al*., 2009).

5.6 ANTIMICROBIAL DRUG SUSCEPTIBILITY

Treatment with an appropriate antibiotic is essential for managing salmonellosis (Rowe *et al*., 1997). Recent increases in antibiotic-resistant bacteria have prompted routine surveillance of microbial populations to determine the extent of the resistance. In this study, 54 *Salmonella* isolates of clinical origin and five (5) from water were examined for their susceptibility to various antibiotics.

In this study, all isolates were susceptible to ciprofloxacin which is one of the most prescribed antibiotics for the treatment of *Salmonella* infections in the St. Dominic hospital. High susceptibility of *Salmonella* species was also observed with norfloxacin in this study. Most *Salmonella* Enterica strains were susceptible to ciprofloxacin among Nigerian patients (Ibrahim *et al*., 2005). In Lagos, Nigeria, Akinyemi *et al.,* (2007) reported 18% reduced susceptibility of *Salmonella* to ciprofloxacin. High susceptibility to ciprofloxacin was also seen in a study by Marks *et al.,*(2010) and by Uwe Groß *et al.,* (2011)**.** High proportions of *Salmonellae was found* to be resistant to ciprofloxacin in Accra, the capital of Ghana (Namboodiri *et al*., 2011). High susceptibility of *Salmonella* to ciprofloxacin and norfloxacin were also found in this study as in the study of Malla *et al.,*(Malla *et al.,* 2005). The high susceptibility of *Salmonella* to quinolones recorded in this study may be connected to the relatively high cost of ciprofloxacin and norfloxacin. Therefore, quinolones are not used indiscriminately because not many can afford them.

A study among patients of a hospital in Bangladesh by Islam *et al.,* (2008) found cephalosporins to be better than fluoroquinolones for the treatment of infection caused by *Salmonella* Typhi. Current reports of multidrug resistance strains of *Salmonella* Typhi

(Adabara *et al.*, 2012), draw the same conclusion. The *Salmonella* isolates were most susceptible to these cephalosporins; cefoxitin, cefotaxime, cefepime and ceftazidime. A 13.6% (8/59) resistance was observed against ceftriaxone. This does not agree with the results of Mills-Robertson *et al.,* (2002) and Schwarz *et al.,* (2010) who had all isolates in their study susceptible to ceftriaxone.

In our study however, the cephalosporins showed high susceptibility as the quinolones. Even though *Salmonella* isolates showed high proportions of susceptibility to quinolones in this study, this group of antibiotics is not approved for use in children due to concerns about cartilage damage (Lynch *et al*., 2009). Cephalosporins such as ceftriaxone are an important line of therapy for children (Weill *et al*., 2004). An increase in resistance among *Salmonella* to extended-spectrum cephalosporins is a significant public health concern because ceftriaxone is a drug of choice for the treatment of severe salmonellosis in children (Rabsch *et al*., 2001).

Antibiogram of these isolates revealed that all the isolates were sensitive to carbapenems (imepenem and meropenem) tested. Nagshetty and colleagues (2010) recorded similar results with Imipenem. These antibiotics are not common in the community and the few available here are again very expensive and not used indiscriminately because not many can afford them.

The variation in the sensitivity pattern to these drugs could be attributed to the common attitude of over-the-counter purchase of the drugs in the areas under study which may result in its widespread usage and hence abuse of these antibiotics.

A high proportion of *Salmonella* isolates were resistant to piperacillin, trimethoprim-sulfamethoxaxole and ampicillin in the current study. Similar results reported in other parts of Ghana by Uwe Groß *et al.,* (2011) and in some parts of Kenya by Kariuki *et al.,* (2005) in their study said that the resistance of Non Typhoidal *Salmonellae* to commonly used drugs including ampicillin rose from 31% in 1994 to 42% in 2003. High resistance to trimethoprim-sulfamethoxaxole (71%) and ampicillin/amoxicillin (70%) has also been recorded (Marks *et al.,* 2010).

Multidrug resistance was found among 41 out of the total 59 *Salmonella* isolates, with high proportions being resistant to ampicillin, Piperacillin, and trimethoprim-sulfamethoxaxole in this study. Similar results were found in Nepal where several *Salmonella* isolates were found to be resistant to at least four antibiotics (Bhatta *et al*., 2007). A study in Ghana found that *S.* Typhi isolates also were multidrug resistant. They reported 50% and 63% of the isolates to be resistant to ampicillin, trimethoprim-sulfamethoxaxole, and chloramphenicol (Marks *et al*., 2010). Another study in Accra reported 87% of *Salmonella sero group B* isolates to be multidrug resistant (Mills-Robertson *et al*., 2003). Recent studies in an African country reported *in-vitro* resistance to these same antibiotics in *Salmonella* infections in Funtua, Nigeria (Abdullahi *et al*., 2012). This may be as a result of indiscriminate use of these antibiotics as well as the overuse of these drugs in human medicine over a long period. Self-medications are another factor that may account for this high level of resistance to these antibiotics as well the development of the MDR organisms.

5.7 ESBL PREVALENCE AMONG *SALMONELLA* ISOLATES

The emergence and spread of resistance in enterobacteriaceae is complicating the treatment of infections and may create species resistant to all currently available antimicrobial agents. *Salmonella* and other enterobacteriaceae may also be ESBL-producers (Kocagoz *et al*., 2006). In this study, we aimed to determine the presence or absence of ESBL-producing *Salmonella* species. No strain was found to produce extended-spectrum beta-lactamase (ESBL). The results here are similar to the findings of Boni-Cisse *et al.,* (2012) who found no ESBL producing *Salmonella* species in their study in Cote d'Ivoire. On the contrary, Mahmood *et al.,* (2012) in Pakistan, found three strains of *Salmonella* which showed extended-spectrum β-Lactamase production by double disk synergy test**.**

Enteric fever and salmonellosis are community-acquired infections (Pitout *et al*., 2005). Since ESBL genes are usually located on mobile genetic elements, the emergence of an ESBL-producing *Salmonella* species can be from the exchange of mobile genetic elements bearing the beta-lactamase gene (Mahmood *et al*., 2012) or get mutated when they come into contact with broad spectrum cephalosporins as done in hospital settings (Asseva *et al*., 2012). The possible absence of ESBL-producing *Salmonella* strains resistant to antibiotics commonly used as treatment may have positively affected the high susceptibility of the isolates found in this study to cephalosporins and quinolones. Although ciprofloxacin and norfloxacin have high *Salmonella* proportions susceptible to them it is important to continue surveying for resistance because quinolones remain the drug of choice after B-lactams in this era of extended-spectrum beta-lactamase production (Gallardo *et al.,* 1999).

5.8 CONCLUSION

The present study indicated that *Salmonella* can infect all age groups. It also indicated that *Salmonella* was most common among age group 6-15 years. The study indicated the presence of *Salmonella* and other faecal contaminants in potable water in Akwatia.

Salmonella Typhi was the most common typhoidal isolates within the study population while *Salmonella* Enterica was the most common non-typhoidal isolate.

No strain of *Salmonella* isolated was resistant to cefepime, cefoxitin, cefotaxime, ciprofloxacin, norfloxacin, meropenem, Imipenem and amikacin and can be used for Empirical treatment. High resistance proportions were found to ampicillin, piperacillin and trimethoprim-sulfamethoxaxole. No isolate produced ESBL.

The common patient complaints were abdominal pains and fever. These complaints may be suggestive of a *Salmonella* infection especially when other disease conditions have been ruled out.

The results revealed that the source of *Salmonella* to the inhabitants of Akwatia may be the well water they drink. Other sources of potable water to the inhabitants, though polluted with faecal isolates, did not yield *Salmonella*.

5.9 RECOMMENDATIONS

1. Establishment of a systematic and regular mechanism for surveillance and monitoring of waterborne pathogens in the drinking water supply is recommended to minimize water-borne disease in Akwatia.
2. Well water drinkers are advised to boil their water and allow to cool especially before drinking. Uncovered wells should also be provided with covers as this may prevent pollution of well water. The production and distribution of commercial sachet-packed water requires attention, monitoring and control by the regulatory agencies of government. Government must also increase resource allocations to relevant regulatory agencies to ensure adequate monitoring and enforcement of drinking water quality in public and private distribution systems. Private enterprises can analyse their samples regularly through certified analytical laboratories.
3. Controlled and judicial antibiotic use in hospital is recommended to help prevent the emergence of ESBL-producing *Salmonellae* species and limit its spread even if it does emerge in the community. Laws restricting over-the-counter dispensing of drugs without prescription also need to be enforced.

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APPENDIX 1: MEDIA USED FOR THE ISOLATION OF *SALMONELLA*

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

- Blood agar
- Brain Heart Infusion
- Buffered peptone water
- Citrate
- Cysteine Lactose Electrolyte Deficient(CLED) Agar
- Kovacs reagent
- MacConkey Agar
- Nutrient Agar
- Peptone Water
- Plate count Agar
- *Salmonella-Shigella* Agar
- Selenite F Broth
- Triple Sugar Iron
- Urea Agar

THE COMPOSITION, PREPARATION AND MODE OF ACTION OF MEDIA

BLOOD AGAR BASE (Liofilchem, Italy)

Beef extract 10.0g

Sodium chloride 5g

Tryptose 10.0g

Agar 15.0g

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

Mode of preparation – Suspend 40g in 1000ml of distilled water and heat to dissolve completely. Sterilize by autoclaving at 121^{o}C for 15minutes. Cool to 50^{o}C and aseptically add 10% sterile defibrinated blood. Mix well and pour.

Mode of action - This is a general purpose enriched and differential solid medium. it supports the growth of most ordinary bacteria. It is therefore used for primary culture. Blood supplies a number of required substances for the growth of fastidious organisms. The haemolytic patterns make it differential.

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

Mode of preparation – Weigh 37g in 1L of distilled water, swirl to mix and warm gently to dissolve completely. Dispense into sterile blood culture bottles and sterilize by autoclaving for 15minutes at 121^0C.

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

CYSTEINE LACTOSE ELECTROLYTE DEFICIENT AGAR (Liofilchem, Italy)

Composition (typical g/L)

Beef Extract 3.0g

Peptone 4.0g

Tryptone 4.0g

L-Cystine 0.128g

Lactose 10.0g

Bromothymol Blue Indicator 0.02g

Agar 15.0g

pH 7.3+/- 0.2 at 25^oC

Mode of preparation –Dispense 36.1g in 1L of distilled water. Swirl to mix, heat to completely dissolve and sterilize by autoclaving for 15 minutes at 121^0C. Pour into sterile Petri dishes.

Mode of action - The medium provides a valuable non inhibitory diagnostic agar for plate culture of organisms especially urinary organisms. It is electrolyte deficient to prevent the swarming of proteus species.

MACCONKEY AGAR (Liofilchem, Italy)

Standard formula in g/l

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

Mode 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^{o}C and pour into sterile Petri plates.

Mode of action - 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. Hence their pale/colourless colonies. The bile salt mixture also inhibits gram positive organisms.

MUELLER HINTON AGAR (LIOFILCHEM, ITALY)

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

Mode of Preparation - Weigh and dissolve 36 grams of the powder in 1 litre of distilled water. Sterilize at 121^{o}C for 15mins.

Mode of action - This medium is recommended for the disc diffusion method of antimicrobial susceptibility testing of bacteria. When enriched with blood, it can be used for Neisseria and Haemophilus species. The starch component ensures the absorption of toxic factors generated during growth of an organism.

NUTRIENT AGAR (Oxoid, England)

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

Mode of preparation – Weigh 28g of powder and disperse in 1 litre of Deionised water. Allow to soak for 10 minutes, swirl to mix then sterilize by autoclaving for 15 minutes at 121^{0}C. Cool to 47^{0}C, mix well and pour plates.

Mode of action - A basic culture media usually for the sub-culture of organisms for purity growths and for the cultivation of non-fastidious organisms.

PLATE COUNT AGAR (Liofilchem, Italy**)**

Compositon-

Tryptone 5.0g

Glucose 1.0g

Yeast Extract 2.5g

Agar 15.0g

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

Mode of preparation - Suspend 23.5 grams of the powder in 1litre of distilled water. Heat until completely dissolved. Autoclaved at 121^{o}C for 15 minutes.

Mode of action - Used to determine the sanitary quality of foods, water and other materials. Enzymatic digest of casein provides the amino acids and other complex nitrogen substances necessary to support bacteria growth. Yeast extract basically supplies the B-complex vitamins whiles dextrose serves as the energy source.

SALINE (PHYSIOLOGICAL SALINE)

Composition

NaCl 8.5g

Distilled water 1 litre

Mode of preparation - Dissolve 8.5g NaCL in water. Autoclaved for 15mins at 121^{o}C. Cool to room temperature.

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

***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 25^{o}C

Mode 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 is inhibitory to 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.

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

Mode of preparation- Take to the boiling 23.0grams dissolved in 1litre of distilled water. Do not autoclave. Aseptically dispense into sterile bijoux bottles.

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

APPENDIX II: BIOCHEMICAL TEST REAGENTS

Buffered peptone water (merck)

Composition

Peptone 10g

NaCl 5g

Na2HPO412H2O 9.0g

KH2PO4 1.5g

pH 7.2 +/- 0.2 at 25^{o}C

Mode of preparation- Weigh and suspend 25.5g in 1litre of distilled water. Dispense into sterile containers in 9ml volume and cap. Autoclave at 121^{o}C for 15mins.

Mode of action a pre- enrichment medium to be used prior to selective enrichment for the isolation of *Salmonella* species. Broth is rich in nutrients and produces high resuscitation rates for injured bacteria. The phosphate buffer system prevents bacterial damage due to changes in the pH of the medium.

TSI Agar (Liofilchem, Italy)

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

Mode of preparation – Suspend 64.5g in one litre of distilled water and boil 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.

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 deionised water. Allow to soak for 10 minutes, swirl to mix and then 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.

Urea Agar Base (Liofilchem, Italy)

Composition (typical g/L) –

Peptone 1.0g

Glucose 1.0g

Sodium chloride 5.0g

Disodium phosphate 1.2g

Monopotassium phosphate 2.0g

Phenol red 0.012g

Agar 15.0g

pH 6.8 +/- 0.2 at 25^{o}C

Mode of preparation – Disperse 2.4g in 95ml of distilled. Swirl to mix and heat to complete dissolution. Sterilize by autoclaving for 15 minutes at 121^{0}C. Cool to 50^{o}C and add 40% of sterile urea salt supplement, X130 or X135. Dispense into final containers and set in a sloped position.

Mode of action - Some organisms have the ability to utilize urea as their only source of nitrogen because they produce the urease enzyme. The enzyme splits urea into ammonia and carbon dioxide. As a result of ammonia production the urea containing medium becomes alkaline and causes the phenol red indicator to become pinkish red.

Indole test

Indicates the ability of the enzyme Tryptophanase to hydrolyse the protein Trptophan generating as a result, indole, pyruvic acid and ammonia. The bacteria feed on the pyruvic acid and ammonia leaving the indole. On the addition of kovac's reagent, a bright red compound on the surface of the medium is a reaction between the kovac's reagent and the indole.

Kovac's reagent

Used in detecting the indole compound.

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.

APPENDIX III: GRAM STAIN AND REAGENTS

Principle

A heat fixed smear is stained with crystal violet, followed by the use of a mordant (iodine), treatment 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 loose the initial dye (crystal violet) and pick up the counter satin- neutral red. This is designated as gram negative.

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.

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 mark it *Toxic*. Store it in a dark place at room temperature. The solution is not good for use if its colour fades.

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.

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: THE BIOCHEMICAL TESTS

The biochemical tests performed with the Vitek 2 Compact automated analyser are as follows:

Biochemical test	**Abbreviation**
5-KETO-D-GLUCONATE	-5KG
ADONITOL	- ADO
Ala-Phe-Pro-ARYLAMIDASE	- APPA
ALPHA-GALACTOSIDASE	-AGAL
ALPHA-GLUCOSIDASE	-AGLU
BETA-Alanine arylamidase pNA	-BAlap
BETA-GALACTOSIDASE	-BGAL
BETA-GLUCORONIDASE	-BGUR
BETA-GLUCOSIDASE	-BGLU
Beta-N-ACETYL-GALACTOSAMINIDASE	-NAGA
BETA-N-ACETYL-GLUCOSAMINIDASE	-BNAG
BETA-XYLOSIDASE	-BXYL
CITRATE (SODIUM)	-CIT
COURMARATE	-CMT
D-CELLOBIOSE	-dCEL
DECARBOXYLASE BASE	-0DEC
D-GLUCOSE	-dGLU
D-MALTOSE	-dMAL
D-MANNITOL	-dMAN
D-MANNOSE	-dMNE

Biochemical test	Abbreviation
D-SORBITOL	-dSOR
D-TAGATOSE	-dTAG
D-TREHALOSE	-dTRE
ELLMAN	-ELLM
FERMENTATION/ GLUCOSE	-OFF
GAMMA-GLUTAMYL-TRANSFERASE	-GGT
Glu-Gly-Arg-ARYLAMIDASE	-GGAA
Glutamyl Arylamidase pNA	-AGLTp
Glycine ARYLAMIDASE	-GlyA
H2S PRODUCTION	-H2S
L-ARABITOL	-lARL
L-HISTIDINE assimilation	-lHlSa
LIPASE	-LIP
L-LACTATE alkalinisation	-lLATk
L-LACTATE assimilation	- lLATa
L-MALATE assimilation	-lMLTa
L-Proline ARYLAMIDASE	-ProA
L-Pyrrolydonyl-ARYLAMIDASE	- PyrA
LYSINE DECARBOXYLASE	-LDC
MALONATE	-MNT
O/129 RESISTANCE (comp.vibrio.)	-O129R
ORNITHINE DECARBOXYLASE	-ODC

Biochemical test	Abbreviation
PALATINOSE	-PLE
PHOSPHATASE	-PHOS
SACCHAROSE/SUCROSE	-SAC
SUCCINATE alkalinisation	-SUCT
Tyrosine ARYLAMIDASE	-TyrA
UREASE	-URE

APPENDIX V: STERILIZATION

Sterilization of glass ware

- To sterilize glassware by dry heat, a temperature of 160^{o}C 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. it was allowed to cool before use.

Media storage

- Plates of culture media were stored at 2–8^{o}C. Media in screw-cap tubes and bottles were also stored in the refrigerator at 2–8^{o}C.

Disposal of waste

Blood

- Blood in brain heart infusion broth are autoclaved at 121oC 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. Dominic Hospital incinerator.

Sharps (needles)

- These were discarded into a puncture resistant sharp bin and incinerated at the St. Dominic Hospital incinerator.

Culture

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

APPENDIX VI: Antimicrobial test results of *Salmonellae* species isolated from blood and stool and water samples

Drug	BLOOD ISOLATES						STOOL ISOLATES														WATER ISOLATES				
	S.Typhi (n=11)			S.Paratyphi B (n=4)			S.Paratyphi B (n=1)			S.Typhimurium (n=13)			S.Enterica (n=16)			S.Enteritidis (n=8)			S.Diarizonae(n=1)		S.Typhi (n=1)		S.Typhimurium (n=4)		
	S	*I*	*R*	*S*	*I*	*R*	*S*	*I*	*R*	*S*	*I*	*R*	*S*	*I*	*R*	*S*	*I*	*R*	*S*	*R*	*S*	*R*	*S*	*I*	*R*
Beta-lactams antibiotic																									
Ampicillin	3 (27.3%)	0	8 (72.7%)	0	0	4 (100%)	0	0	1 (100%)	4 (30.8%)	1 (7.7%)	8 (61.5%)	4 (25%)	1 (6.3%)	11 (68.8%)	1 (12.5%)	0	7 (87.5%)	0	1 (100%)	1 (100%)	0	3 (75%)	0	1 (25%)
Piperacillin	3 (27.3%)	0	8 (72.7%)	0	0	4 (100%)	0	0	1 (100%)	4 (30.8%)	1 (7.7%)	8 (61.5%)	4 (25%)	0	12 (75%)	1 (12.5%)	1 (12.5%)	6 (75%)	0	1 (100%)	1 (100%)	0	3 (75%)	0	1 (25%)
Piperacillin/tazobactam	11 (100%)	0	0	2 (50%)	1 (25%)	1 (25%)	1 (100%)	0	0	12 (92.3%)	0	1 (7.7%)	9 (56.3%)	4 (25%)	3 (18.8%)	7 (87.5%)	0	1 (12.5%)	0	1 (100%)	1 (100%)	0	4 (100%)	0	0
Amoxicillin/clavulanic acid	11 (100%)	0	0	4 (100%)	0	0	1 (100%)	0	0	12 (92.3%)	1 (7.7%)	0	15 (93.7%)	1 (6.3%)	0	7 (87.5%)	1 (12.5%)	0	1 (100%)	0	1 (100%)	0	4 (100%)	0	0
Cephalosporins																									
Cefazolin	11 (100%)	0	0	2 (50%)	1 (25%)	1 (25%)	1 (100%)	0	0	8 (61.5%)	5 (38.5%)	0	15 (93.7%)	1 (6.3%)	0	6 (75%)	2 (25%)	0	1 (100%)	0	1 (100%)	0	4 (100%)	0	0
Cefoxitin	11 (100%)	0	0	4 (100%)	0	0	1 (100%)	0	0	13 (100%)	0	0	16 (100%)	0	0	8 (100%)	0	0	1 (100%)	0	1 (100%)	0	4 (100%)	0	0
Cefotaxime	11 (100%)	0	0	4 (100%)	0	0	1 (100%)	0	0	13 (100%)	0	0	16 (100%)	0	0	8 (100%)	0	0	1 (100%)	0	1 (100%)	0	4 (100%)	0	0
Ceftazidime	11 (100%)	0	0	4 (100%)	0	0	1 (100%)	0	0	12 (92.3%)	0	1 (7.7%)	16 (100%)	0	0	8 (100%)	0	0	1 (100%)	0	1 (100%)	0	4 (100%)	0	0
Ceftriaxone	10 (90.9%)	0	1 (9.1%)	2 (50%)	0	2 (50%)	0	1 (100%)	0	13 (100%)	0	0	11 (68.8%)	1 (6.3%)	4 (25%)	7 (87.5%)	0	1 (12.5%)	1 (100%)	0	1 (100%)	0	4 (100%)	0	0
Cefuroxime	9 (81.8%)	1 (9.1%)	1 (9.1%)	2 (50%)	2 (50%)	0	1 (100%)	0	0	10 (76.9%)	1 (7.7%)	2 (15.4%)	14 (87.4%)	1 (6.3%)	1 (6.3%)	6 (75%)	1 (12.5%)	1 (12.5%)	1 (100%)	0	0	1(100%)	3 (75%)	1 (25%)	0
Cefepime	11 (100%)	0	0	4 (100%)	0	0	1 (100%)	0	0	13 (100%)	0	0	16 (100%)	0	0	8 (100%)	0	0	1 (100%)	0	1 (100%)	0	4 (100%)	0	0
Carbapenem																									
Imipenem	11 (100%)	0	0	4 (100%)	0	0	1 (100%)	0	0	13 (100%)	0	0	16 (100%)	0	0	8 (100%)	0	0	1 (100%)	0	1 (100%)	0	4 (100%)	0	0
Meropenem	11 (100%)	0	0	4 (100%)	0	0	1 (100%)	0	0	13 (100%)	0	0	16 (100%)	0	0	8 (100%)	0	0	1 (100%)	0	1 (100%)	0	4 (100%)	0	0
Non-beta-lactam antibiotic																									
Aminoglycosides																									
Amikacin	11 (100%)	0	0	4 (100%)	0	0	1 (100%)	0	0	13 (100%)	0	0	16 (100%)	0	0	8 (100%)	0	0	1 (100%)	0	1 (100%)	0	4 (100%)	0	0
Gentamycin	10 (90.9%)	0	1 (9.1%)	4 (100%)	0	0	1 (100%)	0	0	11 (84.6%)	0	2 (15.4%)	12 (75%)	0	4 (25%)	8 (100%)	0	0	1 (100%)	0	1 (100%)	0	3 (75%)	0	1 (25%)
Quinolones susceptibilty																									
Ciprofloxacin	11 (100%)	0	0	4 (100%)	0	0	1 (100%)	0	0	13 (100%)	0	0	16 (100%)	0	0	8 (100%)	0	0	1 (100%)	0	1 (100%)	0	4 (100%)	0	0
Norfloxacin	11 (100%)	0	0	4 (100%)	0	0	1 (100%)	0	0	13 (100%)	0	0	16 (100%)	0	0	8 (100%)	0	0	1 (100%)	0	1 (100%)	0	4 (100%)	0	0
Tetracycline	3 (27.3%)	0	8 (72.7%)	3 (75%)	0	1 (25%)	1 (100%)	0	0	11 (84.6%)	0	2 (15.4%)	13 (81.2%)	0	3 (18.8%)	7 (87.5%)	0	1 (12.5%)	0	1 (100%)	1 (100%)	0	4 (100%)	0	0
Nitofurantoin	8 (72.7%)	3 (27.3%)	0	4 (100%)	0	0	1 (100%)	0	0	3 (23.1%)	7 (53.8%)	3 (23.1%)	14 (87.4%)	1 (6.3%)	1 (6.3%)	7 (87.5%)	1 (12.5%)	0	0	1 (100%)	1 (100%)	0	1 (25%)	2 (50%)	1 (25%)
Trimethoprim/sulfamethoxazole	3 (27.3%)	0	8 (72.7%)	0	0	4 (100%)	0	0	1 (100%)	3 (23.1%)	0	10 (76.9%)	5 (31.3%)	0	11 (68.8%)	1 (12.5%)	0	7 (87.5%)	0	1 (100%)	1 (100%)	0	3 (75%)	0	1 (25%)

Data are presented as frequencies and percentages; Note: S=Susceptibility; I=Intermediate and R=Resistance

APPENDIX VII: Susceptibility pattern of *Salmonellae* species isolated from blood and stool and water samples

	BLOOD ISOLATES		STOOL ISOLATES					WATER ISOLATES	
	S.Typhi (n=11)	S.Paratyphi B (n=4)	S.Paratyphi B (n=1)	S.Typhimurium (n=13)	S.Enterica (n=16)	S.Enteritidis (n=8)	S.Diarizonae (n=1)	S.Typhi (n=1)	S.Typhimurium (n=4)
Antibiotics	*NS(PS%)*	*NS(PS%)*	*NS(PS%)*	*NS(PS%)*	*NS(PS%)*	*NS(PS%)*	*NS(PS%)*	*NS(PS%)*	*NS(PS%)*
Beta-lactams antibiotic									
Ampicillin	3 (27.3%)	0 (0%)	0 (0%)	4 (30.8%)	4 (25%)	1 (12.5%)	0 (0%)	1 (100%)	3 (75%)
Piperacillin	3 (27.3%)	0 (0%)	0 (0%)	4 (30.8%)	4 (25%)	1 (12.5%)	0 (0%)	1 (100%)	3 (75%)
Piperacillin/tazobactam	11 (100%)	2 (50%)	1 (100%)	12 (92.3%)	9 (56.3%)	7 (87.5%)	0 (0%)	1 (100%)	4 (100%)
Amoxicillin/clavulanic acid	11 (100%)	4 (100%)	1 (100%)	12 (92.3%)	15 (93.8%)	7 (87.5%)	1 (100%)	1 (100%)	4 (100%)
Cephalosporins									
Cefazolin	11 (100%)	2 (50%)	1 (100%)	8 (61.4%)	15 (93.8%)	6 (75%)	1 (100%)	1 (100%)	4 (100%)
Cefoxitin	11 (100%)	4 (100%)	1 (100%)	13 (100%)	16 (100%)	8 (100%)	1 (100%)	1 (100%)	4 (100%)
Cefotaxime	11 (100%)	4 (100%)	1 (100%)	13 (100%)	16 (100%)	8 (100%)	1 (100%)	1 (100%)	4 (100%)
Ceftazidime	11 (100%)	4 (100%)	1 (100%)	12 (92.3%)	16 (100%)	8 (100%)	1 (100%)	1 (100%)	4 (100%)
Ceftriaxone	10 (90.9%)	2 (50%)	0.0%	13 (100%)	11 (68.8%)	7 (87.5%)	1 (100%)	1 (100%)	4 (100%)
Cefuroxime	9 (81.8%)	2 (50%)	1 (100%)	10 (76.9%)	14 (87.5%)	6 (75%)	1 (100%)	0	3 (75%)
Cefepime	11 (100%)	4 (100%)	1 (100%)	13 (100%)	16 (100%)	8 (100%)	1 (100%)	1 (100%)	4 (100%)
Carbapenem									
Imipenem	11 (100%)	4 (100%)	1 (100%)	13 (100%)	16 (100%)	8 (100%)	1 (100%)	1 (100%)	4 (100%)
Meropenem	11 (100%)	4 (100%)	1 (100%)	13 (100%)	16 (100%)	8 (100%)	1 (100%)	1 (100%)	4 (100%)
Non-beta-lactam antibiotic									
Aminoglycosides									
Amikacin	11 (100%)	4 (100%)	1 (100%)	13 (100%)	16 (100%)	8 (100%)	1 (100%)	1 (100%)	4 (100%)
Gentamycin	10 (90.9%)	4 (100%)	1 (100%)	11 (84.6%)	12 (75%)	8 (100%)	1 (100%)	1 (100%)	3(75%)
Quinolones susceptiilty									
Ciprofloxacin	11 (100%)	4 (100%)	1 (100%)	13 (100%)	16 (100%)	8 (100%)	1 (100%)	1 (100%)	4 (100%)
Norfloxacin	11 (100%)	4 (100%)	1 (100%)	13 (100%)	16 (100%)	8 (100%)	1 (100%)	1 (100%)	4 (100%)
Tetracycline	3 (27.3%)	3 (75%)	1 (100%)	11 (84.6%)	13 (81.3%)	7 (87.5%)	0 (0%)	1 (100%)	4 (100%)
Nitofurantoin	8 (72.7%)	4 (100%)	1 (100%)	3 (23.1%)	14 (87.5%)	7 (87.5%)	0 (0%)	1 (100%)	1 (25%)
Trimethoprim/sulfamethoxazole	3 (27.3%)	0 (0%)	0 (0%)	3 (23.1%)	5 (27.8%)	1 (12.5%)	0 (0%)	1 (100%)	3 (75%)

Data are presented as frequencies and percentages; Note: S. Typhi = Salmonella Typhi; S. Paratyphi B= Salmonella Paratyphi B; S. Enterica= Salmonella Enterica; S. Typhimurium = Salmonella Typhimurium; S. Enteritidis = Salmonella Enteritidis; S. Diarizonae = Salmonella Diarizonae; ***NS*** *= Number susceptible;* ***% PS*** *= Percentage Susceptibility.*