CHARACTERIZATION OF *SALMONELLA* AND OTHER GRAM NEGATIVE BACTERIAL PATHOGENS OBTAINED FROM STOOL AND BLOOD AT CAPE COAST TEACHING HOSPITAL

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DECLARATION

I, Ocansey Wiredu Kofi Dickson hereby declare that this submission is my own work towards the award of MPhil in Clinical Microbiology 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

This work is dedicated first to the Almighty God and secondly to all my family members.

ABSTRACT

Salmonella infections are of serious public health concern since these bacteria have been reported as the most frequently cause of foodborne illness and a common cause of human gastroenteritis and bacteremia worldwide. In addition to the increasing rate and invasiveness of salmonellosis, these bacteria are constantly developing resistance to the commonly tested antibiotics. This study was conducted to characterize *Salmonella* among other isolates from stool and blood cultures at the Cape Coast Teaching Hospital. A total of 971 samples comprising of 463 blood and 508 stool were collected and cultured over a 13 month period (March, 2014 – April, 2015). 17 (3.3%) *Salmonellae* and 3 (0.6%) *Shigella flexneri* were recovered from the stool samples cultured and the blood samples yielded bacteria other than *Salmonella.* There was 69/463 (14.9%) prevalence of bacterial isolates from blood. The blood isolates included *Staphylococcus aureus* (24/69, 34.8%), *Citrobacter freundii* (10/69, 14.5%), *Escherichia coli* (6/69, 8.7%) and *Pseudomonas aeruginosa* (6/69, 8.7%). The *Salmonella* serovars encountered were *Salmonella Typhi* (13/17, 76.5%) and *Salmonella Typhimurium* (4/17, 23.5%). There was 82% (14/17) multidrug resistance among the *Salmonellae* with resistance to ampicillin, co-trimoxazole and tetracycline being 65% each. Among the *Salmonellae,* no drug resistance was observed against cefotaxime, ceftriaxone, gentamycin, amikacin, meropenem, levofloxacin and ciprofloxacin and none produced ESBL. Among the blood isolates, resistance to ampicillin, tetracycline and co-trimoxazole were 96%, 91%, 87% respectively. The PFGE analysis indicated different clones that were closely related among the *Salmonella Typhi.* A similar analysis was observed among the *Salmonella Typhimurium* gel pattern analyzed.

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LIST OF ABBREVIATIONS

ESBL	Extended-spectrum beta-lactamase
CCTH	Cape Coast Teaching Hospital
TS	Typhoidal *Salmonella*
NTS	Non-typhoidal *Salmonella*
PFGE	Pulsed-field gel electrophoresis
OPD	Out-patient department
IPD	In-patient department
LPS	Lipopolysaccharide
WHO	World Health Organization

CDC	Centers for Disease Control
USA	United States of America
XLD	Xylose Lysine Deoxycholate
DCA	Desoxycholate-citrate agar
SS	*Salmonella Shigella*
RES	Reticuloendothelial system
DNA	Deoxyribonucleic acid
NARMS	National Antimicrobial Resistance Monitoring System
MDR	Multidrug resistance
SFB	Selenite F broth
MCA	McConkey agar
BHI	Brain Heart Infusion
TSIA	Triple sugar iron agar
API	Analytical Profile Index
CLSI	Clinical and Laboratory Standards Institute
MIC	Minimum Inhibitory Concentration

CHAPTER ONE

1.0 INTRODUCTION

Salmonellae are enteric gram negative organisms that are widely distributed in nature. They can reside as common commensals in the gastrointestinal tracts of animals and man or cause diseases that range from self-limited diarrhea to bacteremia with enteric fever (Hook, 1990). They sometimes invade vascular structures, bone or other localized sites (Hook, 1990). While some *Salmonellae* are ubiquitous, others are highly host adapted, infecting only a limited number of species (D'Aoust, 1989). The most significant human host-adapted *Salmonellae* are *S. Typhi* which cause typhoid fever. Human beings remain the only known host or reservoir for this serovar (Ziprin & Hume, 2001). Although *S. Choleraesuis* can cause gastroenteritis and enteric fever in man (especially children), it is normally a porcine organism. Other *Salmonellae* such as *S. Typhimurium* have a wide host range and these serovars are responsible for the majority of *Salmonellae* infections in man (Ziprin & Hume, 2001).

1.1 HISTORICAL BACKGROUND

Salmonella was uncovered to cause infection by Dr. Daniel E. Salmon, an American scientist. (Voetsch *et al.,* 2004). In France, the association of human intestinal ulceration reported a transmissible pathogen by clinical pathologists in the early 19th century which was later recognized as *Salmonella*. The findings of *Salmonella* started with Eberth's first detection of the bacterium in 1880 and successive isolation of the bacterium accountable for human typhoid fever by Gaffky (Le Minor, 1984). Further investigations led to the characterization and development of a sero-diagnostic technique for the detection of this human disease causative organism by

European scientists (D'Aoust, 1989; Le Minor, 1984). Afterwards, Dr. E. Salmon isolated the organism then believed to be causative bacterium of hog cholera, but later repudiated (Tindal *et al.,* 2005). In honour of Dr. E. Salmon, Lignieres in 1900 named the organism *Salmonella* (Le Minor, 1984). Further researches resulted in the isolation and characterization of other *Salmonellae*.

A great progression ensued with the sero-detection of somatic and flagellar antigens in the *Salmonella* species in the course of the first 2 decades of the 20th century. White (1926) and Kauffmann (1941) were the first to suggest an antigenic scheme for the classification of *Salmonellae*; currently, there are more than 2,500 serotypes counted in the Kauffmann-White scheme (D' Aoust, 2001, Tindal *et al.,* 2005)

1.2 GENERAL CHARACTERISTICS OF *SALMONELLAE*

Salmonellae are Gram negative, short and plump shaped rods, non-capsulated, non-spore forming, facultative anaerobes and aerobic bacterium of the family Enterobacteriaceae (Freeman, 1985). *Salmonella* classification has changed several times yet still, it's not stable. The genus *Salmonella* was formerly divided into two main species: *Salmonella Bongori* and *Salmonella Enterica.* Nonetheless, a new species, *Salmonella Subterranea* was discovered and validated (Shelobolina *et al.*, 2004; Validation List No: 102, 2005). Amongst these species, *Salmonella Enterica* (S. *Enterica*) is further classified into the six (6) subspecies which are *S. Enterica* subsp. *Enterica* (I), *S. Enterica* subsp. *Salamae* (II), *S. Enterica* subsp. *Arizonae* (IIIa), *S. Enterica* subsp. *Diarizonae* (IIIb), *S. Enterica* subsp. *Houtenae* (IV), and *S. Enterica* subsp. *Indica* (VI). Previously, *S. Bongori* was the subspecies V, but afterward considered as a separate species (Fluit, 2005). Fermentation of certain materials, such as malonate, dulcitol, sorbitol, d-tartrate, mucate, galacturonate, salicine,

ONPG and lactose, as well as manufacturing of certain enzymes such as β-glutamyl-transferase, gelatinase or β-glucuronidase, allow a distinction among the various species and subspecies (Le Minor 1984). Again, the genus comprises over 2500 serovars classified according to three discrete types of surface antigens; somatic (O), flagellar (H) and capsular (Vi) antigens. Approximately
99% of these serovars belong to S. Enterica and almost 60% of them are in *S. Enterica* subsp. *Enterica.* The average DNA sequence similarity between *Salmonella* serotypes is 96-99% (Edwards *et al.*, 2002).

1.3 TAXONOMY AND NOMENCLATURE

The classification of *Salmonella* is very complicated and scientists employ various systems to refer to and commune about this genus. Unfortunately, present usage mostly combines several classification systems that separates the genus into species, subspecies, subgenera, groups, subgroups, and serovars (serotypes), and these applications sometimes results in lots of misunderstanding among scientists. The nomenclature of *Salmonella* has advanced through a sequence of taxonomical and serological characteristics and on the standards of numerical taxonomy and DNA homology (Le Minor and Popoff, 1987). The classification of the genus *Salmonella* has progressed from the preliminary one serotype-one species idea recommended by Kauffmann (1966) on the basis of somatic (O), flagellar (H) and capsular (Vi) antigens (Kauffmann, 1966). At the initial stage of taxonomic system, biochemical reactions were employed to divide *Salmonella* into various subgroups and the Kauffmann White system was the first effort to methodically categorize *Salmonella* employing scientific parameters (Scherer and Miller, 2001). This attempt ended up in the development of five biochemically distinct subgenera (I to V) in which, various serotypes or serovars were given status of a species (Kauffmann, 1966, D' Aoust, 2001, Scherer and Miller, 2001, Le Minor and Popoff, 1987).

The International Association of Microbiologists accepted this system in 1934. Clumping of antibodies exclusive to the different O antigens is used to separate *Salmonellae* into the six serogroups: A, B, C1, C2, D and E. For example, *S. Typhi* and *Salmonella Paratyphi* A, B, C express O antigens of serogroups D, A, B, and C1 correspondingly. Over 99% of *Salmonella* species bringing about human diseases are of the *Salmonella Enterica* subspecies *Enterica* (D' Aoust, 2001). Even though not frequent, cross-reactivity between O antigens of *Salmonellae* and other organisms of group of Enterobacteriaceae sometimes happen (Scherer and Miller, 2001). Hence, further separations of serovars is founded on the antigenicity of the flagellar H antigens being greatly specific for *Salmonella* (Scherer and Miller, 2001).

1.4 *SALMONELLA* EPIDEMIOLOGY

In most parts of Africa where the epidemiology of bacterial infection has been considered, *S. Enterica* appears to be a predominant cause of public acquired bloodstream infections. The relative contributions of nontyphoidal and typhoidal serotypes to infections vary in place (Reddy *et al.*, 2010, Biggs *et al.*, 2014) and time, even within same countries (Gordon *et al.*, 2008, Feasey et al., 2015). Throughout the world, approximately 16 million incidences of sicknesses are caused by *Salmonella* annually, with a resultant 600,000 deaths, rising to roughly 22,000,000 symptomatic illnesses and 220,000 fatalities across the globe annually (Crump *et al*., 2004). Humans are the starting point of *Salmonella Typhi* globally, and spread is mostly by the foodborne and waterborne paths (Feasey et al., 2015). This has immensely contributed to *Salmonellae* being among the most frequent bacterial foodborne pathogens in Africa and worldwide. African countries have dropped back to those in other parts of the world in terms of access to enhanced water and sanitation services. In spite of this, little has been done in researches to ascertain locally vital risk factors for *Salmonellae* spread in both rural and urban locations in Africa. (WHO, 2014).

Salmonella serotypes are important zoonotic pathogens in humans. Contaminated food and meat have become a key route of spread for non-typhoidal *Salmonella* because there are a great number of animal reservoirs (Winokur et al, 2000). Frequent animal reservoirs include turkeys, chickens, cattle and pigs; several other wild and domestic animals also harbour these bacteria (Carli et al, 2001, Winokur et al, 2000). Mishu et al, (1994) reported *Salmonellae* prevalence in animals as: 50% of chickens cultured in Massachusetts, 41% of turkeys inspected in California and 21% of frozen egg whites examined in Spokane.

1.5 CHALLENGES POSED BY *SALMONELLAE* AND OTHER BACTERIAL INFECTIONS

Salmonella pathogens continue to pose great threat to public health. Although several reports have it that *Salmonella* infections has declined significantly, particularly in developed world, current events and many publications show continued difficulties in *Salmonellae* control (Poirel *et al.,* 2008). The first challenge is the extensive dissemination of *Salmonellae* in food. Contaminated food and water has been (and continue to be) one the major routes of salmonellosis transmission (Khan *et al.,* 2007). This bacteria cause significant economic loss as a result of mortality, morbidity and deprived development of infected animals, poultry and human beings (Khan *et al.,* 2007).

The second challenge is antibiotics resistance. Species of *Salmonella Enterica* having multiple drug resistance genes have spread out extensively in many countries over the last few decades. There is appearance and spread of resistance in pathogenic bacterium that subsequently cannot be cured by formerly effective antibiotic regimens (WHO, 2004). According to studies by Van *et al.,* (2007), multi-drug resistance occurs in *Salmonella* serovars. Resistance to extended spectrum cephalosporins and fluoroquinolones which are the antibiotics first considered for cure of severe salmonellosis have also been reported (Bush and Jacoby 2010, Poirel *et al.,* 2008). A rise in the

incidence of resistance to extended-spectrum cephalosporins with a correspondence declined susceptibility to fluoroquinolones in *Salmonellae* causing human diseases have been recorded in Southeast Asian countries and across the globe (Lee *et al.*, 2009).

Another challenge of salmonellosis is capacity building to improve early epidemic detection via regularly *Salmonellae* subtyping using molecular methods and a regular surveillance report especially in the developing world (Bush and Jacoby 2010). In spite of the clinical importance of *Salmonella* diseases, surveillance data globally continue to be inadequate and is more so typified by the deficit of researches and publications from Africa particularly in central, eastern and western Africa (Appiah-Korang *et al.*, 2014)

1.6 PROBLEM STATEMENT

Factual information and statistics on *Salmonella* infection are limited in many African countries, South and Central America as well as Asia where just 1 to 10% of incidences are reported (Portillo, 2000, Hanes, 2003, Hu and Kopecko, 2003). There is limited current documentation on the incidence, antibiotic sensitivity patterns, ESBL production and genetic characterizations of *Salmonella* species in Ghana. No such work has been carried out in Cape Coast. Current literature on *Salmonella* infections and other organisms is scanty.

1.7 JUSTIFICATION

Due to the emerging increase in *Salmonella* infections with a corresponding rise in antibiotic resistance in many parts of Ghana, there is the need to access the state of *Salmonella* infection in Cape Coast. This work will help to know the prevalence of *Salmonella*, their antibiotic resistance patterns and genetic relatedness as well as whether they produce ESBL or not.

This will contribute information to national data on the strains of *Salmonella* in Ghana, their prevalence and antibiotic resistance patterns. Also this study will provide current surveillance resistance patterns of other bacteria pathogens to antibiotics.

1.8 AIMS

The aims of this study is to determine the prevalence of *Salmonellae* and other bacterial infections among patience attending the Cape Coast Teaching Hospital, Cape Coast, Ghana.

1.9.1 Objectives

- To culture stool and blood samples for *Salmonella* and other pathogens at Cape Coast Teaching Hospital (CCTH).
- To determine the prevalence of *Salmonella* and other blood borne bacterial pathogens at CCTH.
- To determine the various serovars of the *Salmonella* isolates obtained.
- To determine the antimicrobial resistance patterns of the isolates obtained.
- To determine if the *Salmonella* isolates produce ESBL.
- To determine the PFGE patterns of the *Salmonella* isolates.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 *SALMONELLA*

Salmonella is the genus name applying to a vast number (more than 2,500) of category of bacteria (Stöppler and Shiel, 2012). It is a gram-negative bacilli shaped and non-spore forming usually motile rods affiliated to the family Enterobacteriaceae (Mejia *et al.*, 2006). It is also a facultative anaerobe with diameters about 0.7 - 1.5 µm, lengths of 2 - 5 µm, and flagella that moves in every direction (Ryan and Ray, 2004). These bacteria are chemo-organotrophs, receiving their strength from oxidation and reduction activities employing organic resources and many subspecies generate hydrogen sulfide. *Salmonella* thrives at temperature range of 2°C to 47 °C, with a quick bacteria increase taking place between 25°C to 43 °C. The lowest temperature for its growth exists at neutral pH but rises exponentially with rising alkalinity or acidity of the suspending medium (D' Aoust, 2001). The optimal pH for *Salmonella* growth ranges from 6.5 to 7.5. Also at a concentrations of ≥ 3% (w/v), sodium chloride usually restrains the growth of *Salmonella* (D' Aoust, 2001).

2.2 *SALMONELLA* SPECIES

The genus *Salmonella* is classified into two main species namely *S. Bongori* and *S. Enterica.*

2.2.1 *Salmonella Enterica*

Salmonella Enterica is devided into six sub-species on the bases of their differential biochemical reactions and additionally sub-divided into serovars on the bases of serology of the

lipopolysaccharide (LPS) (O) somatic and flagellar (H) antigens (Grimont and Weill, 2007, WHO, 2001). Amongst a serovar, there could be strains with variance in virulence (Grimont and Weill, 2007). Many of the *Salmonella* serotypes pathogenic to man are members of the *S. Enterica* subspecies *Enterica*. Examples are: *S. Typhi, S. Paratyphi, S. Enteritidis, S. Typhimurium* and *S. Choleraesuis* (Ibekwe et al., 2008). Apart from *S. Typhi* and *S. Paratyphi*, non-typhoidal illnesses caused by other *Salmonella* serotypes are usually self-limiting, with clinical expressions spanning from minor to severe gastroenteritis. In a few of these incidences, extra complications occur, like gastrointestinal bleeding, bacteraemia and focal infections (Acheson and Hohmann, 2001). Paratyphoid and typhoid fevers are infections caused by *Salmonella Paratyphi* and *Salmonella Typhi* respectively, being conveyed from faeces to ingestion. The spread of typhoid and paratyphoid fevers is prevented through clean water, proper sanitation and hygiene (WHO, 2008, Ibekwe *et al*., 2008).

2.2.2 *Salmonella Bongori*

In 1966, *Salmonella Bongori* was uncovered from a lizard in the town called Bongor, Chad, based on which the name Bongori originated (Le Minor et al., 1969). Initially, it was tagged *Salmonella* subspecies V or *S. Choleraesuis* subspecies *Bongori* or *S. Enterica* subspecies *Bongori*. This specie causes a gastro-intestinal illness referred to as salmonellosis, typified by abdominal cramps and diarrhoea. It is normally seen as a microorganism of cold blooded animals, distinct from other affiliates of the genus, and in many times often found in reptiles (Tortora, 2008). S. Bongori obtained the species state in 2005 after periods of disagreement in *Salmonella* classification (Agbaje *et al.*, 2011)

Its infections in animals, unlike other *Salmonellae*, are normally asymptomatic and does not result in visible consequences (Le Minor *et al.*, 1969). Nonetheless, infections in pets are associated with diarrhoea (Tortora, 2008). Again, infection in human beings have been confirmed, with decisive

accounts from Italy (Giammanco *et al.*, 2002). Most of these incidences occur in children who are below 3 years, who are much exposed to mouth contact with animal faeces. In these incidences, symptoms are manifested by diarrhoea with feverishness, and acute gastroenteritis (Giammanco *et al.*, 2002). Table1 shows *Salmonella* species and subspecies and their respective number of serotypes known (WHO, 2001).

Table 1. Number of *Salmonella* serotypes in each species and subspecies

Salmonella species and subspecies	Number of serotypes
Salmonella Enterica	2,480
S. Enterica subspecies *Enterica*	1,478
S. Enterica subspecies *Salamae*	498
S. Enterica subspecies *Arizonae*	94
S. Enterica subspecies *Diarizonae*	327
S. Enterica subspecies *Houtenae*	71
S. Enterica subspecies *Indica.*	12
Salmonella Bongori	21
TOTAL	2,501

2.3 SEROTYPING

The classification of *Salmonellae* into serovars (serotypes) is done on bases of the antigens lipopolysaccharide (O), flagellar protein (H), and sometimes the capsular (Vi). Among serotypes, there might be strains differing in virulence (Acheson and Hohmann, 2001).

On the grounds of shared characteristics in the content of one or more O antigens, members of *Salmonellae* are put into categories designated A, B, C and so on. Hence, *S. Oranienburg, S.*

Hirschfeldii, S. Choleraesuis and *S. Montevideo* are put into group C1 since all possesses O antigens 6 and 7. *S. Newport* is also categorized C2, because it has O antigens K and 8. For extra categorization, the H antigens or flagellar are used. The two divisions are: phase 1 or specific phase and phase 2 or group phase. Whilst specific phase antigens are found on only a few other species of *Salmonellae*; Group phase may be commonly circulated among a number of serovars. Any growth medium may contain either bacteria in only one phase or bacteria in the two flagellar phases. Phase 1 H antigens are labelled with small alphabets while phase 2 H antigens are labelled with Arabic numerals. Hence, a total antigenic analysis of *S. Choleraesuis* for example is: 6, 7, c, 1, 5, whereby 6 and 7 denotes the O antigens, c denotes phase 1 flagellar antigens and 1 and 5 refers to phase 2 flagellar antigens (Acheson and Hohmann, 2001, WHO, 2001).

2.4 ECOLOGY

Salmonellae universally resides in both warm blooded and cold blooded animals, as well as the general surroundings. These organisms are mostly present on raw egg shells, in birds or poultry, and red meat; they also thrive naturally as part of the normal bacterial flora of Amphibia and reptilians **(**Letellier *et al.,* 1999**)**. *Salmonella* has again been identified in almost all groups of animals belonging to Mammalia, Insecta, Reptilia, Aves and also humans (Hanning *et al.,* 2009). The broad circulation of *Salmonellae* depicts the flexibility of this bacteria to adjust to several different habitats and again its ability as a multi-source zoonotic bacteria for human beings as well **(**Letellier *et al.,* 1999**).**

Although better food processing, adequate cleaning and cooking destroys *Salmonellae*; yet, it may be found in many kitchens. Additional to the regular habitats of *Salmonellae,* infected water is among the main reservoirs of contracting *Salmonella* globally. *Salmonella* multiplication occurs mostly in the animal intestines. The bacterium is excreted in the faecal matter. According to Letellier *et al.,* (1999)**,** *Salmonella* is a vertical contaminant through the various production stages.

2.5 *SALMONELLA* SEROVARS AND THEIR DISTRIBUTION

Salmonella Enterica is one of the frequent associate of human gastro-enteritis and blood borne infections globally (Coburn *et al.*, 2007, Jones *et al.*, 2008, Voetsch *et al.*, 2004), and a vast different animals, especially food animals, have been reported as sources of non-typhoidal *Salmonellae* (Humphrey, 2000). In Southeast Asia, one of the frequent serotype, *Salmonella Enterica* serotype Stanley (S. Stanley) was the 2nd most frequent serotype associated with human salmonellosis in Thailand from 2002 - 2007 (Voetsch et al., 2004). However, this serotype is comparatively rare in Europe (Hendriksen et al., 2012). *S. Enteritidis* and *Salmonella Enterica* serotype *Typhimurium* are the most frequently implicated cause of human *Salmonella* infection in the developed countries (Jones et al., 2008)

Salmonella serovars *Paratyphi* and *Typhi* are widespread pathogens in developing countries although they contribute to less than 1% of *Salmonella* diseases in the US (CDC, 2007) and several of such incidences are linked to international travel. Some rare serovars (like *S. Dublin* and *S. Choleraesuis*) are reported to cause increased severity of illnesses than other non-*Typhi Salmonellae* (Blaser and Feldman, 1981). Nonetheless, substantial variations in the severity of infections amongst the common non-*Typhi Salmonellae* serovars are inadequately understood and possibly undervalued (Blaser and Feldman, 1981).

Salmonella Typhi is host specific and is strictly adapted to only human beings. It causes systemic infections and typhoid fever; resulting in an estimated death of 500,000 per annum globally. It has been reported to cause infection to deeper tissues like spleens, livers and bone marrows than several other *S. Enterica* serotypes. *S. Typhimurium* causes gastroenteritis and unlike *S. Typhi*, it is not host specific hence infect many mammalian species (Wong *et al.*, 2002)

Within the previous 2 decades, *Salmonella Enteritidis* has become one of the most frequent cause of human illnesses in several countries, with chicken eggs being a primary source of these pathogens. This has been ascribed to this serotype's uncommon capability to inhabit ovarian tissue of chickens and be found inside the contents of an unbroken shell eggs (Bouchrif *et al.*, 2009)

2.6 RESERVOIRS AND TRANSMISSION

Salmonellae are extensively distributed in the environment with a good survival in different kinds of foods. Some of the regular vehicles of *Salmonella* infections are eggs, poultry and dairy products. Recently, worries have been expressed over produce including fresh vegetables and fruits as routes of transmission in which contamination may happen at several stages along the food chain (Bouchrif *et al.*, 2009).

Salmonellae contaminated surroundings acts as the infection reservoir since *Salmonella* can thrive in these habitats for a long period. It spreads to vectors like flies, rats and birds where it could be released in their faecal matters for weeks and sometimes months. Mobile mammals like pigs and cattle as well as poultry serves as the significant risk factor for *Salmonella* infections after the direct transmission. Animal vehicles are infected by mouth since *Salmonella* generally originates from infiltrated surrounding and infiltrated feeds. Infections in man occurs via foods or drinks contaminated with *Salmonellae* from animal reservoirs. Unlike other *Salmonellae, S. Paratyphi* A and *S. Typhi* cannot be found in animal reservoirs, hence infection could basically occur via eating inappropriately handled foods by individuals with the infection (Newell *et al.*, 2010).

The bacteria could be introduced at several stages of the food chain including animal feeds, food production, processing and selling, catering and food preparation in at homes (Wong et al., 2002).

Disease surveillances reports, mostly detects poultry as the key sources in the outbreak of *Salmonella* infections. Cox and Pavic, (2010) gave a broad outline on meat manufacturing in poultry linked with *Salmonella* and expanded various means of controlling the bacteria along the whole production sequence. The research stipulated the contamination of poultry could occur from breeder flocks, hatchery surroundings, litters, feeds and water troughs in the coops. Arumugaswamy *et al.,* (1995) gave an account of a total *Salmonellae* contamination of 39.4% in chicken parts, where 35.3% of livers and 44.4% of gizzards were infiltrated in Malaysia.

Many outbreaks in North America and Europe since the 1980s have been attributed to infiltration of eggs contents by *Salmonella Enteritidis* (Bhunia, 2008, Jay *et al.,* 1997). Not long ago, The Centers for Disease Control and Prevention (CDC) of the United State stated that there were closely 1469 diseases linked to eggs infestation by *Salmonella Enteritidis* as concluded in California, Colorado and Minnesota, across 1st May to 31st August, 2010. However, *Salmonella Infantis* was the most frequent serovar in egg industries in Australia (Cox *et al.,* 2002).

Salmonella's distribution could be enhanced by water storing containers, wild animal faeces and also animal dead bodies. Poor environmental hygiene, inappropriate waste disposal and unavailability of clean water facilities results in the spread of typhoid fevers. In places with widespread of typhoid fever, water bodies like lakes and rivers serving as the main source of drinking water to communities are usually infiltrated by untreated waste and are the key reservoirs of illnesses. Dangers to the human wellbeing is additionally threatened by the bacteria's first choice to increase at ambient temperature on fresh produce during sales exhibition (Bordini *et al.,* 2007, Penteado and Leitão, 2004).

Table 2. Examples of reported salmonellosis outbreak

Year	Country	Source	Serotype	Number of cases	Reference
2010	United States	Black and red pepper	Montevideo	272	CDC, 2010
2010	United States	Frozen mamey fruit pulp	Typhi	9	CDC, 2010
2010	United States	Shell eggs	Enteritidis	2,752	CDC, 2010
2009	United States	Alfalfa sprouts	Saintpaul	235	CDC, 2010
2008	United States	Cereal from Malt-O-Meal	Agona	28	CDC, 2010
2007	United States	Dry pet food	Schwarzengrund	62	CDC, 2010
2006	United States	Peanut butter	Tennessee	>288	Montville and Matthews, 2008
2005	Austria	Mixed salad	Enteritidis PT21	85	D'Aoust and Maurer, 2007
2005	England	Kebab	Enteritidis PT1	195	D'Aoust and Maurer, 2007
2005	The Netherlands	Imported raw beef	Typhimurium PT104	165	D'Aoust and Maurer, 2007
2005	Malaysia	Stall food	Typhi	171	Nik and Sharifah, 2005
2004	China	Cake/raw egg topping	Enteritidis	197	D'Aoust and Maurer, 2007
2004	Great Britain	Lettuce	Newport	>350	Montville and Matthews, 2008
2003	Germany	Aniseed herbal tea	Agona	42	D'Aoust and Maurer, 2007
2001	Canada/Australia	Shandong peanuts	Stanley	93	D'Aoust and Maurer, 2007
2001	Norway, Sweden	Fish	Livingstone	60	D'Aoust and Maurer, 2007
2000	Singapore	Dried anchovy	Typhimurium DT104L	33	Ling *et al.*, 2002
1999	Japan	Dried squid	Salmonella spp.	<453	Montville and Matthews, 2008
1996	France	Mont D'or cheese	Salmonella spp.	14	Colak *et al.*, 2007
1994	Switzerland	Potato salad prepared by carrier	Typhi	10	Gruner *et al.*, 1997

2.7 CULTURE AND ISOLATION OF *SALMONELLAE*

Salmonella are Gram-negative bacteria. They are found in the bowel where there is a population of normal flora even in a healthy bowel, so their isolation from stool requires the use of a selective medium. In medical laboratories, *Salmonellae* are normally isolated on *Salmonella Shigella* (SS) agar, Xylose Lysine Deoxycholate (XLD) agar or Deoxycholate-citrate agar (DCA). (Gracias and McKillip, 2004). *Salmonella* cells may be very few in a given sample that, faeces are routinely subjected to enrichment media where a little amount of faeces is inoculated into a selective broth medium, like selenite faecal broth or Rappaport Vassiliadis Soya peptone broth and incubated overnight. These media inhibits to the multiplication of the normal flora of the bowel, whilst enhancing *Salmonella* to increase significantly in quantity. *Salmonella* could then be retrieved by the inoculation of the enrichment broth on one or more of the primary selective agar. The bacteria do not ferment lactose (Ryan and Ray, 2004; Tortora et al, 2001, Gracias and McKillip, 2004).

2.8 GENERAL PATHOLOGY OF *SALMONELLA*

Salmonella serotypes are associated with a range of illnesses from gastroenteritis to typhoid fever, and they are usually distributed via the ingestion of infected foods and water (Hanning *et al.*, 2009). Once they enter the victim cell, there is a rise in the quantities of intracellular free calcium and a reorganization of cytoplasmic inclusions. It also messes up the plasma lemma, which happens to be a critical stage in the entry process (Neidhardt *et al.*, 1996 (ed.)). The highly frequent *Salmonellae* infections (mainly by *Salmonella Enterica* serotype *Enteritidis)* usually result in diarrhoea, feverishness, or abdominal discomfort (Hanning *et al.*, 2009). *Salmonella* infections sometimes self-cure without medication within five to seven days of the infection except when victims become sternly dehydrated or when the disease spreads. Although salmonellosis is usually treated with antibiotics like trimethoprim/sulfamethoxazole, gentamicin, ampicillin and

ciprofloxacin, many of the organism have developed resistance to some of these drugs as an outcome of the usage of antibiotics to enhance the development of feed livestock. Though several persons with this kind of salmonellosis mostly regain health totally, few others acquire Reiter's syndrome resulting in joints pains, eyes irritations, as well as pains when passing urine. This syndrome could persist for months or even years, and could also result in chronic arthritis (CDC, 2007).

2.9 SALMONELLOSIS

Some strains of *Salmonella* cause a disease called Salmonellosis. It is mainly characterized by an acute startup of feverishness, abdominal cramps and discomfort, diarrhoea, nausea and oftentimes vomiting. Signs and symptoms appears 6 to 72 hours from ingestion of *Salmonellae,* and the disease remains 2 to 7 days (WHO, 2013). *Salmonella* bacteria happens to be the most commonly implicated cause of food-borne diseases. *Salmonella* infections are among the primary infectious causes of enteric illnesses in human globally, and in many incidences are very likely to be associated to food products from livestock (Mejia *et al.,* 2006). The majority of human *Salmonellae* diseases result from a restricted number among the over 2,600 *Salmonella* serotypes reported to date. *S. Typhimurium*, *S. Newport* and *S. Enteritidis* are three frequent serovars associated with human illnesses. It has also been reported that the prevalence of certain *Salmonella* serotypes differs by geographical area (Hendriksen *et al.,* 2011, Sirichote *et al.,* 2010, Lee *et al.,* 2009, Hendriksen *et al.,* 2012)

Salmonella carriage to man is basically linked to the ingestion of a several types of infected food materials, though it could also be caused by contact with livestock, infected water and persons (Hanning *et al.,* 2009). Reports state that, a range of one million to one billion *Salmonellae* cells are required in establishing infections though certain researchers propose that others could be infected by far lesser bacterial cells (Lee

et al., 2009). Gastric acid in man could decrease and even exterminate *Salmonellae*. Sometimes certain bacterial cells pass on to the intestine and stick to the wall, later penetrating the cellular lining the intestines. Invasive mechanisms by the bacterium results in cellular death leading to intestinal fluid loss (diarrhea) (Davis and Shiel, 2012). Diseases usually resolve by themselves hence antibiotics medication is not advised for simple illnesses (Gill and Hamer, 2001). However, extra intestinal infection may take place, especially in children, the aged and immunocompromised persons. With such incidences, efficient antimicrobial medication is vital (Cruchaga *et al.*, 2001).

Salmonella infections posse crucial public health worry that, it is assessed to result in 627,200 incidences of illnesses amounting to Can$846.2 million per annum in Canada. This exceeds twice the valued amount for all other food-borne diseases put together (Todd, 1989). The financial weight linked to medical attention and decreased productivity owing to *Salmonella* infections is valued as some billion dollars every year in the USA. Again, this bacteria is amongst the frequently documented enteric pathogens, projected to result in 1.4 million diseases with 400 fatalities per annum in the USA (Voetsch *et al.*, 2004)

Non typhoidal *Salmonella Enterica* basically initiates a self-limiting incident of gastro-enteritis, portrayed by diarrhoea, feverishness, abdominal discomforts, and loss of fluid (Sirichote *et al.*, 2010). Several events are mild, usually not notified to public health facilities. Nevertheless, very severe consequences can occur due to the illness, respective to host factors and *Salmonellae* serovar. Severe illnesses can incident in healthy persons, although it is mostly observed in the immune-compromised, children, and the aged (WHO, 2008). Moreover, a little percentage of incidences in healthy people are complicated by chronic reactive arthritis. Globally, the incident rate of this disease according to estimates is approximately 17 million every year (WHO, 2008, Ibekwe *et al.*, 2008).

2.9.1 Clinical manifestation

Salmonella's infectivity is dependent on factors such as serotype, strains of bacterium, growth conditions and hosts' susceptibility. Nonetheless, host factors regulating susceptibility to infections are intestinal tract conditions, age, underlying disease and immune-deficiencies. *Salmonella* infectivity dose widely ranges from 1 - 109 cfu/g. Nevertheless, a single food source outbreaks means as few as 1 - 10 bacterial cells may result in *Salmonella* infection. (Yousef and Carlstrom, 2003, Bhunia, 2008).

2.9.1.1 Enteric fever

S. Typhi and *S. Paratyphi* A, B and C are associated with typhoid fevers and para-typhoid fevers respectively, where symptoms of the later are milder with less mortality rate than the former. Both serovars are host specific and occur only in man. Diseases mainly happen as a result of ingesting foods and water contaminated with human wastes. Currently, antimicrobial resistant strains have been reported in many widespread/endemic regions, especially South-east Asia, India, Pakistan and Middle-East (Scherer and Miller, 2001).

Typhoid fever (enteric fever) due to *S. Typhi* is a widespread infection in both the tropics and subtropics and has been a key public health burden in developing countries of the world with an extrapolated yearly occurrence of 540 for each 100,000 persons.

Approximately ten percent of infected individuals might relapse, die or experience severe complications like typhoid encephalopathies, gastro-intestinal bleedings and intestinal perforations. Relapses are the most frequently encountered likely because of the persistence of the bacteria in the reticuloendothelial system (RES). Typhoid encephalopathies, usually with shocks, are linked with high fatality/mortality. Minor gastro-intestinal bleedings normally solve without

blood transfusion though one to two percent of incidences could end in fatality when bigger blood vessels are affected. Intestinal perforations might result in abdominal pains, increased pulse and decreased blood pressure of patient, posing danger in 1% - 3% of admitted persons (Hu and Kopecko, 2003; Parry, 2006).

2.9.1.2 Gastroenteritis

Non-typhoidal *Salmonellae* infections or enterocolitis is associated with about 150 *Salmonellae* serovars but the most predominant ones are *S. Typhimurium* and *S. Enteritidis*. Diseases are mainly the consequence of ingesting water and/or foods contaminated with livestock wastes instead of human wastes. The development of multi-drug resistant *S. Typhimurium* DT104 with implicated outbreaks linked to beef contamination, has caused double the admission rates compared to other food-borne *Salmonella* infections (Yousef and Carlstrom, 2003).

2.9.1.3 Bacteremia and other complications of non-typhoidal salmonellosis

Closely eight percent of unattended incidences of *Salmonella* illnesses end up as bacteremia. Bacteremia is a critical situation where bacterial cells move into bloodstream via penetration of the barriers of the intestines. This is connected to more invasive serovars such as *S. Cholearaesuis* and *S. Dublin*. Bacteria in blood by *Salmonellae* must be considered in events of feverishness of unknown source. Individuals having bacteremia and other complications must receive antimicrobial medication (Scherer and Miller, 2001; Hanes, 2003).

2.9.1.4 Chronic carrier state

Salmonella infections may be distributed by chronic carriers with potential infestation to several people, particularly persons working in food related facilities. Circumstances enhancing chronic

carriage, still remain partially clarified. Non-typhoidal serovars persist in the gastro-intestinal system for roughly 6weeks-3months, subject to the type of serovar. Approximately 0.1% of nontyphoidal *Salmonellae* incidents are excreted in faecal substances up to more than a year. Again 2% - 5% of unresolved typhoid illnesses end up in chronic carrier states. Furthermore, about 10% of unresolved convalescent typhoid incidents sheds *S. Typhi* in faeces for one to three months and between one percent to four percent turn chronic carriers to excrete the organism over a year (Scherer and Miller, 2001; Parry, 2006).

2.9.2 Seasonality of salmonellosis

There is seasonality in the occurrence and frequency of *Salmonella* infections in Ghana. *Salmonella Typhi* infections were seen to follow the rainfall pattern with the peak in July (Marbell *et al.,* 1974). Moreover, the isolation of *Salmonella* serotypes was more in the cold periods (October-December) than in the warm periods (July-September) in Spain (Martinez-Urtaza *et al.*, 2004).

Kariuki *et al.*, (2006a, 2006b) in two different studies in Kenya also noticed that a higher number of samples were obtained in May and June (it mostly rains during these months). Seasonal trends were again observed in nontyphoidal *Salmonella* infections (*Enteritidis*) in man at East Asia (*Typhimurium*) and India where the highest infections occurred in the colder periods (November-December) (Ekdahl *et al.*, 2005). A similar study at Korea reported that nontyphoidal *Salmonella* diseases surfaced mostly at the summer season (June-August) (Cho *et al.*, 2008).

2.10 GEOGRAPHY AND INVASIVENESS OF *SALMONELLAE*

Geographic distributions is highly significant in the proportion of invasiveness due to a specific serotype of *Salmonella* (Fernandes *et al.*, 2006). Many of the serotypes considered invasive at US happens to be invasive worldwide; nevertheless, others currently invasive at US are not resulting in invasive diseases in other regions of the globe (Gemma *et al.*, 2009). For instance, the invasive index of serotype *Salmonella Schwarzengrund* is 15.4% at US and Brazil (Fernandes *et al.*, 2006) but is 25% in Taiwan (Lauderdale et al., 2006). Again, in many sub-Saharan African regions, Nontyphoidal *Salmonella* are currently the most frequent causes of bacteremia (Gordon *et al.*, 2008). In such places, events of invasiveness are normally associated with serotypes *S. Typhimurium* and *S. Enteritidis*, occurring in widespread of illnesses brought by antimicrobial resistant strains related to HIV status and age (Gordon *et al.*, 2008).

In their research, Gemma *et al*, 2009 further indicated that, severity of *Salmonella* diseases due to distinct serotypes are liked not only to host' factors in man (Gordon *et al.*, 2002) but also to bacterial phylogeny (Kariuki *et al.*, 2006a).

2.11 INFANTS AND *SALMONELLA* INFECTIONS

Salmonellosis is very common amongst infants than the overall populace (Voetsch *et al.*, 2004, Jones *et al.*, (2006), with a low incidents amongst breastfed babies, implying that breastmilk may have certain constituents inhibiting *Salmonellae.* Studies conducted by Liu *et al.*, (2012), concluded that human milk contains mucins that are vital in the foundational development of new oral prophylactics and therapeutic substances preventing infant illnesses due to *Salmonellae* and liked pathogenic organisms (Bo Liu *et al.*, 2012)

Nontyphoidal *Salmonellae* is amongst the highly significant entero-pathogens implicating bacteria in blood in the young child in several regions worldwide. Extra-intestinal illnesses due to *Salmonellae* strains mostly result in high morbidities and mortalities amongst young children in the tropics of African regions (Enwere *et al.*, 2006; Hill *et al.*, 2007, Berkley *et al.*, 2005). In the developed world, nontyphoidal *Salmonellae* mostly cause mild gastro-enteritis, whilst extraintestinal infections are rare (Graham *et al.*, 2000). Conversely, current researches in sub-Sahara African regions, document nontyphoidal *Salmonellae* as constantly amongst the most frequently implicated paediatrics' bacteraemia (Hill *et al.*, 2007)

Nontyphoidal *Salmonellae* illnesses are related to malaria and anaemia, oftentimes confusing diagnosis hence delaying the right management/treatment (Berkley *et al.*, 1999; Graham *et al.*, 2000). In an instance, Nontyphoidal *Salmonellae* were the highest isolates from blood in children who continued to be feverish after malaria treatment in Ghana (Commey *et al.*, 1994).

2.12 PREVALENCE OF *SALMONELLA* INFECTION

An intercontinental report summary by Thorns, (2000) gave an estimate incidence rate of *Salmonella* infections in every 100, 000 persons for 1997: 14 in United States of America, 38 in Australia, and 73 in Japan. The European Union, recorded an estimate ranging from 16 incidents in every 100, 000 (Netherlands) to 120 incidents in every 100, 000 in some areas of Germany (Thorns, 2000).

There are inadequate information on the incidence rates of nontyphoidal *Salmonellae* among African countries, and distinct geographical areas sustain diverse serotypes, with different serotypes predominating at distinct seasons. Nonetheless, the World Health Organization review among every age category bothering on the worldwide spread of *Salmonellae* between 2000 and 2002 indicated that amongst

pathogens of humans, *Salmonella Enteritidis* appeared the most frequent serotype, contributing to 65% of all isolates with *Salmonella Typhimurium* and *Salmonella Newport* following having 12% and 4% respectively (Galanis *et al.,* 2006). However, similar studies in certain African regions including Morocco, Cameroon, Senegal, Mali and Tunisia in 2002 reported that *Salmonella Enteritidis* and *Salmonella Typhimurium* were evenly distributed among all the age categories (Galanis *et al.,* 2006). The dissimilarities in the patterns of nontyphoidal *Salmonellae* diseases could be the result of ecology i.e. animal reservoirs or variations geography in the African sub-region and globally.

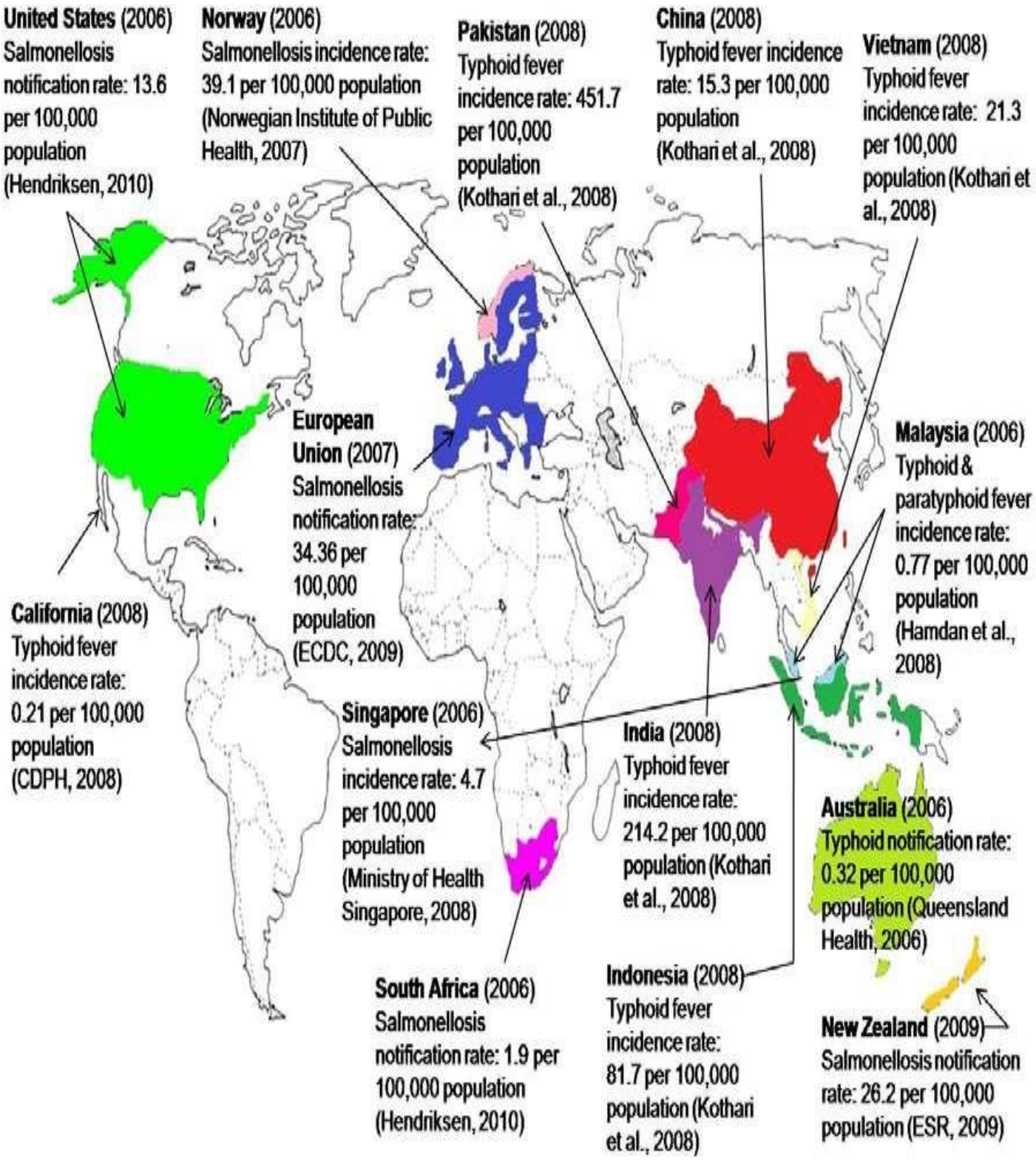
Typhoid events are steady having small numbers in the industrialized world, while non-typhoidal *Salmonella* infections have risen globally. Among the developing countries, typhoid fevers mostly cause death in 5% - 30% of typhoid infested persons. World Health Organization approximates 16 -17million incidences per annum, causing roughly 600 000 loss of lives. Mortality rate varies from area to area, though may be as high as 5% - 7% irrespective of the usage of the right antimicrobials. Conversely, non-typhoidal incidents result in 1.3billion events where 3million die. In the US, closely 2 - 4million incidents of *Salmonellae* gastro-enteritis happen where 500 die yearly. Exact numbers of these illnesses are hard to estimate since generally only a vast outbreak gains investigation whilst small and periodic events are under reported. Statistics on these diseases are limited in several Asian, African, Southern and Central American countries where just 1% -10% incidents reports are made (Hanes, 2003; Portillo, 2000; Hu and Kopecko, 2003).

Typhoid fevers are endemic with persistence in the Middle-East, certain East and South-European countries as well as Central and South-American countries. It is largely an illness of returningtravelers in the United States and many parts of Europe. Typhoid occurrences among widespread regions is basically less in the early years of life, peaking in school going aged children and young adults, finally dropping in middle aged persons (Scherer and Miller, 2001; Parry, 2006).

In a study by Usman *et al.*, 2007 which considered Molecular epidemiology of public acquired invasive NTS in children between 2 – 29 months in Gambian rural areas, *S. Enterica* serotype *Enteritidis* was the most frequent (80.6%), and then *S. Enterica* serotype *Typhimurium* (8.0 %). From 1995-1998, a survey was carried out in southern Latin America on food-borne outbreaks caused by bacterial pathogens. Is was reported that *Salmonellae* were the most accountable (36.8%) among all incidents in the area (Parry, 2006). *Salmonella* strains were the pathogenic organisms mostly found in outbreaks in the European province, accounting for 77.1% of the outbreaks documented where the causative microbes were verified (WHO, 2001).

Figure 1. Distribution of *Salmonella* infections in different parts of the world (CDC, 2007).

Figure 2. Some of the incidence and notification rate of sallmonellosis and enteric fever in different parts of the world (Pui *et al.*, 2011).


United States (2006) Salmonellosis notification rate: 13.6 per 100,000 population (Hendriksen, 2010)
Norway (2006) Salmonellosis incidence rate: 39.1 per 100,000 population (Norwegian Institute of Public Health, 2007)
Pakistan (2008) Typhoid fever incidence rate: 451.7 per 100,000 population (Kothari et al., 2008)
China (2008) Typhoid fever incidence rate: 15.3 per 100,000 population (Kothari et al., 2008)
Vietnam (2008) Typhoid fever incidence rate: 21.3 per 100,000 population (Kothari et al., 2008)
European Union (2007) Salmonellosis notification rate: 34.36 per 100,000 population (ECDC, 2009)
Malaysia (2006) Typhoid & paratyphoid fever incidence rate: 0.77 per 100,000 population (Hamdan et al., 2008)
California (2008) Typhoid fever incidence rate: 0.21 per 100,000 population (CDPH, 2008)
Singapore (2006) Salmonellosis incidence rate: 4.7 per 100,000 population (Ministry of Health Singapore, 2008)
India (2008) Typhoid fever incidence rate: 214.2 per 100,000 population (Kothari et al., 2008)
Australia (2006) Typhoid notification rate: 0.32 per 100,000 population (Queensland Health, 2006)
South Africa (2006) Salmonellosis notification rate: 1.9 per 100,000 population (Hendriksen, 2010)
Indonesia (2008) Typhoid fever incidence rate: 81.7 per 100,000 population (Kothari et al., 2008)
New Zealand (2009) Salmonellosis notification rate: 26.2 per 100,000 population (ESR, 2009)

2.13 OTHER GRAM NEGATIVE BACTERIAL PATHOGENS

The family Enterobacteriaceae is the largest and most heterogeneous collection of medically significant gram-negative rods and are commonly isolated from clinical specimens. There are over fourteen genera that been described to cause human infection but by far the most important single species is *Escherichia coli*. *Salmonella* are a major cause of gastroenteritis. Other important genera include *Shigella, Proteus*, *Klebsiella, Enterobacter, Citrobacter, Serratia* and *Yersinia.* (Health Protection Agency 2010, Ryan *et al.*, 2004)

2.13.1 Epidemiology of gram negative pathogens

Enterobacteriaceae are found in soil, water, and vegetation and are part of the normal enteric flora of all animals including humans. Some members of the family e.g. *Shigella, Salmonella, Yersinia pestis* (the plague bacillus) are almost always associated with disease when isolated from humans i.e., they are never "normal" flora, whereas others e.g. *Escherichia coli, Klebsiella pneumoniae*, and *Proteus mirabilis* are members of the normal commensal flora and usually cause opportunistic infections (Navarro *et al.*, 2002, Levinson, 2010).

Infections caused by the Enterobacteriaceae can originate from an animal reservoir, infected human beings with illness or by endogenous spread of organisms in a susceptible patient. Infections can involve virtually any body site. More than 5% of hospitalized patients develop hospital acquired (nosocomial) infections, with members of the Enterobacteriaceae responsible for a large proportion of these infections (Levinson, 2010).

2.13.2 Pathogenesis of gram negative pathogens

Endotoxin is synonymous with the Gram-negative cell wall, in particular the LPS component. It is responsible for many of the toxic manifestations of infections with Gram-negative bacilli. The intravascular presence of endotoxin stimulates a cascade of pro-inflammatory cytokine production that produces the dreaded clinical picture of "Gram-negative septicemia" manifest by fever, leukopenia, disseminated intravascular coagulation, shock and often death. Hair-like projections on the surface of the bacilli called pili or fimbriae mediate attachment to host cells. (Navarro *et al.,* 2002, Levinson, 2010)

2.13.3 Escherichia coli

Escherichia coli are present in the gastrointestinal tract of all people in large numbers and are frequently associated with infections of the urinary tract or the abdominal cavity when gastrointestinal tract integrity has been compromised. Most infections are endogenous. The exceptions are toxin mediated diseases associated with particular serotypes (strains) of *E. coli*. The antigenic composition of *E. coli* is complex, with more than 170 cell membrane (O) antigens, 56 flagellar (H) antigens and numerous capsular (K) antigens described. The serological differentiation of *E. coli* isolates is not routinely performed but may be useful for epidemiological purposes. The exception is in the active searching for *E. coli* O:157 H:7 in stool from patients with diarrhea. (Russell and Jarvis, 2001, Ryan *et al.,* 2004)

2.13.3.1 Clinical manifestations of E. coli

E.coli is the cause of a large majority of urinary tract infections. It is also an important organism in any infective process involving disruption of the GI tract, almost always as part of a polymicrobial process. Several types of toxin mediated diseases caused by specific serotypes of E.

coli including Enterohemorrhagic *E. coli*, Enterotoxigenic *E. coli* and Enteroinvasive *E. coli* (Russell and Jarvis, 2001, Ryan *et al.*, 2004)

2.13.4 Yersinia

Yersinia pestis is the etiologic agent of plague. People usually get plague from being bitten by a rodent flea that is carrying *Y. pestis* or by handling an infected animal. During a five year period in the middle of the fourteenth century epidemic plague (the Black Death) claimed 25,000,000 people – almost one quarter of the European population. There are two clinical forms – bubonic and pneumonic. The bubonic plague is characterized by painful, inflamed, swollen lymph nodes in the groin or axillae with fever and bacteremia. The pneumonic form is often lethal, is much more infectious and was responsible for the rapid epidemic spread seen in the squalid conditions of the European Middle Ages. (Zhou *et al.*, 2006)

Yersinia enterocolitica is a well-recognized but uncommon cause of gastroenteritis. Mesenteric adenitis, a condition that can often be mistaken for acute appendicitis is also a manifestation of infection with this organism (Zhou *et al.*, 2006).

2.13.5 Serratia, Proteus, Citrobacter and Enterobacter

These four genera are all members of the family Enterobacteriaciae that, collectively, are sometimes referred to as “SPICE” enteric Gram-negative bacilli. These genera are organisms of particular concern in hospitals and are inherently resistant to antibiotics. Selection of antibiotics for hospital-acquired infections is influenced by the types of enteric Gram-negative bacilli that are found in particular institutions. (Ryan *et al.*, 2004)

2.13.6 Vibrio and Campylobacter

The genus Vibrio is comprised of many species of motile, aerobic Gram-negative rods that are found in aquatic environments. Three species are commonly pathogenic for humans, the most important being *V. cholerae* – the etiologic agent of epidemic cholera. While some cases are mild, the profuse, watery diarrhea of severe cases of cholera can cause death by dehydration in hours. The presence of cholera is a marker of inadequacy of water and sewage treatment and thousands of people still die annually of this preventable illness. (Ryan *et al.*, 2004, Connerton *et al.*, 2011)

2.13.7 Pseudomonas aeruginosa

Pseudomonas aeruginosa is a ubiquitous organism found in soil, vegetation and water. It is the most important of a large group of environmental organisms commonly referred to as "pseudomonads" that share many phenotypic characteristics. They only utilize oxidative means of respiration and are not able to metabolize substrates via fermentative pathways. Sometimes pseudomonads and similar, but genetically unrelated Gram-negative rods such as *Achromobacter spp*. and *Alcaligenes spp.* are referred to as "non-fermenters" in contrast to the Enterobacteriaciae. (Anupurba *et al.*, 2006)

P. aeruginosa is the prototypic opportunistic pathogen in that it very rarely causes disease in immunocompetent people but can cause very serious infection of almost any tissue in people with impaired immune function. It is a particularly worrisome organism in patients undergoing cancer chemotherapy and in the Intensive Care Unit. It is inherently resistant to many antibiotics. (Anupurba *et al.*, 2006)

2.14 STOOL BACTERIAL PATHOGENS

The transmission of infectious pathogens such as bacteria, viruses, protozoa and helminths are generally via the faecal-oral route (Nimri and Meqdam, 2004). These pathogens are collectively referred to as entero-pathogens which are defined as being capable of causing infections in the intestinal tract (El Sheikh and El Assouli, 2001). The entero-pathogens enter the faecal–oral route of the transmission pathway via faeces as a result of inadequate sanitation and hygiene as well as food and water that may be contaminated with human and animal faecal matter (Howard *et al.*, 2002, Nimri and Meqdam, 2004)

The common bacterial entero-pathogens aside *Salmonella* are diarrhoegenic *Escherichia coli* (DEC), *Vibrio cholerae* types (*V. cholerae* types) and *Shigella spp.* These bacteria can further be classified as entero-toxigenic *V. cholerae* and entero- toxigenic *E. coli* (ETEC), entero- invasive (*S. dysenteriae, S. Typhi* and entero- invasive *E. coli* (EIEC)), enteropathogenic [(entero- pathogenic *E. coli* (EPEC)] and entero-haemorrhagic (enterohaemorrhagic *E. coli* (EHEC)) bacteria (Howard et al., 2002, Nimri and Meqdam, 2004).

Shigella species are the causative agents of shigellosis or bacillary dysentery (Torres, 2004). Infection of individuals with *Shigella spp*. manifests with bloody diarrhoea, fever and stomach cramps (Keusch *et al.*, 1972). There are 4 strains of *Shigella* namely; *Shigella dysenteriae, Shigella flexneri, Shigella sonnei* and *Shigella boydii* (Todar, 2007). The route of transmission for *Shigella spp*. is usually via the faecal-oral route due to consumption of faecally contaminated water (Torres, 2004). *S. dysenteriae* causes deadly epidemics in developing countries around the world annually. With a very low infectious dose of 10-200 organisms by ingestion, the pathogenecity of *Shigella spp*. is activated when the bacteria invade the cells lining the colonic

mucosa, wherein they multiply and kill the cells *S. dysenteriae* secretes a toxin that plays a role in the destruction of the tissues (Torres, 2004; Day and Maurelli, 2002).

2.15 TREATMENT OF *SALMONELLA* INFECTIONS

Several mechanisms have been attributed to the report of the rise in the problems of diseases caused by bacteria. These include (1) antibiotics treatments failures or complications, that turns more tangible as the figure of antimicrobials in the resistance patterns escalates; (2) rise in virulence, brought by the connection of virulence determinants with genetic factors coding for resistance; and (3) reduced colonization resistance of intestinal microorganisms due to past antibiotics medication, which enhances infections with a resistant specie of bacterium (Barza, 2002).

Therapy for *Salmonellae* diseases is mainly antimicrobial drugs, though there are resistant serovars. Though antimicrobials may effectively cure the illness, the medication sometimes impart normal microflora in the intestines, hence causing risks of secondary enteric disturbance (Savino *et al.,* 2011). According to Linden *et al.,* (2008), the treatment for *Salmonella* infection includes regulation of cellular signals and transcriptions and modulation of the attachment of bacterial cells to the epithelial lining of the intestines, with attaching by both mutualistic and pathogenic microbes.

Chloramphenicol was applied effectively as the first line drug in treating typhoid fevers between the 1950s and 1970s (Crump *et al*., 2004). Emergence of resistance to this drug occurred in 1972, attributed to self-transmissible *Inc*HI plasmids. Trimethoprim/sulfamethoxazole and ampicillins were resorted to, but specie resistance to all 3 antimicrobials developed quickly in the 1980s and 1990s in South-Asia and Southeast-Asia, the Middle-East and Africa (Crump *et al*., 2004, Lee *et al*. 2003). In overriding this problem, fluoroquinolones were introduced as drugs of choice.

Notwithstanding, in the few years, ciprofloxacin resistant species have emerged in the Asia (Crump *et al*., 2004).This problem has warranted the usage of ceftriaxone and cefotaxime as alternates in curing enteric fevers (Lee *et al*. 2003).

In the treatments of gastroenteritis, ciprofloxacin is usually given at the early indications severe gastro-enteritis whilst ceftriaxone is offered to children having systemic *Salmonella* illness. In industrial livestock such as pigs, therapy is mostly contraindicative, and when need be, it may be administered by injections with many therapeutic alternates on the basis of factors like withdrawaltime. Antimicrobial therapies are generally not considered apart from sporadic events since it may extend bacterial presence in faeces (Yousef and Carlstrom, 2003, Gray and Fedorka-Cray, 2002).

Furthermore, D'Aoust states that, *Salmonella* disease therapies are basically restricted to fluids and electrolytes replacement (D'Aoust, 2001). Antimicrobials are not the best resort since they extend recurrent shedding of *Salmonellae* as well as the carrier state following an acute-phase of *Salmonella* infection. Antimicrobial usage again reduces endogenous gut microorganism numbers which contend with *Salmonellae* for mucosal attaching sites (D'Aoust, 1989).

2.15.1 Vaccines

Salmonella Vaccines are difficult because of the different variety of serotypes and the limitations in specificity to a single or a small number of closely linked serotypes. Commercially produced vaccines are in existence in attenuated forms for human and poultry. An oral Vivotif Berna vaccine contains an avirulent *S. Typhi* specie, providing defense up to 7years in human. Recently, important progression in the production of attenuated-vaccines of *S. Enterica* serotype *Typhi* species have been made. Clinical vaccination trials of these have proven to have much tolerance

and immunogenicity. An instance is the attenuated *S. Enterica* serotype *Typhi* strain CVD 908htrA (aroC aroD htrA), Ty800 (phoP phoQ) and chi4073 (cya crp cdt) which are more capable possible typhoid vaccines. Again, clinical tryouts have shown that *S. Enterica* serotype *Typhi* vaccines having heterologous antigens, like that of tetanus toxin-fragment C, could stimulate immune response to the available antigens in human volunteers. In several instances, difficulties linked with the expression of antigens among *Salmonellae* have effectively been handled making the development of *Salmonella* vaccines in times ahead highly promising (Garmory *et al.,* 2002, Zhang, *et al.,* 2013).

In past years, large researches have indicated that, the attenuated *Salmonellae* vaccines vectors are maximal carriers for transporting passenger-antigens to the mucosa walls to stimulate humoral, cellular, and mucosal immunities. These immunities gives defense against problems with the wildtype pathogens from which the passenger-antigens were obtained. Countless researches have proven that, uses of attenuated *Salmonella* vaccines in recombinant multivalent vaccines development have several merits (Zhang, *et al.,* 2013).

2.16 ANTIBIOTIC SUSCEPTIBILITY AND RESISTANCE

Highly infectious diseases due to bacteria which have developed resistance against frequently used antimicrobials have gained key worldwide health care concern in the 21st century (Alanis, 2005). Main dangers concerning antimicrobial usage is the appearance and distribution of resistance among bacterial pathogens which subsequently could not be cured by formerly effective therapies (WHO, 2001). Contrast with illnesses due to antibiotic-susceptible microbes, illnesses due to microbes resistant to one or more antimicrobial have been linked to greater rate of morbidity and/or mortality. Aside facts that there is natural resistance amongst all or many strains of such species

(intrinsic resistance), several others obtain resistance due to mutation in the existing DNA of the bacteria or may obtain new DNA (Montville and Matthews, 2008, Barza, 2002).

Other factors responsible for antibiotic resistance include contact with sub-optimal degrees of antibiotics, broad spectrum antimicrobials and microorganisms harbouring resistant DNA, insufficient hygienic surroundings in health facilities, noncompliance with treatment, antimicrobials for viral diseases, selling drugs without clinical directions and the use of antibiotics in foods/agriculture have all contributed to the provision of selective pressure that have resulted in antibiotic resistant bacteria and resistance genes (Barza, 2002).

Resistance of *Salmonellae* to a single antimicrobial was initially documented within early 1960s (Montville and Matthews, 2008). From thence, rates of isolating *Salmonella* serotypes having resistance to one or many antimicrobials have risen globally. This is attributed to the rise and unregulated usage and effortless access to antimicrobials within several countries of the globe (Yoke-Kqueen *et al.,* 2007). Evolving *S. Typhi* resistance have been reported particularly in African and Asian countries and the emergence of *S. Typhimurium* DT104 during the 1980s brought key public-health concerns, thus bringing threats to the lives of infested people (Montville and Matthews, 2008). According to studies by Van *et al.,* (2007), multi-drugs resistance occurs amongst *Salmonellae* serovars like *Albany, Anatum, Havana, London* as well as *Typhimurium.*

Extended spectrum cephalosporin and fluoroquinolone are the choicest antibiotics therapy for severe Salmonellosis. According to WHO, those medications limit the appearance of resistance to the extended spectrum cephalosporin and fluoroquinolone and are hence classified as critically important antimicrobials (Collignon *et al.,* 2009). However, there have been reported cases of emerging resistance of *Salmonellae* to these antimicrobials. In *Salmonellae*, vast numbers of

plasmid borne genes which codes for enzymes deactivating extended-broad spectrum cephalosporin have been reported up to now (Bush and Jacoby 2010). Within plasmids mediated quinolone resistance (PMQR) genes reported up to now, qnr genes embody the biggest set and are spread globally (Poirel *et al.*, 2008).

Increasing appearance of resistance to extended spectrum cephalosporin with reducing susceptibility to fluoroquinolone among *Salmonellae* serotypes which cause human illnesses have been documented in some Southeast Asia regions (Lee *et al.*, 2009). Definitive phage type 104 (DT104) being a frequent *S. Typhimurium* phage type, mostly show resistance to ampicillins, streptomycin, chloramphenicol, sulphamethoxazole, as well as tetracycline (Khakhria *et al.*, 1998). Furthermore, *S. Typhimurium* (none DT104) strains resistant to kanamycin instead of chloramphenicol, have evolved (Poirel *et al.*, 2008, Lee *et al.*, 2009)

The resistance to the conventional first line antimicrobials like ampicillin, chloramphenicol and trimethoprim/sulfamethoxazole outlines multi-drugs resistance (MDR) in *S. Enterica* (Crump *et al.,* 2011). This raises a lot of worry since several diseases with MDR *Salmonellae* are obtained via eating contaminated food from animals like pigs and poultry eggs. There is a report that S. *Choleraesuis* serovars showing resistance to cephalosporin and/or fluoroquinolone have been found in pigs in Taiwan and Thailand (Asai *et al.,* 2010). Aside these, antibiogram tests by Singh *et al.,* (2010) reported that *Salmonellae* serovars from chicken eggs in the market and poultry farms in Northern India showed resistance to bacitracin, polymyxin B as well as colistin

A study in Gambia had 33% of the invasive NTS isolates demonstrating resistance to all 8 antibiotics used, which included ampicillin (74.2 %), cotrimoxazole (64.5%) and tetracycline (63

%) (Usman *et al.*, 2007). The susceptibility trend of nontyphoidal *Salmonella* serotypes within the same research indicated a great percentage susceptible to cefotaxime (100%), ciprofloxacin (100%), nalidixic acid (100%), chloramphenicol (80.6%) and gentamicin (54.8%). But, cotrimoxazole (35%), tetracycline (37%) and ampicillin (26%) were little less (Usman *et al.*, 2007). ***2.16.1 Extent of drug resistance in Salmonellae***

High rates of antimicrobial resistance (>50% to 100%) to chloramphenicol, trimethoprim/sulphamethoxazole and ampicillin have been reported from Africa, Asia, and South America (Pegues *et al*., 2005). Of much worry is the worldwide emergence of multidrug resistant *S. Typhimurium* DT104 with resistance to a minimum of 5 antibiotics (ampicillins, chloramphenicol, streptomycin, sulphonamides, and tetracyclines) (Humphrey, 2001). Review statistics indicated an alarming rise in the total antibiotics resistance amongst *Salmonellae* from 20% to 30% during the 1990s to a soaring 70% among certain countries of the world at the turn of the century (Su *et al*., 2004).

2.17 *SALMONELLA* AND EXTENDED SPECTRUM BETA – LACTAMASE (ESBL).

According to Arlet *et al.*, 2006, fluoroquinolone and beta-lactam are the choicest medications for invasive *Salmonellae* diseases. Notwithstanding, resistance have evolved against different groups of antimicrobials in several regions of the globe with a corresponding distribution of resistant species. (Orman *et al.*, 2002, Arlet *et al.*, 2006).

Salmonellae resistant to cephalosporin are mainly because of the production of extended spectrum beta lactamases (ESBLs). Many ESBLs among *Salmonellae* are derived from TEM and SHV beta lactamases categories. Other sets, like PER and CTX-M types, have lately been reported (Bradford, 2001; Bonnet, 2004). Furthermore, beta lactamases of the groups Ambler class B (metallo beta lactamases) and class A, like KPC-2, capable of hydrolyzing carbapenem, have also

been reported (Miriagou *et al.,* 2003). Of particular worry is the finding that, CTX-M type ESBLs show levels of resistance to cefotaxime (Ctx) and ceftriaxone (Cro) appreciably greater than resistance to ceftazidime (Caz) (Bradford, 2001), though Poirel *et al.,* (2008) have documented certain CTXMs, like CTX-M-15, having excellent activities against Caz.

Since 1988, NTS isolates with resistance to ESBLs, have been documented in countries in Northern and Western Africa, Southern America, Middle-East, Eastern Europe as well as Eastern Asia, India, Russia, Turkey, Greece and US (Parry, 2003). Differences in geographical distribution of ESBLs may be based on different treatment and prophylactic protocols (Szabó *et al*., 1997).

In time past, just CTX-M-1, CTX-M-2 and CTX-M-9 had been reported among *Salmonellae* serovars. (Miriagou *et al.,* 2004). Outbreaks caused by CTX-M-2 ESBLs producing *Salmonellae* in Argentina have been reported (Orman *et al.,* 2002). Other outbreaks includes: *Salmonella Typhimurium* producing CTX-M-4 or CTX-M- 5 in Belarus, Russia, Hungary and Latvia reported between 1996 to 1999 (Edelstein *et al.,* 2004, Tassios *et al.,* 1999). Rotimi *et al.,* (2008) also documented outbreaks caused by CTX-M-15 production in *Salmonellae* serotypes in Kuwait and the United Arab Emirates. Moreover, periodic diseases in people, associated with CTX-M-15 ESBL producing *Salmonellae,* have already been documented in Lebanon as well as other places (Moubareck *et al.,* 2005; Kim *et al.,* 2007; Morris *et al.,* 2006).

2.18 *SALMONELLA* AND PULSED FIELD GEL ELECTROPHORESIS (PFGE)

Traditional electrophoresis makes use of a single electrical field which result in the migration of bio-molecules via a matrices based on their mass-to-charge ratio; the distance of migration of the bio-molecules are suggestive of their mass and size (Klotz and Zimm, 1972). These standard

electrophoresis could efficiently separate gene (DNA) fragments up to ~20kb. Nonetheless, bigger fragments would co-migrate appearing as big bands at the top of the gel when imaged. To overcome this problem, Schwartz and Cantor in 1984, developed the pulsed field gel electrophoresis (PFGE). This design resolves DNA through an alternation of the electrical field between spatially distinct pairs of electrodes. This system separates DNA fragments of up to ~10Mb based on their reorientations and movements at different speeds via the holes of an agarose gel.

Pulsed field gel electrophoresis (PFGE) has been widely applied as a molecular technique for subtyping *Salmonella* serovars. Many researches have indicated the utilization of PFGE for surveillances as well as investigations of *Salmonella Enterica* serovar *Typhi* and other *Salmonellae* serovars (Lee *et al.*, 1998, Tsen *et al.*, 1999, Navaro *et al.*, 1996).

CHAPTER THREE

MATERIALS AND METHODS

3.1 STUDY AREA AND POPULATION

The study was carried out at the Central Regional Hospital, Cape Coast which serves as the biggest and the referral hospital in the Cape Coast Metropolis and in the Central Region of Ghana. The Cape Coast Metropolitan which houses Cape Coast (its capital), is the smallest district in the country and lies within latitudes 5^0.07' to 5^0.20' north of the Equator and between longitudes 1°.11' to 1˚.41' west of the Greenwich. This Metropolitan is one of the 20 Metropolitans, Municipals and Districts found in Ghana's Central Region.

It shares boundaries with the Gulf of Guinea (south), Komenda/Edina/Eguafo/Abrem Municipality (west), Abura/Asebu/Kwamankese District (east) and Twifu/Hemang/Lower Denkyira District (north). It has a total area of 122square kilometers. According to the 2010 population census, Cape Coast has a township population of 169,894 individuals.

3.2 INCLUSIVE CRITIRIA

- Patients who reported at the laboratory (both out-patients and in-patients) for stool and blood cultures were considered for the study. Participants included patients of all sexes and age groups.

3.3 EXCLUSIVE CRITIRIA

- For the blood sampling, patients who were severely anaemic or are considered by the physician-in-charge to be seriously ill or mentally incapable of giving informed consent were excluded.

- Patients who attended the CCTH severally with the same complaint or disease were not considered for the research.
- Patients declining to be involved in the research were not considered.
- Patients who had started medication of antimicrobials were not included.

3.4 ETHICAL CLEARANCE ISSUES

Ethical clearance for the research was granted by the joint Committee on Human Research Publications and Ethics of the School of Medical Sciences and the Cape Coast Teaching Hospital, Cape Coast.

3.5 SAMPLE COLLECTION

Samples were collected from 971 participants of all sexes and age groups. The study population constituted patients (both out-patients and inn-patients) who report at the Cape Coast Teaching Hospital, Cape Coast. This number was made up of 508 stool samples and 463 blood sample. The whole research was sectioned into two main stages. The first stage involved collection of samples, isolations, identifications and confirmations of *Salmonellae* and other pathogens based on their colonial morphologies or cultural characteristics, stains reactions, motility as well as biochemical and serological properties. The second stage involved antibiotics susceptibility and resistance pattern determination of isolates, *Salmonellae* screening for Extended Spectrum Beta Lactamases (ESBLs) production and finally genetic analysis employing Pulsed Field Gel Electrophoresis (PFGE) technique.

3.5.1 Stool sample collection and processing

Stool samples for culture were collected in clean, dry, leak-proof, disinfectant-free and widenecked containers. About 3-5g of each sample was collected. The samples were appropriately labelled with pathological numbers corresponding to the details of the patient.

3.5.1.1 Stool culture

Approximately 1-2g of every stool specimen was inoculated into 10mls of newly prepared Selenite F-broth (SFB) and incubated between 35 - 37ºC for 18-24hours aerobically in bacteriological incubator. For watery samples, 1-2mls were inoculated into the SFB. The specimens were also plated on *Salmonella Shigella* (SS) agar and MacConkey agar (MCA) aerobically the same day.

3.5.2 Blood sample collection and processing

Blood specimens were obtained from persons who reported with suspected enteric fever and bacteremia in general at the hospital for culture using a strict aseptic technique. Samples of inpatients were also received from the various wards in the hospital. About 3-5 mls of blood were taken aseptically from each patient and introduced into 30mls of Brain-Heart Infusion (BHI) broths in glass bottles. The blood was then mixed thoroughly with the broth by tilting it several times.

3.5.2.1 Blood culture

The blood was inoculated into brain-heart infusion broth on the first day and sub-cultured every other day on blood agar, chocolate agar and MacConkey agar. The plates were incubated at 35 – 37ºC for 18 – 48hours. The inoculation and incubation was done up to the seventh day. The plates were observed for bacteria growth and for *Salmonella* using the colonial or morphological characteristics/appearance, gram staining, biochemical tests, etc. as described below.

3.6 BACTERIA ISOLATION

The media were observed and analyzed critically for the presence of characteristic bacterial colonies of *Salmonella* and other pathogens. The colonial morphology /cultural characteristics considered were colony size, elevation, edge, surface, consistency, emulsifiability and medium change (fermentation). For the stool cultures, three to four single colonies from suspected pathogens were sub-cultured separately on SS agar and MacKonkey agar. Both media are incubated aerobically at 35 - 37ºC for 18-24hours. The pure cultures obtained were then taken through identification, storage, confirmation, antibiotic susceptibility tests, and finally ESBL screening and PFGE analysis for the *Salmonella* isolates.

3.7 BACTERIA IDENTIFICATION

Initial identification was carried out with the use of the colonial morphologies and cultural characteristics of the bacteria on the agar. Single colonies were picked for gram staining, and then biochemical testing (Urea, Triple Sugar Iron Agar (TSIA), Citrate and Indole, Oxidase, Catalase and Coagulase) and motility test.

3.7.1 Gram's staining

The suspected *Salmonella* colonies and other pathogens in stool as well as all the blood isolates were stained to verify their gram reactions, shapes and arrangement using Gram's staining method.

3.7.2 Biochemical tests

Biochemical tests comprising urea, triple sugar iron agar (TSIA) slant reaction, citrate and indole were performed to affirm the specific biochemical profile of the gram negative isolates. Other biochemical tests performed on the isolates were catalase, oxidase and coagulase.

3.7.2.1 Urea

Using a sterile bacteriological loop, three to four isolated colonies from a pure culture were picked and streaked over the surface of the urea agar slant without stabbing the butt. It was incubated aerobically at 35 - 37°C overnight with a loosened cap. A positive urease reaction was indicated by a colour change from pale to red-pink. Colonies of *Klebsiella pneumoniae* and *E. coli* were used as a positive and negative controls correspondingly.

3.7.2.2 The Triple Sugar Iron Agar (TSIA)

Using a sterile bacteriological straight wire, pure colonies were picked, stubbed into the butt and streaked on the slant observing all aseptic techniques. It was incubated aerobically at 35 - 37°C for 18 – 24hours with a loosened cap. Pink slopes and yellow butts (with or without blackening as a result of H2S) were reported as positive results for *Salmonellae. Proteus vulgaris* was utilized as positive control bacteria with *Pseudomonas aeruginosa* being negative control.

3.7.2.3 Citrate

Isolated colonies from pure growths were introduced by aseptic mean into a sterile Simmons citrate agar and lightly streaked on the surface of the slant/slope. It was incubated aerobically at 35 - 37°C overnight with a loosened cap. A positive citrate result is displayed by a colour change from green to blue. Colonies of *Klebsiella pneumoniae* and *E. coli* were respectively used as a positive and negative controls.

3.7.2.4 Indole

From a pure culture, isolated colonies were picked and emulsified in peptone water aseptically using a bacteriological loop. The resultant mixture was incubated aerobically at 35 - 37°C overnight. Indole production was detected by adding Kovac's reagent. Positive indole test produced a pink coloured rink at the top of the broth while a negative indole test produced no

colour change. *E. coli* and *Pseudomonas aeruginosa* were correspondingly employed as a positive and negative controls.

3.7.2.5 Motility test

To perform the test, 2-3 bacterial colonies were picked with a sterile straight wire and emulsified into sterile peptone water. It was then placed in the incubator for approximately 8hours. A drop of the broth was put onto a slide and then a cover slip placed on it. Using the x40 objective lens, the setup was observed under the microscope for motility. The true motility was differentiated from Brownian motion by making sure that random movement of bacteria due to the energy of the microscopic field was not mistaken for the purposeful bacterial movement due to flagella.

3.7.2.6 Catalase test

About 2mls of 3% hydrogen peroxide solution was pour into a test tube. One end of a sterile capillary tube was used to pick 2-3 colonies from a pure culture of the test organism and immersed into the hydrogen peroxide solution. An immediate release of bubbles indicated a positive result. *Staphylococcus epidermidis* was utilized as a positive control and *Streptococcus agalactiae* was also utilized as a negative control.

3.7.2.7 Oxidase test

A drop of catalase reagent was applied to a piece of clean filter paper. Using a piece of stick, 1-2 colonies from a pure culture were picked and streaked on the filter paper where the reagent was applied. It was then observed for colour differences. A change from colourless to violet implied a positive result while no colour change implied a negative result. Positive and negative controls were *Pseudomonas aeruginosa* and *E. coli* respectively.

3.7.2.8 Coagulase test

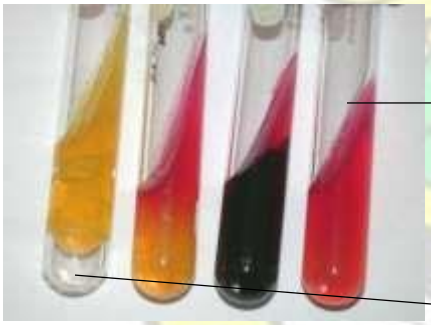
About 2 colonies of the test organism was emulsified in 0.8mls of peptone water and 0.2mls of plasma added. The solution was thoroughly mixed and incubated at 35-37°C overnight. A visible clot observed indicated a positive result while no clot indicated a negative result. Known *Staphylococcus aureus* was employed as a positive control whilst known *Staphylococcus epidermidis* was employed as a negative control.

Figure 3: The various reactions of the biochemical tests.

a. Indole test Positive Negative

b. Citrate test Positive Negative

c. Urea test Positive Negative



None glucose, none lactose fermenter

None lactose fermenter + H_2S gas

None lactose fermenter

Glucose and lactose fermenter + Gas

d. TSIA test

3.8 STORAGE

Organisms which depicted all the characteristics of *Salmonella* according to the identification methods used above were stored for confirmation and further characterization. All the blood isolates and two *Shigella* isolates from stool were also stored for confirmation and susceptibility testing.

3.8.1 Bacteria broth

From a pure culture, isolated colonies were picked and emulsified in peptone water aseptically and incubated aerobically at 35 - 37°C for 18 – 24hours. Using a permanent marker, sterile cryogen tubes were labelled with the date and the pathological numbers of the samples from which the suspected *Salmonellae* were obtained. With a micropipette, 150 µl of sterile glycerol was introduced into the cryogen tubes. With a new micropipette tip, 850 µl of the bacterial culture (peptone water + bacteria) was transferred to the same tube. The tubes were covered and turned upside down severally, to completely mix the glycerol and the bacteria. The mixture was then stored in a regular -20°C freezer.

3.9 FINAL CONFIRMATION OF ISOLATES

The stored isolates were carried in ice-chest with ice packs to the Noguchi Memorial Research Laboratory in Accra, Ghana for final confirmation. The confirmations were done using The Analytical Profile Index (API) system.

3.9.1 The Analytical Profile Index (API) system

During the analysis, 3.0mls of sterile saline having a concentration of 0.45 with a pH of 4.5 was introduced into a clean clear plastic test tubes by aseptic means. A sterile swab-stick was utilized in transferring 2-3 bacterial colonies into the saline and thoroughly mixed to obtain an even mixture. Using a VITEK2 Densi CHEKTM, the density of the suspension was altered to 0.5McFarland standard. It was then placed in the desired VITEK2 identification card. Employing a computer user interface, specimens' unique numbers were entered. The VITEK2 identification

cards were loaded into the analyzer and the tests started. After incubating between 18 - 24hours at 37°C within a humidity chamber, the various colour reaction outcomes were read. The outcomes of the tests together with oxidase test which was performed separately, were converted to a seven digits code. The code derived per specimen was compared with a database-book which gives the genus and species identification of the organism tested.

3.10 *SALMONELLA* SEROTYPING

3.10.1 Slide agglutination for O antigen and Vi grouping

Two to three bacteria colonies were suspended in 0.5 ml of normal saline and used as antigenic suspension for group O. A drop each of polyvalent antiserum and normal saline (30 µl) as a control were put at the ends of a clean glass slide. About 5 - 10 µl of the antigenic suspension for group O was added onto both the polyvalent antiserum and normal saline on the glass slide. It was mixed by tilting the glass slide sideways within 60seconds and the agglutination checked. Gross agglutinations were observed employing fluorescent light. It was first checked that, no agglutination was observed on the reaction with antigenic suspension and normal saline since that served as a negative control. Only heavy agglutinations observed within a minute in the reaction with polyvalent antiserum was considered positive. Agglutinations that delayed or were weak, were considered negative. Specimens that tested positive with the polyvalent antiserum were taken through the same protocol using monovalent antiserum.

3.10.2 Slide agglutination test for H antigen serotyping

Two to three bacteria colonies were suspended in 0.5 ml normal saline and used as antigenic suspension for H serotyping. A drop each of H-serum and normal saline (30 µl) as a control were put at the ends of a clean glass slide. About 5 - 10 µl antigenic suspension for H serotyping were added each onto the H-serum and normal saline on the glass slide. It was then mixed by turning

the glass slide back and forth within a minute and the agglutinations checked. Agglutinations were grossly observed using transmitted light including fluorescent light. An initial check was made that, no agglutination is seen in the reaction of antigenic suspension and normal saline since that served as a negative control. Only heavy agglutinations observed within a minute in the reaction with polyvalent antiserum was considered positive. Agglutinations that delayed or were weak, were considered negative.

Figure 4. *Salmonella* serotyping using slide agglutination method

3.11 ANTIMICROBIAL SUSCEPTIBILITY TESTING

Suspension of test organisms were prepared by picking pure colonies from a purity plate with a sterile wire loop and suspending it in sterile peptone water. The density of suspensions to be inoculated were determined by comparing with 0.5 McFarland standards. A sterile cotton swab was utilized and the extra fluid was removed by gentle rotation of the cotton swab against the surface of the tube and then spread evenly over the Muller Hinton agar plate.

The antimicrobial discs tested were ampicillin (10µg), co-trimoxazole (25µg), tetracycline (30µg), cefuroxime (30µg), gentamicin (10µg), levofloxacin (5µg), ceftriaxone (30µg), chloramphenicol (10µg),

cefotaximr(30µg), ciprofloxacin (5µg), meropenem (10ug) and amikacin (30µg). The plates were then incubated within 18 - 24hours at 35 – 37ºC. The inhibition zone around each disc was measured in millimeters, using a pair of calipers and a ruler, and interpreted as sensitive and resistance following the method of CLSI, 2006.

3.12 ESBL SCREENING OF *SALMONELLA* ISOLATES

The ESBLs screening was done in two phases.

3.12.1 Initial Screening of Salmonella for ESBL production

The initial screening for ESBL in the *Salmonella* isolates was done using single discs of cefpodoxime (10µg), ceftazidime (30µg), cefotaxime (30µg) and ceftriaxone (30µg). Two -three bacterial colonies were inoculated into peptone water and the turbidity adjusted to 0.5McFarland standards. A sterile cotton swab was immersed into the inoculum and streaked on the Mueller Hinton agar. A sterile forceps was used to pick and position the antimicrobial discs onto the Mueller-Hinton agar at 20mm apart and incubated for 18-24 hours at 35 – 37ºC. The inhibition zones were measured using a pair of calipers and ruler and compared with a standard chart to determine whether the isolates were ESBL producers or not.

3.12.2 Phenotypic confirmatory ESBL testing

The outcome of the initial screening was confirmed phenotypically using the combination disc methods. In this test, pairs of antibiotic discs that contain an extended spectrum cephalosporin (cefotaxime, ceftazidime) with and without clavulanic acid are positioned facing each other on the same plate. In this study the combination discs below was used.

- cefotaxime(30µg); cefotaxime/clavulanic acid (30µg/10µg)
- ceftazidime (30µg); ceftazidime/clavulanic acid (30µg/10µg)

Two to three bacterial colonies were inoculated into peptone water and the turbidity adjusted to

0.5McFarland standards. Sterile cotton swab was immersed into the inoculum and streaked on the Mueller Hinton agar. Sterile forceps was utilized in picking and positioning the antimicrobial discs onto the Mueller Hinton agar at 20mm apart and incubated for 18-24 hours at 35 – 37ºC. The inhibition zones were measured using a pair of calipers and ruler. Any inhibition zone diameter greater than or equal to 5mm for each antibiotic tested in combination with clavulanic acid versus its inhibition zone when tested alone = ESBL positive.

3.13 PULSED FIELD GEL ELECTROPHORESIS (PFGE)

PFGE was carried out employing the PulseNet international standardized protocol. Bacterial colonies were inoculated into cell suspension buffer (100 mM Tris, 100 mM EDTA, pH 8.0), and the density was adjusted to between 0.48-0.54. Proteinase K was added to a final concentration of 1mg/ml and 500µl of cell suspensions was added to 500µl of 1% SeaKem agarose. Using a pipette, 300µl of the agarose mixture was picked and put into reusable plug molds. The solidified agarose plugs were transferred into a 5ml of lysis buffer solution (50mM Tris, 50mM EDTA, 1%Sarkosyl [pH 8.0]) and 25µl of proteinase K (20mg/ml) and then incubated in a shaking water bath for 2hours at 54°C. Type 1 water was applied to wash the plugs 2times for 15minutes each time and 4times with 0.01M Tris EDTA for 15minutes each time in a shaking water bath. Agarose embedded DNA plugs were cut (2mm) and restricted with 50U of XbaI for 2hours at 37°C. The digested DNA plugs were loaded onto the combs and a 1%SeaKem agarose gel was made with the use of 0.5× Tris-buffered EDTA buffer and electrophoresed using a CHEF Mapper with switch times of 2.12seconds - 63.8seconds at 6V/cm for 18hours at 14°C. The gels were stained with ethidium bromide (1mg/ml) and unstained using 2 deionized water washes. With the use of UV trans-illumination, Gel Doc 1000 imager was applied to obtain images.

Analysis of PFGE patterns was performed using Molecular Analyst Fingerprinting Plus software with data sharing tools. *Salmonella* serovar Braenderup was utilized as a reference standard organism.

3.14 DATA MANAGEMENT AND ANALYSIS

The data obtained was entered into Microsoft Excel spreadsheet. It was then imported into SPSS Version 21.0 software (Chicago, Ill, USA) for statistical analysis. Descriptive data analysis was done. Confidence level of 95% was adopted and p values <0.05 were considered significant.

CHAPTER FOUR

4.0 RESULTS

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

A total of 971 samples consisting of 463 blood and 508 stool were collected over a 13 month period (March, 2014 – April, 2015). There were more female participants than males (62.3%). Their ages range from 1 day to 78 years and the modal age group was 0-20 year (44.7%). The sex and age distribution of participants are shown in Table 3.

Table 3. Sex and Age distribution of participants.

		Number	Percentage (%)
Sex	Female	605	62.3
	Male	366	37.7
Total		971	100.0
Age group	0 - 20 years	434	44.7
	21 - 40 years	386	39.8
	41 - 60 years	92	9.5
> 60 years	59	6.0	
Total		971	100.0

There were 69/463 (14.9%) blood samples which yielded bacteria but none of the isolates was *Salmonella*. On the other hand, 17 (3.3%) *Salmonellae* were recovered from the stool samples cultured. The *Salmonella* serotypes were *Salmonella Typhi* (13/17, 76.5%) and *Salmonella*

Typhimurium (4/17, 23.5%) (Table 6.). Other *Salmonella* serotypes were not identified. Three *Shigella flexneri* isolates (3/508, 0.6%) were also obtained from the stool samples.

4.1.1 Blood isolates

Staphylococcus aureus was the most prevalent isolate 24/69 (34.8%). This was followed by *Citrobacter freundii* (10/69, 14.5%), *Escherichia coli* (6/69, 8.7%) and *Pseudomonas aeruginosa* (6/69, 8.7%). The details and other isolate types obtained from blood were presented on Table 4.

Table 4. Isolates obtained from blood cultures

Isolate	Number	Percentage (%)
S. aureus	24	34.8
Citrobacter freundii	10	14.5
Escherichia coli	6	8.7
Pseudomonas aeruginosa	6	8.7
Enterobacter sp.	5	7.3
Staphylococcus epidermidis	5	7.3
Streptococcus agalactiae	4	5.8
Klebsiella pneumoniae	3	4.3
Klebsiella oxytoca	2	2.9
Streptococcus pyogenes	2	2.9
Proteus vulgaris	1	1.4
Proteus mirabilis	1	1.4
Total	69	100.0

Table 5. Demographic characteristics of participants stratified by the isolates obtained in blood samples.

Organism		*Citrobacter freundii* (N = 10)	*Enterobacter sp* (N = 5)	*E.coli* (N = 6)	*Kleb. oxytoca* (N = 2)	*Kleb, pneumoniae* (N = 3)	*Proteus mirabilis* (N = 1)	*Proteus vulgaris* (N = 1)	*P. aeruginosa* (N = 6)	*Staph. Aureus* (N = 24)	*Staph. epidermidis* (N = 5)	*Strep. agalatiae* (N = 4)	*Strep. pyogenes* (N = 2)	Total (N = 69)
Sex	Female	5	2	3	1	2	0	1	4	13	1	2	1	35 (51%)
	Male	5	3	3	1	1	1	0	2	11	4	2	1	34 (49%)
Patient	IP	10	5	5	2	3	0	1	5	23	5	4	2	65 (94%)
	OP	0	0	1	0	0	1	0	1	1	0	0	0	4 (6%)
Age	0 – 20	3	4	5	2	2	1	1	6	21	5	4	0	54 (78%)
	21 – 40	7	1	1	0	0	0	0	0	2	0	0	0	11 (16%)
	41 – 60	0	0	0	0	1	0	0	0	1	0	0	0	2 (3%)
	>60	0	0	0	0	0	0	0	0	0	0	0	2	2 (3%)

Key: **IP** – In Patient, **OP** – Out Patient

4.1.2 Stool isolates

There were 17 (3.3%) *Salmonellae* isolated. The *Salmonella* species were distributed among all age groups and sexes of patients from whom samples were obtained. However, more females (10/17, 59%) were infected than males. The age groups mostly infected were 21- 40years (10/17, 59%) and 0-20 (6/10, 35%). The most presented complaints among these patients from whom *Salmonella* was isolated were diarrhoea (n = 6, 35%) and gastroenteritis (n = 6, 35%) (Table 7). The 3 *Shigella flexneri* were isolated from 2 females (2/3, 67%) and 1 male (1/3, 33%)

Table 6. *Salmonella* isolates obtained from stool cultures

Isolate	Number	Percentage (%)
Salmonella Typhi	13	76.5
Salmonella Typhimurium	4	23.5 Total
17	100.0	

Table 7. Details of patients with *Salmonella* infection

Age	Sex	Clinical presentation	Consistency	Department	*Salmonella* serovar
22	M	Gastroenteritis	Semi formed	In-patient	*Salmonella Typhi*
45	M	Fever/headache	Semi formed	Out-patient	*Salmonella Typhi*
9	M	Malaria/enteric fever	Formed	Out-patient	*Salmonella Typhi*
20	F	Diarrhoea	Loose	Out-patient	*Salmonella Typhi*
7	F	PUO	Loose	Out-patient	*Salmonella Typhi*
32	F	Cyesis/Gastroenteritis	Loose	Out-patient	*Salmonella Typhi*
28	F	Diarrhoea	Loose	In-patient	*Salmonella Typhi*
30	F	Gastritis	Loose	In-patient	*Salmonella Typhi*
30	F	Diarrhoea	Loose	Out-patient	*Salmonella Typhi*
1	F	PUO	Loose	Out-patient	*Salmonella Typhi*
29	F	Diarrhoea	Loose	Out-patient	*Salmonella Typhi*
25	M	Gastroenteritis	Formed	In-patient	*Salmonella Typhi*
18	M	Gastroenteritis	Semi formed	In-patient	*Salmonella Typhi*
25	F	Chronic diarrhoea	Watery	In-patient	*Salmonella Typhimurium*
25	F	Diarrhoea	Watery	Out-patient	*Salmonella Typhimurium*
2	M	Gastroenteritis	Loose	Out-patient	*Salmonella Typhimurium*
36	F	Gastroenteritis	Semi formed	Out-patient	*Salmonella Typhimurium*

4.2 IN-PATIENT AND OUT-PATIENT DISTRIBUTION OF PARTICIPANTS

There was an even distribution of patients recruited for the studies in terms of the two departments in the hospital. In-patients constituted 494/971 (50.9%) while out-patients constituted 477/971 (49.1%). However, considering only the blood samples, there were more in-patients (408/463,

88.1%) than out-patients (55/463, 11.9%). On the other hand, the stool samples involved more outpatients (422/508, 83.1%) than in-patients (86/508, 16.9%)

For the blood pathogens isolated, as high as 65/69 (94%) were from in-patients. The 17 *Salmonellae* isolates were recovered from 11/17 (64.7%) out-patients and 6/17 (35.3) in-patients while all the *Shigella* isolates were recovered from out-patients.

4.3 ANTIBIOTIC RESISTANCE PATTERNS

4.3.1 Resistance patterns of blood isolates

Although most of the isolates exhibited high resistance, none of them was resistant to all antibiotics used. Resistance to ampicillin was the most common (66/69, 96%), followed by tetracycline (63/69, 91%), co-trimoxazole (60/69, 87%) and cefuroxime (49/69, 71%). The antibiotics with least bacterial resistance were amikacin (2/69, 3%), meropenem (5/69, 7%), levofloxacin (7/69, 10%) and ciprofloxacin (12/69, 17%)

Among the isolates, 98.6% were multi-drug resistant. They were resistant to 2 or more and up to 10 antibiotics. In all the tested species, only one *Enterobacter* isolate was sensitive to all 12 antibiotics.

Two *Citrobacter freundii* and two *Escherichia coli* isolates as well as one isolate of *Pseudomonas aeruginosa* and one isolate of *Staphylococcus epidermidis* were resistant to 9 antibiotics. All the ten (10) *Citrobacter freundii* isolates showed 100% resistance to co-trimoxazole, ampicillin and tetracycline. All the *Klebsiella sp* (5) had a 100% resistance to cefuroxime, tetracycline, chloramphenicol, ampicillin and co-trimoxazole.

Table 8. Antibiotics resistance patterns of isolates obtained from blood.

ANTIBIOTICS												
ISOLATES	CXM	TET	CHL	AMP	LEV	CTR	CIP	CTX	AMK	GEN	COT	MEM
Citrobacter freundii (n = 10)	60%	100%	60%	100%	0%	60%	10%	80%	10%	10%	100%	20%
Enterobacter sp (n = 5)	60%	80%	40%	80%	0%	80%	0%	80%	0%	40%	80%	20%
E. coli (n = 6)	67%	83%	33%	100%	17%	67%	67%	67%	0%	17%	67%	0%
Kleb. Oxytoca (n = 2)	100%	100%	100%	100%	0%	50%	0%	100%	0%	50%	100%	0%
Kleb. pneumoniae (n = 3)	100%	100%	100%	100%	0%	33%	0%	0%	0%	0%	100%	0%
Proteus mirabilis (n = 1)	100%	100%	0%	100%	0%	100%	0%	100%	0%	0%	100%	0%
Proteus vulgaris (n = 1)	0%	100%	100%	100%	0%	100%	0%	100%	0%	0%	100%	0%
P. aeruginosa (n = 6)	50%	100%	83%	100%	33%	67%	17%	33%	17%	0%	100%	0%
Staph. aureus (n = 24)	79%	83%	46%	96%	8%	83%	13%	58%	0%	38%	88%	4%
Staph. epidermidis (n=5)	80%	100%	80%	100%	20%	80%	40%	80%	0%	40%	100%	20%
Strep. agalatiae (n = 4)	50%	100%	50%	67%	33%	0%	33%	67%	0%	0%	50%	0%
Strep. pyogenes (n = 2)	100%	100%	100%	100%	0%	50%	0%	0%	0%	50%	50%	0%
TOTAL (n = 69)	71%	91%	58%	96%	10%	68%	17%	62%	3%	25%	87%	7%

Key: GEN – gentamycin **COT** – co-trimoxazole **TET** – tetracycline **CIP** – ciprofloxacin **AMP** – ampicillin **LEV** – levofloxacin **CTX** – cefotaxime **CTR** – ceftriaxone **CHL** – chloramphenicol **AMK** – amikacin **CXM** – cefuroxime **MEM** - meropenem

Table 9. Multidrug resistance pattern of isolates obtained from blood.

Isolates	Number of Antibiotics								
	2	3	4	5	6	7	8	9	10
Citrobacter freundii (n = 10)		1	1	3	1	2		2	
Enterobacter sp. (n = 4)				1	1		2		
Escherichia coli (n = 6)		1	1		1	1		2	
Klebsiella oxytoca (n = 2)					1		1		
Klebsiella pneumoniae (n = 3)				2	1				
Proteus mirabilis (n = 1)					1				
Proteus vulgaris (n = 1)					1				
Pseudomonas aeruginosa (n = 6)			1	1	2	1		1	
Staphylococcus aureus (n = 24)		1	3	4	6	5	3		2
Staphylococcus epidermidis (n = 5)					1	2	1	1	
Streptococcus agalactiae (n = 4)	1		1		1	1			
Streptococcus pyogenes (n =2)				1	1				

4.3.2 Antimicrobial resistance pattern of Salmonella isolates obtained from stool

None of the 17 isolates was resistant to all the antibiotics tested. Resistance to ampicillin, cotrimoxazole and tetracycline were (11/17, 65%) each. None of the isolates was resistance to cefotaxime, ceftriaxone, gentamycin, amikacin, meropenem, levofloxacin and ciprofloxacin.

All *Salmonella Typhi* isolates except one, were resistant to at least 2 antibiotics. This pattern was different from that of the *Salmonella Typhimurium* isolates where one of the isolates was sensitive to all tested antibiotics. There was high multi-drug resistance among isolates with the highest being

6 antibiotics (1/17, 6%), followed by 5 antibiotics (3/17, 18%) and 4 antibiotics (7/17, 41%). The percentage of *Salmonella Typhi* which showed multi-drug resistance was 92% (12/13) while that of *Salmonella Typhimurium* was 50% (2/4).

Table 10. Antibiotic resistance pattern of *Salmonella* isolates

	Number (%) of resistant strains						
Type of antibiotic.	*S. Typhi* (N = 13)			*S. Typhimurium* (N = 4)			Total (N = 17)
	R (%)	IS (%)	S (%)	R (%)	IS (%)	S (%)	
Tetracycline	9 (69%)	1 (8%)	3 (23%)	2 (50%)	0 (0%)	2 (50%)	11 (65%)
Co-trimoxazole	11 (84%)	1 (8%)	1 (8%)	0 (0%)	1 (25%)	3 (75%)	11 (65%)
Meropenem	0(0%)	0 (0%)	13 (100%)	0(0%)	0 (0%)	4(100%)	0 (0%)
Ampicillin	11 (85%)	2 (15%)	0 (0%)	0 (0%)	0 (0%)	4 (100%)	11 (65%)
Cefuroxime	0 (0%)	3 (23%)	10 (77%)	0 (0%)	0 (0%)	4 (100%)	0 (0%)
Chloramphenicol	2 (15%)	1 (8%)	10 (77%)	0 (0%)	2 (50%)	2 (50%)	2 (12%)
Cefotaxime	0 (0%)	0 (0%)	13 (100%)	0 (0%)	0 (0%)	4 (100%)	0 (0%)
Ceftriaxone	0 (0%)	0 (0%)	13 (100%)	0 (0%)	0 (0%)	4 (100%)	0 (0%)
Gentamycin	0 (0%)	0 (0%)	13 (100%)	0 (0%)	0 (0%)	4 (100%)	0 (0%)
Amikacin	0 (0%)	0 (0%)	13 (100%)	0 (0%)	0 (0%)	4 (100%)	0 (0%)
Levofloxacin	0 (0%)	0 (0%)	13 (100%)	0 (0%)	0 (0%)	4 (100%)	0 (0%)
Ciprofloxacin	0 (0%)	0 (0%)	13 (100%)	0 (0%)	0 (0%)	4 (100%)	0 (0%)

Key: S, susceptible; **IS**, intermittently susceptible; **R**, resistant

4.3.3 Antimicrobial resistance pattern of Shigella isolates from stool

There was multidrug resistance in all the 3 *Shigella* isolates with the highest number of antibiotic multiple resistance being 5. Resistance to tetracycline and co-trimoxazole were 100% each and

that of ampicillin was 67%. No antimicrobial resistance was recorded against cefuroxime, cefotaxime, ceftriaxone, gentamycin, amikacin, meropenem, levofloxacin and ciprofloxacin.

Table 11. Antibiotic resistance pattern of *Shigella* isolates

	Number (%) of resistant strains		
Type of antibiotic	*Shegella flexneri* (N = 3)		
	R (%)	IS (%)	S (%)
Tetracycline	3 (100%)	0 (0%)	0 (0%)
Co-trimoxazole	3 (100%)	0 (%)	0 (0%)
Meropenem	0 (0%)	0 (0%)	3 (100%)
Ampicillin	2 (67%)	0 (0%)	1 (33%)
Cefuroxime	0 (0%)	0 (%)	3 (100%)
Chloramphenicol	2 (67%)	0 (0%)	1 (33%)
Cefotaxime	0 (0%)	0 (0%)	3 (100%)
Ceftriaxone	0 (0%)	0 (0%)	3 (100%)
Gentamycin	0 (0%)	0 (0%)	3 (100%)
Amikacin	0 (0%)	0 (0%)	3 (100%)
Levofloxacin	0 (0%)	0 (0%)	3 (100%)
Ciprofloxacin	0 (0%)	0 (0%)	3 (100%)

Key: S, susceptible; **IS**, intermittently susceptible; **R**, resistant

4.4 ESBL TEST RESULTS ON *SALMONELLA* ISOLATES

All the 17 *Salmonella* isolates were sensitive to cefpodoxime (10µg), ceftazidime (30µg), cefotaxime (30µg) and ceftriaxone (30µg). Upon further confirmation with combine discs of cefotaxime (30µg); cefotaxime/clavulanic acid (30µg/10µg) and ceftazidime

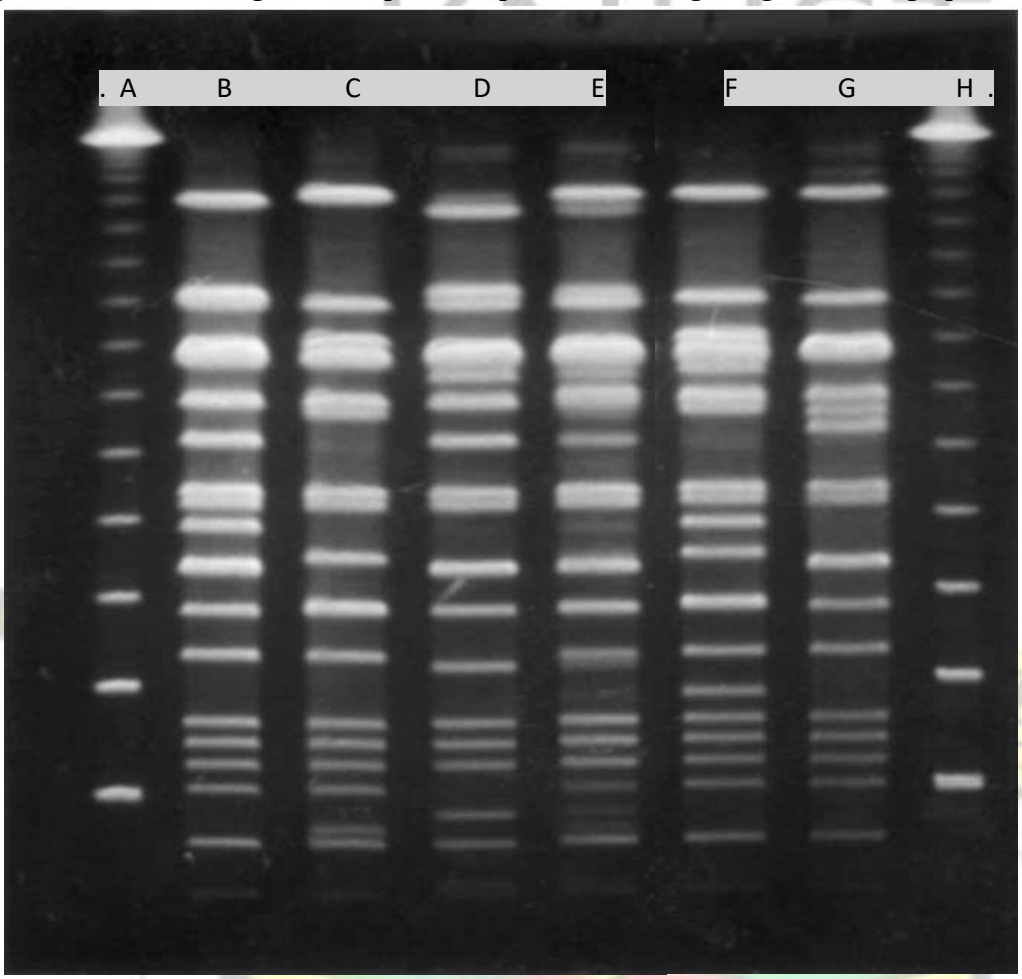
(30µg); ceftazidime/clavulanic acid (30µg/10µg), none had a zone diameter difference of ≥5mm. Hence none of the *Salmonellae* was an ESBL producer.

4.5 PULSED-FILLED GEL ELECTROPHORESIS (PFGE)

In the genetic analysis using pulsed-field gel electrophoresis, there was indistinguishable closeness in the fingerprints among the *Salmonella Typhi*. Lanes A and H were *Salmonella Braenderup* controls. Lanes B and E appear to have similar fragment patterns, so may be closely related clones. Lanes C and F also appear to be similar, so may be related clones, but lane C was different from all the others. The details of these observations could only be determined by the BioNumeric software but this software was not available for this study. These observations suggested that the *Salmonella Typhi* isolates were of different clones circulating in Cape Coast Municipality.

No notable difference was observed among the *Salmonella Typhimurium* gel pattern analyzed. Lane A was the *Salmonella Braenderup* control organism. The strains tested were in lanes B and C (were the same strain) and D and E were also the same strain. This observation indicates that, there was a close genetic relatedness within the two strains of *Salmonella Typhimurium* analyzed. The gel pictures are presented in Figures 4 and 5.

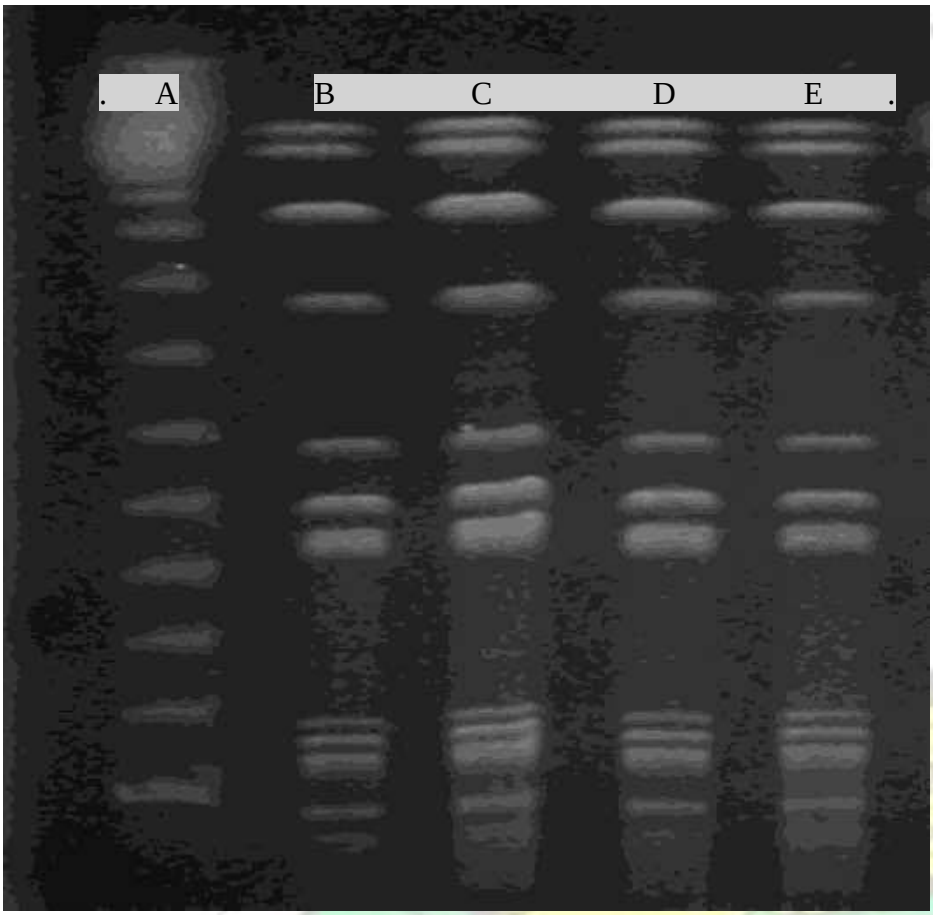
Figure 5. Pulsed-field gel electrophoresis picture showing the genome fingerprint of *S. Typhi*



A, H – *Salmonella Braenderup* (control organism)

B, C, D E, F, G – *Salmonella Typhi* (test organism)

Figure 6. Pulsed-field gel electrophoresis picture showing the genome fingerprint of *S. Typhimurium*



A –*Salmonella Braenderup* (control organism)

B, C, D, E – *Salmonella Typhimurium* (test organism)

CHAPTER FIVE

5.0 DISCUSSIONS

This study, was meant to characterize *Salmonella* and other isolates obtained from blood and stool samples at the Cape Coast Teaching Hospital.

5.1 PREVALENCE OF *SALMONELLA*

5.1.1 Frequency of Salmonella isolation from blood cultures

Invasive serovars of *Salmonellae* (TS and NTS) have emerged as a major public health problem in sub-Saharan Africa (Berkley *et al.,* 2005, Kariuki *et al.,* 2006, Gordon *et al.,* 2008). NTS have has been reported as the most common cause of invasive bacterial disease in some parts of Africa such as Ivory Coast (21/319, 6.6%), Kenya (170/26986, 0.6%) and Malawi (2439/27581, 8.8% and 2517/35197, 7.2%) (Berkley *et al.,* 2005, Gordon *et al.,* 2008). However, in this current study, there was no *Salmonella* among the 69/463 (14.9%) blood isolates obtained within the 13 months period of this study. A surveillance study conducted in the Gambia by Kwambana-Adams *et al.,* (2015) which studies elsewhere reported a 0.8% prevalence of *Salmonella* in blood cultures and Mohanty *et al.,* (2006) in India who reported 0.69% prevalence. Contrary to this finding, AppiahKorang *et al.,* (2014) reported a 6.5% (181/2768) prevalence of *Salmonella* bacteraemia at the Korle-Bu Teaching Hospital, Accra-Ghana. The variations could be attributed to the relative contributions of typhoidal and nontyphoidal serovars to infections since they vary in both place (Biggs *et al.,* 2014) and time, even within countries (Gordon *et al.,* 2008, Feasey *et al.,* 2015).

5.1.2 Frequency of Salmonella isolation from stool cultures

There was *Salmonella* prevalence of 3.3% (17/508) among the patients whose stool samples were cultured. This corresponds to the finding of Babu *et al.*, (2010) in India which reported a prevalence of 4.2% and Tesfaye *et al.*, (2014) in Ethiopia with 1.08% prevalence in stool cultures. Mikhail *et al.*, (1990) also reported a prevalence of 3% in Djibouti. Others prevalence are: Babylon province 5.66%, Hydar (2009); Baghdad 3.5%, AL-Magdi, (1986); 0% Moges, (2010); 2.5% Getamesay *et al.*, (2014) and 1.6% (Huruy *et al.*, (2011). In Tanzania, Temu *et al.*, (2008) also reported a prevalence as low as 0.8%. The low prevalence might be due to the increasing awareness of the people about personal and environmental hygiene made by the health institutions and other partners.

In contrast to this current finding, studies done at different parts of the world have reported higher *Salmonellae* prevalence in stool: Bangladesh (16%) (Nesa *et al.*, 2011), Switzerland (14.4 %) (Loosl *et al.*, 1985) and Ethiopia (11.5%) (Reda *et al.*, 2011), (15.4%) (Mache, 2001).

Typhoidal infection typically occurs due to ingestion of contaminated food or water (Scherer and Miller, 2001). The low isolation rate in this study may therefore be due to the availability of portable and clean drinking water as well as improved environmental hygiene.

5.1.3 Frequency of Shigella isolation from stool cultures

In this study, three *Shigella flexneri* (3/508, 0.6%) were isolated from the stool samples. A similar finding of 0.4% in urban areas and 0.5% in rural areas was reported by Seung-Hak *et al.*, (2006) in Korea. However, higher *Shigella* prevalence have been reported elsewhere across the globe. Alam *et al.*, (2003) in their study at Karachi, Pakistan reviewed a total of 16379 stool samples where the isolation rate for *Shigella flexneri* was (6.2%). In Nigeria, prevalence was reported to be

14% in urban areas and 9% in rural areas (Chikwelu et al., 1997). Others reported include 7.7% in Djibouti (Mikhail, *et al.,* 1990), 8% in Bangladesh (Fitzroy, 2008), 4% in Nepal (Bhattacharya *et al.,* 2005) 35% in Uganda (Legros *et al.,* 1998), and 20% in Ethiopia (Mache, 2001). The low rate of isolation as observed in the present study may be due in part to continuing educational programs at elementary schools, aggressive infection-control measures in our hospital and healthcare centres and possibly under-reporting of shigellosis cases by general practitioners.

5.1.4 Prevalence of Salmonella serovars

In this study, Typhoidal *Salmonellae* (TS); 76.5% was more prevalent than Non-Typhoidal *Salmonellae* (NTS) 23.5%. This contrasts a study in Gambia by Kwambana-Adams *et al.,* (2015), where NTS prevalence was as high as 86% as against TS (14%). Others contrast studies reported NTS (88%) against TS (12%), Babu *et al.;* (2010) and NTS (76.1%) against TS (23.9%), BoniCissé *et al.,* (2012). The observed differences in serovars distribution are affirmed in several researches which concluded that *Salmonella* serovars differs by geographical region (Sirichote *et al.,* 2010, Lee *et al.,* 2009, Hendriksen *et al.,* 2011)

5.2.0 ANTIBIOTIC RESISTANCE PATTERN

5.2.1 Antibiotic resistance pattern of Salmonellae

To successfully combat new resistances in *Salmonellae* infections, fluoroquinolones were proposed as the drug of choice. However, during the past decade, ciprofloxacin resistant strains have been reported in the Asian subcontinent (Crump *et al*., 2004). In this study, all the *Salmonella* serotypes were susceptible to ciprofloxacin, levofloxacin, amikacin, gentamycin, meropenem, ceftriaxone and cefotaxime. This agrees to a study by Groß *et al.,* (2011) who also reported similar

Salmonella susceptibility pattern to these antimicrobials. High susceptibility of *Salmonella* to ciprofloxacin and norfloxacin were also found in the study of Malla *et al.,* (2005). The current finding agrees with the proposal that in the treatment of gastroenteritis, ciprofloxacin should be administered at the first sign of severe gastroenteritis whereas ceftriaxone is given to children with systemic salmonellosis (Yousef and Carlstrom, 2003). The high susceptibility to these antibiotics in Cape Coast could be attributed to appropriate prescription and proper use of these drugs in salmonellosis. It could also be due to the expensive nature of these drugs making them less assessable to the community hence less indiscriminate use.

A study in Accra, Ghana (Namboodiri *et al*., 2011) reported high proportions of *Salmonellae* isolated to be resistant to ciprofloxacin. The emergence of resistance to ciprofloxacin and other fluoroquinolones has resulted in the use of ceftriaxone or cefotaxime as alternatives for treatment of enteric fever (Lee *et al.,* 2003). However, similar trends of *Salmonellae* resistance against extended spectrum cephalosporin and fluoroquinolone which are the drugs of choice for treating severe *Salmonella* infections have been documented worldwide (WHO, 2013, Bush and Jacoby 2010). In this study, no antibiotic resistance was recorded against ciprofloxacin, levofloxacin, amikacin, gentamycin, meropenem, ceftriaxone, cefotaxime, cefoxitin and ceftazidime.

A study carried out by Islam *et al.,* (2008) in Bangladesh, reported that cephalosporins were better than fluoroquinolones in their application in treating Salmonella Typhi infections. A report of multi-drug resistance strains of *Salmonella Typhi* (Adabara *et al.*, 2012), also had the similar findings. Again, the rise in occurrence of resistance to extended spectrum cephalosporins as well as the decline in susceptibility to fluoroquinolones in *Salmonella* isolates causing infections in man, have been documented in Southeast Asian countries (Lee *et al.*, 2009). But the findings in this study indicates that both groups of drugs still remain drugs of choice in treating salmonellosis

in Cape Coast. This agrees to a study by Guerra *et al.*, (2000) in Spain who analyzed the resistance profile for 15 antimicrobial agents of 333 *Salmonella* strains and reported that all the strains were susceptible to amikacin, ceftazidime and ciprofloxacin.

The high resistance pattern demonstrated by the *Salmonellae* against ampicillin (65%), tetracycline (65%), and co-trimoxazole (65%) in this study corresponds to several studies such as that of Tesfaye *et al.,* (2014) and Crump *et al.,* (2011). High rates of antimicrobial resistance (>50% to 100%) to chloramphenicol, trimethoprim/sulphamethoxazole and ampicillin have been reported from Africa, Asia, and South America (Pegues *et al*., 2005). In another study in Kenya, Kariuki *et al.,* (2005) demonstrated that the prevalence of NTS multiple drugs resistant to all the commonly used antimicrobials such as ampicillin, co-trimoxazole and chloramphenicol increased from 31% in 1994 to 42% in 2003. Again, Parry (2003) in his study concluded that antimicrobial resistance in *Salmonella* to chloramphenicol, ampicillin and co-trimoxazole is common in Africa. This observed trend could be attributed to increased and uncontrolled use as well as easy accessibility to these antibiotics. Other factors may include prescriptions not taken for a total duration of therapy, administration of antibiotics for viral infections, antibiotics sold without medical supervision and the use of antibiotics in foods/agriculture (Barza, 2002)

There was high multi-drug resistance among isolates. The percentage of *Salmonella Typhi* which showed multi-drug resistance was 92% (12/13) while that of *Salmonella Typhimurium* was 50% (2/4) culminating in a total of 82% (14/17). Similar results were found in Nepal where several *Salmonella* isolates were found to be resistant to at least four antibiotics (Bhatta *et al*., 2007) and in Ghana by Mills-Robertson *et al*., 2003. Appiah-Korang *et al.,* (2014) also reported a 44.8% *Salmonellae* multidrug resistant in their study in Accra-Ghana.

5.2.2 Antibiotic resistance pattern of Shigella isolates

In this study, resistance to tetracycline and co-trimixazole were 100% each and that of ampicillin was 67%. No antimicrobial resistance was recorded against cefuroxime, cefotaxime, ceftriaxone, gentamycin, amikacin, levofloxacin and ciprofloxacin. Fitzroy, (2008) reported a similar finding in his study at Bangladesh where there was increased antimicrobial resistance to tetracycline and co-trimoxazole but no resistance against gentamycin, cefuroxime and ciprofloxacin. Also Ashkenazi *et al.*, (2002) in central Israel, identified a significantly increased resistance to tetracycline (from 23% to 87%) and ampicillin (85%) and emerging resistance to quinolones including ciprofloxacin (0.5% to 2%).

5.2.3 Antibiotic resistant pattern of blood isolates

Although most of the isolates exhibited high multi-drug resistance, none of them was resistant to all antibiotics used. Resistance to ampicillin was the most common (66/69, 96%), followed by tetracycline (63/69, 91%), co-trimoxazole (60/69, 87%) and cefuroxime (49/69, 71%). The antibiotics which recorded least bacterial resistance were amikacin (2/69, 3%), meropenem (5/69, 7%), levofloxacin (7/69, 10%) and ciprofloxacin (12/69, 17%). There was 98.6% multidrug resistance among isolates with the highest multiple resistance being 10 antibiotics (2/69, 3%), followed by 9 antibiotics (6/69, 9%), 8 antibiotics (8/69, 11%) and 7 antibiotics (13/69, 19%). Similar trends are presented in the sensitivity patterns of the *Salmonella* isolates as discussed above.

5.3 SEX AND AGE DISTRIBUTION OF PATIENTS WITH *SALMONELLA* ISOLATES

The *Salmonella* species isolated in this study were distributed among all sexes and age groups.

However, more females (n = 10, 59%) were infected than males. A higher prevalence in females than males was obtained in a report by Umeh and Agbulu (2010) who documented a 58.0% prevalence of *Salmonella Typhi* in females. The same study also recorded a higher prevalence of *S. Paratyphi* in females than in males. Again, Dakora (2014) in her study in Ghana reported that, of the 54 *Salmonella* isolated, 55.6% (30/54) were from females and 44.4% (24/54) from males.

Several researches have reported that, there is a high *Salmonellae* infection rates in children of school-going age and young adults (Feasey *et al.,* (2010) and Abdullahi *et al.,* (2012)). This is consistence with this study which recorded a prevalence of 59% in participates ≤25 year. This observation could be attributed to the under developed immune system of this age category which render them more susceptible to enteric pathogenic bacteria (Ja'afar *et al*., 2013).

5.4 ESBL PREVALENCE AMONG *SALMONELLA* ISOLATES

Findings of extended spectrum beta-lactamases (ESBLs) among *Salmonellae* is a newly emergent threat globally. Both healthcare related outbreaks as well as community outbreaks have been documented (Arlet *et al.,* 2006). In this study, we aimed at determining the presence or absence of ESBL producing *Salmonella* species. No strain was found to produce extended-spectrum betalactamase (ESBL). This finding agrees to that of Boni-Cisse *et al.,* (2012) who found no ESBL producing *Salmonella* species in their study in Cote d'Ivoire. A study in Ghana by Dakora (2014) who screened 54 *Salmonella* isolates found none to produce ESBL. On the contrary, a rise in the occurrence of resistance to extended spectrum cephalosporins and a decline in the susceptibility to fluoroquinolones in *Salmonellae* causing human illnesses have been documented in Southeast Asian countries (Lee *et al.,* 2009). Since 1988, NTS isolates with resistance to ESBLs have been reported in Northern and Western African countries, Southern America, the Middle-East, Eastern

Europe and East Asia, Russia, India, Turkey, Greece and the United States (Parry, 2003). Other countries where ESBL producing *Salmonellae* have been reported include Argentina (Orman *et al.*, 2002), Belarus, Hungary and Latvia (Edelstein *et al.*, 2004; Tassios *et al*., 1999), Kuwait and United Arab Emirates (Rotimi *et al.*, 2008), Lebanon and elsewhere (Moubareck *et al.*, 2005; Morris *et al.*, 2006; Kim *et al.*, 2007). Differences in geographical distribution of ESBLs may be based on different treatment and prophylactic protocols (Szabó *et al*., 1997)

5.5 GENETIC RELATEDNESS OF *SALMONELLAE* ISOLATED

The pulsed-field gel electrophoresis analysis showed a close genomic relatedness within the *Salmonella* species analyzed. The genomic fingerprint sequence analysis revealed that all the *Salmonella spp.* were related within species denoting that they are from a common origin.

CHAPTER SIX

6.1 CONCLUSION

There is low prevalence of *Salmonella* at Cape Coast from the samples cultured. No *Salmonella* was isolated from blood. *Salmonella* prevalence in the stool samples was 3.3%. There were 17 *Salmonella* isolates in the study. There were 13 *Salmonella Typhi* isolates giving a prevalence 76.5% and 4 *Salmonella Typhimurium* with a prevalence of 23.5%. The *Salmonella* species isolated were distributed among all sexes and age groups with a high infection rate in the 1-25years age group. Prevalence of other pathogens isolated were *Staphylococcus aureus* (24/69, 34.8%), *Citrobacter freundii* (10/69, 14.5%), *Escherichia coli* (6/69, 8.7%) and *Pseudomonas aeruginosa* (6/69, 8.7%) from blood and *Shigella flexneri* (3/508, 0.6%) from stool

There was high proportion of *Salmonella* isolates, 82% (14/17) being multidrug resistant. In spite of the high percentage of *Salmonellae* being resistant to the commonly tested antibiotics, no resistant strain was recorded against ciprofloxacin, levofloxacin, amikacin, gentamycin, ceftriaxone, meropenem, cefotaxime, cefoxitin and ceftazidime.

Among the other blood isolates, resistance to ampicillin, tetracycline, co-trimoxazole and cefuroxime were 96%, 91%, 87% and 71% respectively. The antibiotics with less bacterial resistance were amikacin (2/69, 3%), meropenem (5/69, 7%) levofloxacin (7/69, 10%) and ciprofloxacin (12/69, 17%). There was 98.6% multidrug resistance among the blood isolates with resistance to 2 or more and up to 10 antibiotics.

6.2 RECOMMENDATIONS

1. There should be a continued surveillance of incidence, resistant pattern and ESBL of *Salmonella* across the country to determine when ESBL producing *Salmonella* arises.
2. There should be more education on controlled and judicial use of antibiotic in the various health facilities to help prevent the emergence of ESBL-producing *Salmonellae* species. Legislations restricting over-the-counter dispensing of drugs without prescription should also be enforced.
3. It is recommended that clinical laboratories be equipped and the staff trained on *Salmonella* speciation and phenotypic testing for ESBL to effectively monitor drug resistance patterns.

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APPENDIX 1: THE COMPOSITION AND PREPARATION OF CULTURE MEDIA

BLOOD AGAR BASE (Biomark, India)

Beef heart infusion from (Beef extract) 500.00g

Sodium chloride 5.00g

Tryptose 10.00g Agar

15.00g pH 7.3 +/- 0.2

at 25ºC

Preparation – Suspend 40g in 1000ml of distilled water and heat to dissolve completely. Sterilize by autoclaving at 121ºC for 15minutes. Cool to 50ºC and aseptically add 5% v/v sterile defibrinated blood. Mix well and pour into sterile Petri dishes. Flame the top to remove air bubbles.

CHOCOLATE AGAR (Biomark, India)

Beef heart infusion from (Beef extract) 500.00g

Sodium chloride 5.00g

Tryptose 10.00g Agar

15.00g pH 7.3 +/- 0.2

at 25ºC

Preparation – Suspend 40g in 1000ml of distilled water and heat to dissolve completely. Sterilize by autoclaving at 121ºC for 15minutes. Cool to 75ºC and aseptically add 5% v/v sterile defibrinated blood. Mix well and pour into sterile Petri dishes. Flame the top to remove air bubbles.

XYLOSE LYSINE DEOXYCHOLATE (XLD) AGAR (Biomark, India)

Composition

Lactose 7.5g

Sucrose 7.5g

Sodium Thiosulfate 6.8g

L-Lysine 5.0g

Sodium chloride 5.0g

Xylose 3.75g

Yeast Extract 3.0g

Sodium Deoxycholate 2.5g

Ferric Ammonium Citrate 0.8g

Agar 15.0g pH 7.3 +/- 0.2 at

25ºC

Preparation - Suspend 55 grams of dehydrated medium in 1000 ml purified or distilled water. Heat with frequent agitation until the medium boils. Do not autoclave. Allow to cool to 50ºC and pour into sterile petri dishes.

BRAIN HEART INFUSION BROTH (Biomark, India)

Composition (typical g/L)

Calf brain, infusion 200.00g

Beef infusion 250.00

Proteose peptone 10.00g

Dextrose 2.0g

Sodium Chloride 5.00g

Disodium Hydrogen Phosphate 2.50g pH

7.4+/- 0.2

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 121ºC.

MAC CONKEY AGAR (Biomark, India)

Standard formula in g/l

Peptic digest of animal tissue 20.00g

Sodium taurocholate 5.00g

Lactose 10.00

Neutral red 0.04g

Agar 20.00g pH

7.1+/- 0.2 at 25ºC

Preparation – Suspend 55g in 1000ml distilled water and heat to dissolve the medium completely. Sterilize by autoclaving at 121ºC for 15 minutes. Cool to 45-50 ºC and pour into sterile Petri plates.

MUELLER HINTON AGAR (Biomark, India)

Composition

Beef infusion 300.00g

Casein acids hydrolysate 17.50g

Starch, soluble 1.50g

Agar 17.00g pH 7.3

+/- 0.1 at 25ºC

Preparation - Weigh

and dissolve 38

grams of the powder

in 1 litre of distilled

water. Sterilize at

121ºC for 15mins. Cool to 45-50 ºC and pour into sterile Petri plates.

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

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

121ºC. Cool to 47ºC, mix well and pour into plates.

SALMONELLA – SHIGELLA **AGAR (**Biomark, India)

Composition

Peptic digest of animal tissue 5.00g

Beef extract 5.00g

Lactose 10.00g

Sodium citrate 10.00g

Bile salt mixture 8.50g

Sodium citrate 10.00g

Sodium thiosulphate 8.50g

Ferric citrate 1.00g

Brilliant green 0.00033g

Neutral red 0.025g

Agar 15.0g pH 7.0

+/- 0.2 at 25ºC

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

SELENITE F BROTH (Biomark, India)

Composition

Casein enzymic vhydrolysate 5.00g

Lactose 4.00g

Sodium Phosphate 10.00g Sodium

hydrogen selenite 4.00g pH 7.0

+/- 0.2 at 25ºC

Preparation- Weigh and suspend 23.0grams in 1litre of distilled water. Heat to dissolve completely and dispense into bijoux bottles. Sterilize in a boiling water bath or free flowing steam for 10minutes. Do not autoclave.

SALINE (PHYSIOLOGICAL SALINE)

Composition

NaCl 8.5g

Distilled water 1 litre

Mode of preparation - Dissolve 8.5g NaCL in distill water. Autoclaved for 15mins at 121ºC.

Cool to room temperature.

APPENDIX II: BIOCHEMICAL TEST REAGENTS

Peptone water (Biomark, India)

Composition

Peptic digest of animal tissue 10.00g

Sodium chloride 5.00g pH 7.2 +/-

0.2 at 25ºC

Preparation- Weigh and suspend 15g in 1litre of distilled water. Dispense into sterile containers and cap. Autoclave at 121ºC for 15mins.

TSI Agar (Biomark, India)

Formula in g/l

Peptic digest of animal tissue 10.00g

Casein enzymic hydrolate 10.00g

Yeast extract 3.00g

Beef extract 3.00g

Lactose 10.00g

Sucrose 10.00g

Dextrose 1.00g

Sodium chloride 5.00g

Sodium thiosulphate 0.30g

Ferrous sulphate 0.20g

Phenol red 0.024g

Agar 12.00g pH 7.3

+/- 0.2 at 25oC

Preparation – Suspend 65g in one litre of distilled water and boil to dissolve the medium completely. Dispense into test tubes and sterilize by autoclaving at121ºC for 15 minutes. Allow to cool in a slanted position such that deep butts are formed.

Mode of action – The Triple Sugar Iron Agar (TSIA) medium was used to distinguish between enteric bacteria based on their physiological ability (or lack thereof) to: a. metabolize lactose and/or sucrose, b. conduct fermentation to produce acid, c. produce gas during fermentation, d. generate H_2S. The medium contains 1.0% each of sucrose and lactose and 0.1% glucose. If only glucose is fermented, acid produced in the butt will turn it yellow, but insufficient acid products are formed to affect the methyl red in the slant. However, if either sucrose or lactose are fermented, sufficient fermentation products will be formed to turn both the butt and the slant yellow. If gas is formed during the fermentation, it will show in the butt either as bubbles or as cracking of the agar. If no

fermentation occurs, the slant and butt will remain red. The medium also contains ferrous sulfate. If the bacterium forms H2S, this chemical will react with the iron to form ferrous sulfide, which is seen as a black precipitate in the butt (a black butt).

Simmons Citrate Agar (Biotec, UK)

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

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 121ºC for 15 minutes. Allow to set as slopes.

Mode of action – Simmons citrate agar tests the ability of organisms to utilize citrate as a carbon source. Simmons citrate agar contains sodium citrate as the sole source of carbon, ammonium dihydrogen phosphate as the sole source of nitrogen, other nutrients, and the pH indicator bromthymol blue. Organisms which can utilize citrate as their sole carbon source use the enzyme citrase or citrate-permease to transport the citrate into the cell. These organisms also convert the ammonium dihydrogen phosphate to ammonia and ammonium hydroxide, which creates an

alkaline environment in the medium. At pH 7.5 or above, bromthymol blue turns blue. At a neutral pH, bromthymol blue is green.

Urea Agar Base (Biomark, India)

Composition (typical g/L)

Peptic digest of animal tissue 1.00g

Dextrose 1.00g

Sodium chloride 5.00g

Disodium phosphate 1.20g

Monopotassium phosphate 0.80g

Phenol red 0.012g

Agar 15.00g pH 6.8

+/- 0.2 at 25ºC

Preparation –

Disperse 24g in

950ml of distilled.

Swirl to mix and heat

to complete

dissolution. Sterilize

by autoclaving for 20

minutes at 121ºC.

Cool to 50ºC and

aseptically add 50mls

of sterile 40% of urea

solution and mix

well. Dispense into

sterile final

containers and set in

a sloped position.

Mode of action - This medium is used in the detection of urease activity in gram-negative organisms. Urea Agar contains urea and phenol red as the pH indicator. Organisms capable of hydrolyzing urea form ammonia as a byproduct, thus turning the medium alkaline. The pH indicator turns from pale yellow to pink-red in color in these conditions.

Indole test

Indole test was used to determine the ability of an organism to spilt amino acid tryptophan to form the compound indole. Tryptophan is hydrolysed by tryptophanase to produce three possible end products – one of which is indole. Indole production is detected by Kovac's reagent which contains 4 (p)-dimethylamino benzaldehyde. This reacts with indole to produce a bright red coloured compound on the surface of the medium between the kovac's reagent and the indole.

.**Kovac's reagent**

Used in detecting the presence of 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 a mordant (iodine). It is then decolourized with acetone alcohol and finally counter stained with neutral red. The air dried fixed smear of bacteria, when stained with crystal violet picks up the stain and retains its when the mordant is applied hence giving it a purple colour. The mordant iodine enhances the union between the dye and the internal content of the organism. On the other hand acetone alcohol may cause the bacteria to lose the initial dye (crystal violet) and pick up the counter satin- neutral red. This is designated as gram negative.

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 screwcap 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: STERILIZATION

Sterilization of glass ware

To sterilize glassware by dry heat, a temperature of 160ºC 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)

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

All media for culture and isolation were stored in a refrigerator at 2–8ºC.

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 CCTH incinerator.

Stool in Selenite F 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.

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 V: Demographic characteristics of participants stratified by the isolates obtained in blood samples

ORGANISM	*CITROBACTER* (N = 10)	*ENTEROBACTER SP* (N = 5)	*E. COLI* (N = 6)	*KLEB. OXYTOCA* (N = 2)	*KLEB, PNEUMONIAE* (N = 3)	*PROTEUS MIRABILIS* (N = 1)	*PROTEUS VULGARIS* (N = 1)	*P. AERUGINOSA* (N = 6)	*STAPH. AUREUS* (N = 24)	*STAPH. EPIDERMIDIS* (N = 5)	*STREP. AGALATIAE* (N = 4)	*STREP. PYOGENES* (N = 2)	TOTAL (N = 69)
SEX FEMALE	5	2	3	1	2	0	1	4	13	1	2	1	35 (51%)
MALE	5	3	3	1	1	1	0	2	11	4	2	1	34 (49%)
DEPARTMENT													
IPD	10	5	5	2	3	0	1	5	23	5	4	2	65 (94%)
OPD	0	0	1	0	0	1	0	1	1	0	0	0	4 (6%)
AGE 0 – 20	3	4	5	2	2	1	1	6	21	5	4	0	54 (78%)
21 – 40	7	1	1	0	0	0	0	0	2	0	0	0	11 (16%)
41 – 60	0	0	0	0	1	0	0	0	1	0	0	0	2 (3%)
>60	0	0	0	0	0	0	0	0	0	0	0	2	2 (3%)

Key: GEN – gentamycin **COT** – cotrimoxazole **TET** – tetracycline **CIP** – ciprofloxacin **AMP** – ampicillin **LEV** – levofloxacin **CTX** – cefotaxime **CTR** – ceftriaxone **CHL** – chloramphenicol **AMK** – amikacin **CRX** – cefuroxime **MEM** - meropenem

APPENDIX VI: Antibiotics resistance patterns of isolates from blood cultures

ORGANISM / ANTI - BIOTIC		*CITROBACTER* (N = 10)	*ENTEROBACTER SP* (N = 5)	*E. COLI* (N = 6)	*KLEB. OXYTOCA* (N = 2)	*KLEB, PNEUMONIAE* (N = 3)	*PROTEUS MIRABILIS* (N = 1)	*PROTEUS VULGARIS* (N = 1)	*P. AERUGINOSA* (N = 6)	*STAPH. AUREUS* (N = 24)	*STAPH. EPIDERMIDIS* (N = 5)	*STREP. AGALATIAE* (N = 4)	*STREP. PYOGENES* (N = 2)	TOTAL (N = 69)
CXM	R	6	3	4	2	3	1	0	3	19	4	2	2	49 (71%)
	S	4	2	2	0	0	0	1	3	5	1	2	0	20 (29%)
TET	R	10	4	5	2	3	1	1	6	20	5	4	2	63 (91%)
	S	0	1	1	0	0	0	0	0	4	0	0	0	6 (9%)
CHL	R	6	2	2	2	3	0	1	5	11	4	2	2	40 (58%)
	S	4	3	4	0	0	1	0	1	13	1	2	0	29 (42%)
AMP	R	10	4	6	2	3	1	1	6	23	5	3	2	66 (96%)
	S	0	1	0	0	0	0	0	0	1	0	1	0	3 (4%)
LEV	R	0	0	1	0	0	0	0	2	2	1	1	0	7 (10%)
	S	10	5	5	2	3	1	1	4	22	4	3	2	62 (90%)
CTR	R	6	4	4	1	1	1	1	4	20	4	0	1	47 (68%)
	S	4	1	2	1	2	0	0	2	4	1	4	1	22 (32%)
CIP	R	1	0	4	0	0	0	0	1	3	2	1	0	12 (17%)
	S	9	5	2	2	3	1	1	5	21	3	3	2	57 (83)
CTX	R	8	4	4	2	0	1	1	2	14	4	3	0	43 (62%)
	S	2	1	2	0	3	0	0	4	10	1	1	2	26 (38)
AMK	R	1	0	0	0	0	0	0	1	0	0	0	0	2 (3%)
	S	9	5	6	2	3	1	1	5	24	5	4	2	67 (97%)
GEN	R	1	2	1	1	0	0	0	0	9	2	0	1	17 (25%)
	S	9	3	5	1	3	1	1	6	15	3	4	1	52 (75%)
COT	R	10	4	4	2	3	1	1	6	21	5	2	1	60 (87%)
	S	0	1	2	0	0	0	0	0	3	0	2	1	9 (13%)
MEM	R	8	4	6	2	3	1	1	6	23	4	4	2	64 (93%)
	S	2	1	0	0	0	0	0	0	1	1	0	0	5 (7%)

Key: GEN – gentamycin **COT** – cotrimoxazole **TET** – tetracycline **CIP** – ciprofloxacin **AMP** – ampicillin **LEV** – levofloxacin **CTX** – cefotaxime **CTR** – ceftriaxone **CHL** – chloramphenicol **AMK** – amikacin **CRX** – cefuroxime **MEM -** meropenem