PREVALENCE AND ANTIBIOTIC SUSCEPTIBILITY OF *SALMONELLA* SPECIES ISOLATED FROM ASYMPTOMATIC FOOD HANDLERS IN WESTLANDS DIVISION, NAIROBI, KENYA

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DECLARATION

This thesis is my original work and has not been presented in any other institution for the award of a degree or any other award.

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DEDICATION

To my father Dr. M. K. A. Yegon for his constant inspiration and support.

ABSTRACT

Salmonella are Gram-negative facultative intracellular anaerobic bacteria that cause a wide range of diseases in diverse hosts ranging from gastroenteritis, enteric fever and bacteraemia. These infections can lead to a convalescent lifetime carrier status/asymptomatic carriers. The antimicrobials that are currently widely regarded as most favourable for the management of *Salmonella* infections by the World Health Organisation (WHO) in adults is the fluoroquinolone group of antibiotics while in young children the third generation cephalosporin is used for serious infections. However, the occurrence of emerging resistant strains is evident. The aim of this study was to isolate, characterize and determine the antibiotic susceptibility of the *Salmonella* isolates. Being an epidemiological study, a cross sectional laboratory based design was employed, involving stratified random sampling. This study was based at the Kenya Medical Research Institute microbiology centre for microbiology research (CMR) laboratories in Nairobi Kenya. A total of 400 stool samples were used and the study was run for a period of four months. The samples were cultured in Xylose Lysine Deoxycholate agar (XLD) and Mac Conkey agar both are selective and differential media for isolation of *Salmonella sp;* serotyping and biochemical test was conducted for the confirmation of the isolated bacterial strains. The Kirby-Bauer disc diffusion method was used to test susceptibility of the isolated *Salmonella* to antibiotics commonly used in animal and human health. Data was analyzed using analysis of variance (ANOVA) and descriptive statistical methods were used for statistical analysis. A total of 6 serotypes were isolated with a total prevalence of 2%, namely; *Salmonella* para*typhi* A, *S.* para*typhi* B, *S. enteritidis* with a percentage of 0.5%, *S. typhimurium, S. typhisuis* both with a percentage of 0.25%. Among the isolates, a significant variation in inhibition zone sizes of *salmonella* under the commonly used drugs was observed $F=19.48$ while $p \leq 0.05$, thus significant difference in their effectiveness. A drug susceptibility of 81.8% was observed. Multi-drug resistance of 37.5% was observed with 4 antimicrobial resistance profiles. Ampicillin and Amox-clav showed more resistance among the *Salmonella* isolates. Although a prevalence of 2% can be considered an insignificant figure, it indicates a probability of *Salmonella* infection being passed to the food consumers by the asymptomatic handlers. Furthermore, should there be a case of infection by asymptomatic food handler to consumer, and the chances of the consumer being infected by the drug resistant *Salmonella* serotypes will be high. Therefore, the ministry of public health should make it mandatory for hotel owners to employ certified food handlers to break this cycle of infections.

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LIST OF ABBREVIATIONS AND ACRONYMS

ACSSUT- Ampicillin, Chloramphenical, Streptomycin, Sulphonamides and Tetracycline type of resistance

ANOVA- Analysis of Variance

ATCC- American Type Culture Collections

CDC- Centre of Disease Control

CLSI- Clinical Laboratory Standard Institute

CMR- Centre for Microbiology Research

KEMRI- Kenya Medical Research Institute

MDR- Multi-drug resistance

MH- Muller Hinton agar

NARST- Nalidixic acid resistant *S. typhi*

NCCLS- National Council of Clinical Laboratory Science

NTS- Non-typhoidal *Salmonella*

USA- United States of America

WHO- World Health Organization

XLD- Xylose lysine deoxycholate agar

LIST OF DEFINITIONS

Zoonotic infections- a disease that normally exists in animals but that can infect humans

Salmonellosis- infection with *Salmonellae* marked by intestinal problems and fever and caused especially by eating certain improperly stored or undercooked foods.

Food borne infections- any infectious illness whose cause is the ingestion of food tainted by a bacteria, virus, worms, or other organisms

Morbidity- the incidence or prevalence of a disease or of all diseases in a population

Mortality rate- the relative frequency of deaths in a specific population; death rate

Exogenous risk factors- risks over which we have no control and which are not affected by our actions

Sporadic cases- Occurrences on occasions or in a scattered, isolated, or seemingly random way

Pathogenesis- the development of morbid conditions or of disease

Bacteremia- an invasion of the bloodstream by bacteria

Enterocolitis- inflammation of the small intestine and colon

Gastroenteritis- irritation of the digestive tract, particularly the stomach and intestine

Septicemia- blood poisoning from any of the various diseases caused by microorganisms in the blood

Focal infections- bacterial infection localized in a specific part of the body

Virulence- the degree of pathogenicity of a microorganism as indicated by the severity of disease produced and the ability to invade the tissues of the host

Systemic infections- an infection in which the pathogen is distributed throughout the body rather than concentrated in one area

Febrile illness- any disease associated with or characterized by fever

Atherosclerotic plaques- a deposit of fat and other substances that accumulate in the lining of the artery wall

Epidemics-an outbreak or unusually high occurrence of a disease or illness in a population or area

Endemic- a disease or pathogen that is found in or confined to a particular location

Reservoir- an organism that is the host for a parasitic pathogen or that directly or indirectly transmits a pathogen to which it is immune

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background information

Salmonella are Gram-negative, facultative, intracellular, anaerobic bacteria that cause a wide range of diseases in human beings, ranging from gastroenteritis to enteric fever caused by paratyphoid and typhoid serotypes, respectively. These infections mostly lead to a convalescent lifetime carrier status called asymptomatic carrier state. There are two types of *Salmonella* associated with the human host, non-typhoid *Salmonella* and typhoid *Salmonella.*

Non-typhoid *Salmonella* is more frequently experienced in developing countries as compared to the developed countries and can affect as many as 21.5 million (WHO, 2003) individuals each year especially where unhygienic conditions are likely to occur. Salmonellosis caused by non-typhoid *Salmonella* is considered as one of the most widespread and common food borne diseases. At the moment, and in most countries chiefly in the sub Saharan regions, it represents a major public health burden. Although salmonellosis is a self-limiting infection, antibiotics may cut short the length of illness (Washington *et al.*, 2006). Millions of human incidents are reported worldwide to cause thousands of deaths each year (WHO, 2003) especially among the young, immuno-compromised persons and the elderly whose immune systems are weak.

Salmonellosis infections in humans are habitually contracted through the consumption of contaminated food of animal origin such as meat products, poultry products and milk. In addition a variety of other foods such as green vegetables planted using untreated organic manure or food handled improperly through infected persons have

been indicated in the spread of *Salmonella* infection (Washington *et al.*, 2006).The causal organisms go through food stages to households or food-service establishments and institutions. Human incidents have been observed to occur where persons have had contact with infected animals, including domesticated animals such as cats and dogs which most likely acquired the infection in similar ways as humans (WHO, 2005).

Typhoid fever is known to be spread via faecal oral route through the contamination of water sources or food by faecal material of an infected person (Madigan *et al.,* 2009). Therefore, asymptomatic food handlers and employees in catering departments of institutions can pose great danger of infecting their clientele if they are already harbouring the bacteria and also if good production procedures during food handling and preparation are not followed.

There is broad scientific consensus that the use of antibiotics in food animals on some occasions has detrimental effects on human health (DANMAP, 2000). Food animals exposed to additives such as the antibiotics used for growth promotion may serve as a reservoir of resistant bacteria and resistance genes that may spread to the human population, thereby limiting the medical value of antimicrobial drugs (Aarestrup *et al.,* 2001).

The antimicrobials currently widely regarded as most favourable for the management of *Salmonella* infections in adults is the group of fluoroquinolone. In young children the third generation cephalosporin which are given by injection are widely used for severe infections. Chloramphenicol, Ampicillin and Amoxicillin and Trimethoprim-Sulfamethoxazole is occasionally used as alternative drugs (WHO, 2005). Resistance by *Salmonella* to fluoroquinolones has emerged in several countries as a result of

using antibiotics for human treatment in the treatment of animals which are later used as a food source (Kariuki *et al.,* 2004). In addition, under dosage and misuse of antibiotics in the treatment of human infections has led to mutations in the bacterial genome, enabling the *Salmonella* to gain resistance to antibiotics that were once effective, posing a public health concern. In some cases, multi-drug resistance by bacteria is transferred through one coherent piece of DNA, referred to as a plasmid (WHO, 2005).

Multi-drug resistant (MDR) strains of *Salmonella* are now encountered frequently and the rates of multi-drug resistance have increased considerably in recent years (WHO, 2005). Even worse, some variants of *Salmonella* have been observed to develop multi-drug resistance as an integral part of their genetic material (Kariuki *et al*., 2000). These are likely to retain their drug resistant genes even when antimicrobial drugs are no longer used, a situation where other resistant strains would typically lose their resistance (WHO, 2005). Selective pressure from the use of antimicrobials is a major driving force behind the emergence of resistance, but other factors also need to be taken into consideration. For example, some *Salmonella* serotypes have been shown to be prone to resistance. A recent example is the global spread of a multi-drug resistant *S. typhimurium* phage type DT104 in animals and humans (Jones *et al.,* 2007). While the spread of DT104 may have been facilitated by the use of antimicrobials, international and national trade of infected animals is thought to play a major role in international spread (Helms *et al*., 2005, WHO, 2005, Chiu *et al*., 2006).

The emergence of MDR *Salmonella* strains with resistance to fluoroquinolone and third-generation cephalosporin's is a serious development, which results in severe limitation of the possibilities for effective treatment of human infections, hence a human health scare. A public health concern is the consequence of emergence of

drug-resistant *Salmonella* following the non-human use of antimicrobial agent, and the possible spread of these agents by the infected asymptomatic food handlers to the public through contamination of food by improper handling. This study is therefore important as it will analyze, assess, the probability of exposure or transfer of *Salmonella spp.* and their antimicrobial resistance profiles from asymptomatic food handlers to unsuspecting population. The results may constitute a source of information on the efficiency of hazard assessment during application of food safety management systems in food outlets, as well as to access the possible risk of transmission of antimicrobial resistant *Salmonella spp.* from asymptomatic food handlers to consumers.

1.2 Statement of the problem

Salmonella when consumed constitute a major source of food-borne illnesses in humans such as salmonellosis, bacteremia and enteric fever which can be fatal. In recent times, the incidence of infections by *Salmonella* among food handlers and consumers has been on the rise both in terms of occurrence and severity of the cases. Most of these infections have been indicated to be a result of food contamination during preparation. Compounded with the emergence of drug resistance due to the misuse of antibiotics by man. This is indicated by the WHO fact sheet on *Salmonella* infections and drug resistance (WHO, 2005). The antibiotic resistance tendency of *Salmonella* is of global concern, because of the possibility for transfer of these resistant pathogens to the other consumers by asymptomatic food handlers, and because of their resistance to common antibiotics. Thus, these bacteria may cause infections with limited therapeutic options. This may lead to treatment failure and may have serious consequences for the patient. Therefore, it is of utmost importance to assess the risk to consumers that may arise from asymptomatic food handlers

working in food kiosks, hotels, cafeterias among other food outlets, and access the antibiotic resistant probability of the *Salmonella* isolates from faeces of these food handlers.

1.3 Justification of the study

Food handlers are an important group in the food processing chain. They are also important in the progression of foodborne illnesses. This can be a consequence of contamination by an asymptomatic food handler during food processing, hence posing risk to consumers. Food safety is of utmost importance to consumers, regulatory agencies and governments.

In cities around the world, food outlets are an important part of the society and the readymade food from food establishments, manufacturing and processing industries are fast rising to be popular to consumers in developing countries such as Kenya. Therefore, the chances of infection of consumers by asymptomatic food handlers are also on the increase. From the early 1990s, strains of *Salmonella* which are gaining resistance to a range of antimicrobials have been noted to be emerging in Kenya (Kariuki *et al*., 2004). This also includes first-choice remedy for humans and these are aggressively threatening to become a serious public health crisis, thus the importance of this research in ascertaining the prevalence of the asymptomatic infections among the food handlers constantly posing risk of infecting their food consumers. In addition to this, Kenya has limited prevalence and resistance surveillance studies that have been published. Therefore, there is no adequate data to comparatively analyze and assess the probability of exposure or transfer of *Salmonella* contaminants, and their antimicrobial resistance profiles from food handled by asymptomatic food handlers. This study will be essential for performing risk assessment and management and

determining the current *Salmonella* prevalence and antibiotic efficiency of the available drugs.

1.4 General Objective

To determine the prevalence and antibiotic susceptibility of *Salmonella* serotypes isolated from the asymptomatic food handlers in Westlands division, Nairobi.

1.4.1 Specific Objectives

i. To determine the antibiotic susceptibility of the isolated *Salmonella* serotypes.

ii. To determine the prevalence o*f Salmonella* serotypes among the asymptomatic food handlers.

1.5 Hypothesis

i. There is no antibiotic susceptibility observed among the isolated *Salmonella* serotypes.

ii. There is no prevalence of *Salmonella* serotypes among the asymptomatic food handlers.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 The organism

Salmonella belongs to the family enterobacteriaceae. Members of this genus are rod-shaped Gram negative and cause typhoid fever, paratyphoid fever and food borne illness (Ryan and Ray, 2004). Members of this genus have also been reported to produce hydrogen sulphide on Triple Sugar Iron Agar (TSI) except for *S.* Para*typhi* A (Cheesbrough, 2000).

2.2 History

Salmonella bacteria were named after Daniel Elmer Salmon, an American veterinary pathologist, who together with Theobald Smith first isolated the bacterium from pigs in 1885 (Ryan and Smith, 2004). Most cases of *Salmonella* infection involve consumption of undercooked meat, chiefly poultry meat (Atlas, 1995). Other sources other than meat have been implicated in infections by *S. enterica*.

2.3 Microbiology

In a clinical laboratory, *Salmonella* are usually isolated on selective and differential media such as Mac Conkey agar, Xylose Lysine Deoxycholate (XLD) agar or Deoxycholate-citrate agar (DCA) agar; this is followed by serotyping and biochemical culture to ascertain the identity of the isolate (Cheesbrough, 2000). The populations of *Salmonella* in a stool sample may be too low for the samples to be routinely cultured, necessitating subjection to enrichment culture involving a small sample (pea size) of the stool specimen being incubated in a selective broth media such as selenite F broth (Madigan et al., 2009). These inhibit the growth of normal flora found in a healthy

human bowel, while allowing increase in numbers of *Salmonella* prior to primary culture of the sample.

Mac Conkey agar is used when isolating enteric bacteria; it contains bile salts and crystal violet that inhibits Gram positive bacteria and certain Gram negative bacteria respectively (Madigan *et al*., 2009). This media selects and differentiates the lactose fermenters from non-lactose fermenters in a sample. Non lactose fermenters on Mac Conkey appear colourless and are pathogenic organisms such as *Salmonella* (Tortora *et al.,* 2001, Ryan and Smith, 2004).

Xylose lysine Deoxycholate (XLD) media contains Xylose and lysine. It is used in the isolation of bacteria that do not ferment lactose but ferment Xylose sugar to produce an acid, such as *Shigella* and *Salmonella*. This media is both selective and differential for bacterial identification. In XLD, *Shigella* (is a non-Xylose fermenter) appears as small, rounded and colourless colonies, coliforms appear as yellow to orange colonies and *Salmonella* (is a Xylose fermenter) appear as small pink colonies with or without black centres formed as a result of production of H_2S from the metabolism of thiosulphate present in the media (Tortora *et al.,* 2001, Ryan and Smith, 2004).

Serotyping is based on the immunologic reactivity of two surface structures polysaccharide O antigen; flageline protein and the VI antigen which are specific to *Salmonella.* This separates the unconfirmed cases of salmonella from other enteric bacteria before the biochemical tests are carried out. Biochemical tests comprise the Triple sugar test (TSI) and Indole, Methyl Red, Voges-Proskauer and citrate tests (IMViC) (Cheesbrough, 2000).

TSI is an important test in isolation and identification of *Salmonella*. It is composed of three different sugar types, glucose 0.1%, lactose 1% and sucrose 1% and is used to detect carbohydrate fermentation at different levels by an isolate both aerobic and anaerobic, gas production and hydrogen sulphide gas production (Cheesbrough, 2000). Carbohydrate fermentation is indicated by the presence of gas and a visible colour change (from red to yellow) of the pH indicator phenol red. Most bacteria that ferment sugar in the anaerobic butt are enteric bacteria. The production of hydrogen sulphide is indicated by the presence of a precipitate that blackens the medium at different levels (Cheesbrough, 2000). *Salmonella* is unable to ferment all carbohydrates at both phases of the media, positive *Salmonella* result show a visible colour change producing a yellow butt and a red slant indicating that glucose fermented with gas and hydrogen sulphide being produced.

IMViC test, IMViC reactions are a set of four useful reactions that are commonly employed in the identification of members of family enterobacteriaceae (Cheesbrough, 2000). The four reactions are: Indole test, MR test, lysine decarboxylase, Voges Proskauer test and Citrate utilization test (Cheesbrough, 2000).

2.4 Nomenclature

The taxonomy of *Salmonella* is considered complicated (Tindall *et al*., 2005). Traditionally *Salmonella* was considered to have many serotypes that were initially said to be members of a single species *Salmonella enterica.* However, currently there are three recognized species, *S. enterica, the species, S. bongori*, and *S. subterranean* with six main subspecies in *S. enterica* (WHO, 2007). *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), *S. enterica* subsp. *indica* (VI).

Named according to where they were first isolated or the disease they are known to cause**.**

Based on the immuno-reactivity of O-lipopolysaccharide (LPS) and H-flagella protein antigens (Kauffman-White scheme), which are the most commonly used methods of *Salmonella* classification, as of 2007, 2,557 serovars of *S. enterica* and 22 serovars of *S. bongori* have been recognized. All found in disparate environments and are associated with many different diseases (WHO, 2007). Majority of human clinical isolates, include *Salmonella* serovariant's Enteritidis, Typhimurium, and Typhi.

2.5 Incidence of *Salmonella* infection among food workers

Foodborne illnesses are widespread and an ever growing public health crisis in both developed and developing countries. World health organisation (WHO) indicates that the global incidence of foodborne illness is difficult to estimate. According to WHO report in 2005, 1.8 million people died from diarrhoeal foodborne infections. A great proportion of these cases could be attributed to contamination of food and drinking water. In industrialized countries, the percentage of those suffering from foodborne infections yearly was reported to be up to 30% (WHO, 2005). The numbers in developing countries have not been established. They are the majority who suffer the brunt of foodborne infections including those caused by parasites (WHO, 2007).

Salmonellosis is the major foodborne illness in most countries (WHO, 2007). Studies by Medus *et al.,* (2006) evaluated the impact of surveillance on the detection of outbreaks in restaurants from the year 1997 to 2004, and found that 110 out of 4,976 (2.2%) confirmed *Salmonella* cases reported were identified as food workers. In the United States, approximately 40,000 cases of salmonellosis are reported every year, though the actual number of infections may be thirty or more times greater if the

milder cases were diagnosed and reported (CDC, 2005). In a separate study (Murakami and Horikawa, 2007) isolated *Salmonella* from 106 of 331,644 (0.032%) faecal samples from food handlers, with *S.* Serovar *enteritidis* being the most common. A different study by Yanping *et al*., (2009), found that 29 of the 305 (9.5%) asymptomatic food handlers sampled from a hospital cafeteria were *Salmonella* carriers. The twenty nine isolates were grouped into five serotypes; Agona, Derby, Enteritidis, Infantis and Senftenberg. Through these studies, *S. enteritidis* has been indicated as the major cause of foodborne illnesses. This serotype is associated with significant morbidity and mortality worldwide and is reported to be responsible for approximately 17% of all human *Salmonella* infections in the USA (Younus *et al.*, 2006).

These results indicate that food workers should be considered an important source of *Salmonella* transmission. Those identified through surveillance should raise a high index of suspicion of a possible outbreak at their place of work. Hence food service managers need to be alert to *Salmonella*-like illnesses among food workers to facilitate prevention and control efforts.

2.5 Drug resistance in *Salmonella* species

Recently, there has been continuous emergence of multi-drug resistant (MDR) *Salmonella* strains globally with resistance to Fluoroquinolone and third-generation cephalosporin (WHO, 2005). Multi-drug resistant strains of *S. enterica* serovar Typhimurium was first reported in the mid-1980s (Threlfall *et al*., 1994, Ribot *et al*., 2002), and was typically identified by penta-drug resistance to Ampicillin, Chloramphenicol, Streptomycin, Sulfonamides, and Tetracycline (ACSSuT type of resistance). Yanping *et al*., (2009) observed that all the isolates grouped into five

groups Agona, Derby, Enteritidis, Infantis and Senftenberg were resistant to Chloramphenicol, Nalidixic acid and Tetracycline. Kariuki *et al*., (1996) noted that, of the *Salmonella* isolated from cattle and food handlers, only 16% of non typhoidal *Salmonella* (NTS) were sensitive to all ten antibiotics tested, 20% were resistant to one agent mostly Streptomycin or Tetracycline and 17% were resistant to two agents usually Streptomycin and Tetracycline or Tetracycline and Ampicillin. In general, 47% of the isolates in the study were resistant to three or more antimicrobials and the most frequent resistance pattern was to Ampicillin, Cefuroxime, Chloramphenicol, Co-trimoxazole, Streptomycin and Tetracycline which are the current readily available therapeutics in developing countries. In a different study done after an outbreak of typhoid fever infection, *S. Enterica* serovar Typhi isolated from samples in three different parts of Kenya, establish that only 13.7% were fully susceptible to drugs, whereas another 82.4% were resistant to each of the five commonly available drugs: Ampicillin, Chloramphenicol, Tetracycline, Streptomycin, and Co-trimoxazole (Kariuki *et al.,* 2004). Guerra *et al*., (2000) analysed the resistance profile for 15 antimicrobial agents of 333 *Salmonella* strains and reported that though all the strains were susceptible to Amikacin, Ceftazidime and Ciprofloxacin, resistance to Ampicillin and Chloramphenicol ranged between 22%-46% of the isolates. Dobardzic (1996) found the resistance of Chloramphenicol, Ampicillin and Co-trimoxazole vary between 18% and 50%. Sporadic cases of Ciprofloxacin treatment failure in typhoid fever have been reported in Europe and more recently, in Asia (Parry *et al*., 2003, Butt *et al*., 2003). Another study found that antimicrobial resistance in *Salmonella* to Chloramphenicol, Ampicillin and Co-trimoxazole is common in Africa (Parry, 2003).

Njinkeng *et al.,* (2005) noted that MDR *S. typhi* and Nalidixic acid resistant *S. typhi* (NARST) strains were found in Cameroon, Central Africa. NARST have also been

reported in East Africa (Kariuki *et al.,* 2004). These conclude that the resistance of *Salmonella* species to drugs is a present concern which is ever increasing to several critical antimicrobials used to treat *Salmonella* infections in resource poor countries. Such drug resistant *Salmonella* infections could become progressively untreatable.

2.6 Pathogenesis

Salmonella serotypes are associated with three distinct human disease syndromes, bacteremia, typhoid fever, and enterocolitis (Fang and Fierer, 1991) which can manifest itself as gastroenteritis, enteric fevers, septicaemia, focal infections and an asymptomatic state. Virulence in *Salmonella* requires the harmonized expression of intricate array of virulence factors (V-factors) allowing the bacterium to escape the immune system of the host (Ohl and Miller, 2001). *Salmonella* attack the host by inducing their uptake by the host intestinal epithelium cells. Serotypes linked to causing gastroenteritis initiate an intestinal inflammatory and secretory reaction, whereas those that causes enteric fever cause systemic infection through their capacity to exist and reproduce in mononuclear phagocytes (Ohl and Miller, 2001).

2.7 Clinical manifestation of *Salmonella*

Salmonella symptoms in humans vary with the serovar causing the infection, infectious dose, the nature of the contaminated food, and the host immune status. Certain serovars are highly pathogenic to humans. Still, strains of the same serovar are known to differ in their pathogenicity. Infants, immuno-suppressed patients, and those affected with blood disease are more susceptible to *Salmonella* infection than the healthy adults (CDC, 2005).

2.8 Typhoid fever

Typhoid is strictly a human disease. The bacteria enter the human digestive tract penetrate the intestinal mucosa to the mesenteric lymph nodes where bacterial multiplication and dispersion occurs. Lipopolysaccharide (LPS) endotoxin is released into the bloodstream resulting in septicemia (Madigan *etal*., 2009). The secondary illness after bacterial dispersion is responsible for causing fever and clinical illness manifested by persistent headache, fever and chills, unproductive cough, rose-spots on the trunk, abdominal tenderness, malaise, epitasis and unpredictable mood (Gianella *et al.*, 1979; Nester *et al.*, 2001). Complications correlated with enteric fever include copious haemorrhage in the intestines and perforation of the intestines (Finlay *et al.,* 1989) and this infection is fatal if not treated.

2.9 Salmonella enteritidis Infection (salmonellosis)

Salmonellosis ranges clinically from the common *Salmonella* gastroenteritis infections; it manifests itself as diarrhoea, abdominal cramps, and fever to enteric fevers including typhoid fever which are life-threatening febrile systemic illnesses requiring prompt antibiotic therapy (CDC, 2005). Focal infections and an asymptomatic carrier state occur as a result of salmonellosis. The most common form of salmonellosis is a self-limited, uncomplicated gastroenteritis (Madigan *et al.*, 2009).

2.10 Foodborne *Salmonella* toxic infections

These infections are caused by ingestion of preformed *Salmonella* toxin by omnipresent *Salmonella* serovars such as *Typhimurium*. Symptoms appear about 12-24 hours following ingestion of contaminated food containing a sufficient amount of *Salmonella* and they include diarrhoea, vomiting and fever and last 2-5 days,

spontaneous cure usually occurs hence no need of medical intervention among patients with non-compromised immune system (Medus *et al*., 2010).

2.11 Asymptomatic carriers

An asymptomatic carrier is an individual who serves as host for an infectious agent but who does not show any apparent signs of the illness and may serve as a source of infection for others (Atlas, 1995). Asymptomatic carriers are potentially dangerous when they go unnoticed, because they do not display any disease symptoms. *Salmonella* excretion by patients may continue long after clinical cure. About 5% of patients clinically cured from typhoid remain carriers for months or even years (Madigan *et al*., 2009). Antibiotics are usually ineffective on *Salmonella* carriers even if *Salmonella* are susceptible to them because the site of carriage may not allow penetration by the antibiotic. A classic case of the dangers of asymptomatic food handlers is “Typhoid Mary” who caused uproar in the United States between the years 1900-1915. “Typhoid Mary” is known to have infected an estimated fifty-four people while she worked as a cook out of whom three confirmed individuals died (Shanson, 1989; Atlas, 1995).

2.12 Treatment of *Salmonella* infections

The antibiotics that form the mainstay of therapy for typhoid patients in developing countries are Ampicillin, Co-trimoxazole and Chloramphenicol (WHO, 2005). Due to the increasing resistance to traditionally used antibacterials used for therapy, the use of Fluoroquinolone, such as Ciprofloxacin and Ofloxacin, for the treatment of typhoid has become more common in the Asian countries and subsequently in the developing countries (Parry, 2004).

2.13 Control of *Salmonella* infection

Salmonella is linked to all kinds of foods, such as the contamination of meat from cattle, pigs, goats, chicken among others may be said to originate from animal salmonellosis. Most often, this results from the contamination of muscles with intestinal contents of the animal during slaughter and hauling of carcasses. Also the handling of contaminated meat may result in contamination of hands, tables, kitchenware, towels and other foods during preparation, hence in this case the cleaning of hands before and during the handling of food is necessary. However, when contaminated meat is ground, multiplication of *Salmonella* is facilitated and if cooking is superficial then *Salmonella* is spread.

Prevention of *Salmonella* infection relies mainly on the avoidance of contamination by the improvement of hygiene. Preventing multiplication of *Salmonella* in food by the practice of constant storage of food at 4°C, use of pasteurized and sterilized egg, milk and milk products and all types of foods should be prepared in a clean environment. Cooking should be thorough, vegetables and fruits may carry *Salmonella* when contaminated with fertilizers of faecal origin, or when washed with polluted water.

Also segregation from work and social activities should be considered for symptomatic and asymptomatic persons. To avoid the spread of the disease, and as it is said, prevention is better than cure. All measures should be taken to ensure that the healthy population does not contract the disease through education, vaccination and maintenance of a clean working and living environment (WHO, 2005).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Site

The study samples were obtained from asymptomatic food handlers within Westland division. Westlands is located 3.1 kilometers by road, northwest of the central business district (CBD) of Nairobi. With latitude: 1.2700; Longitude: 36.8100. Other than being a commercial centre, it is among the eight administrative divisions of Nairobi that consists of six subdivisions/locations namely; Parklands, Kitisuru, Highridge, Kangemi, Kilimani and Lavington. Westland initially in the colonial era was a residential neighbourhood of Nairobi. In the late 1990's and early 2000's, more businesses relocated to Westland where land and office space was more readily available and less expensive compared to the CBD. Among these are star rated and normal hotels, cafes, restaurant, bistros, fast foods and food kiosks. Most of the food workers for these food establishments live in a shanty town known as the Deep Sea Settlement. This has a shanty settlement of roughly 7,000 inhabitants found within Westland division.

3.2 Study Design

The sampling design used was multi-stage sampling; in this case a four stage random sampling was employed. The study area was Westland's division as this is where most of the food outlets taking part in the study were located (first stage). This was divided according to the sub-divisions available in Westland's (second stage). The hotels taking part in the surveillance study by KEMRI CMR, within the divisions in Westland's, were then identified and numbered. The first 20 food outlets were selected randomly using the table of random samples (3^{rd} stage). Counting started

with the first food handler interviewed, every 2nd food handler interviewed was chosen for the study (4th stage). The number of food handlers in each hotel was determined and ratios made by comparing the number of food handlers in each institution, this enabled the research to determine the number of food handlers to be picked in each institution.

3.3 Inclusion criteria

i. Only stool samples of food handlers that were asymptomatic and who worked in food preparation.

ii. Were above 18 years (Adult).

iii. Willingly consented to taking part in the research.

iv. Participants working in food establishments within Westlands division, Nairobi.

v. Participants who had not been on any form of antibiotics for at least one month before sample collection.

vi. Samples that arrived in the laboratory while still well labelled and properly capped with no spilling.

3.4 Sample Size Estimation

The minimum sample size was determined using the following formula (Fishers *et al.*, 1998).

$$N = \frac{Z^2 P (1-P)}{\delta^2}$$

Where;

N is the sample size.

P is the estimated value of prevalence

δ is the level of significance (5 %)

Z- Standard normal deviate that corresponds to 95 % confidence interval (1.96)

According to a study by Senthilkumar and Prabakaran, (2005) the prevalence of *Salmonella* among healthy food handlers was 17.4 %. Assuming a prevalence rate of 17.4 % at 95 % confidence level the minimum estimated sample size was given as 221 using the formula given below.

Therefore; $n = \frac{(1.96)^2 \times 0.174 (1-0.174)}{(0.05)^2}$

Minimum sample size = 221.

17.4 % = 221

If 100 % = Maximum sample size

To get the maximum sample size;

$\frac{(221X100)}{17.4} = 1270$

Maximum sample size=1270

In this study the sample size used was 400 as per the availability of resources. Which is between the minimum and the maximum sample size and the study took a period of four months.

3.5 Preliminary experimental design

3.5.1 Laboratory experimental design for bioassays

Test groups included selected organisms (American Type Culture Collection (ATCC) strains and clinical isolates from a previous study) and the antibiotic disks at the recommended concentrations. This test was to pre-determine whether the antibiotic disks and medium being used were active.

Controls: This constituted both *Salmonella* ATCC 25822 and *E. coli* ATCC 25922. This was to establish if the test organisms being used were susceptible to the common drugs of choice or resistant. *Klebsiella pneumonia* and *Proteus mirabilis* ATCC 12453 were tested to determine viability and to ascertain if the media of choice was viable for the test.

All experiments were carried out in triplicates for replication purposes in order to minimize experimental error.

3.5.2 Sampling Procedures

Salmonella bacteria are usually transmitted via the faecal oral route, for these reason faecal samples were considered for this study other any other samples. Asymptomatic food handlers from the various hotels and institutions, some of whom were at the time participating in a surveillance study by KEMRI. For faecal ova and cyst count for the determination of parasitic infections among food handlers, these were identified sampled and educated on the importance of the study. An inclusion criterion was

issued with the consent papers to read and sign. Those who consented to the study were issued with clean and labelled poly pots in which to collect their stool samples. The poly pots were then transported to the laboratory in a clean cool box at 4^{0} C within the first 8 hours of sample collection for bacterial isolation. Samples that were not cultured within the first 24 hours of sample collection were refrigerated at temperatures below -4^{0} C.

3.6 Laboratory Procedures

3.6.1 Good laboratory practice (GLP)

Good laboratory practice was observed during the study to ensure the study was planned, performed, monitored, recorded and reported and achieved accordingly. Appropriate storage and disposal of used materials was observed.

3.7 Preparation of control test organisms

Stocked bacterial control strains were sub-cultured on Muller Hinton agar prepared according to the manufacturer's instructions and incubation of the freshly inoculated plates was done at 24^{0} C for 12 – 18 hours to obtain young freshly growing culture which was used as the control microorganisms.

3.7.1 Isolation of *Salmonella*

The stool samples were first inoculated in selenite F enrichment media for the first 24 hours. Isolation of *Salmonella* strains from the stool samples was done by culturing the enriched samples in selective and differential media and incubated for 24 hours at 37^{0} C. The media of choice in this case was Xylose Lysine Deoxycholate agar (XLD) and Mac Conkey agar for confirmatory cases where a result from the XLD was unsatisfactory. The plates were then visually examined to presumptively identify

Salmonella colonies after 24 hours. Pure cultures were obtained by sub-cultivating a single *Salmonella* colony on Muller Hinton agar plate after a Gram stain had been done. The needle end of a sterile loop was touched to a well isolated *Salmonella* colony and streaked across a third of the Muller Hinton plate while being flamed after each subsequent streak. The plates were then incubated at 37^{o}C for 24 hours from which a single colony was selected for confirmative identification of *Salmonella* through serotyping.

3.7.2 Identification of *Salmonella*

Isolates for serotyping were selected from there differential and selective medias used in this study. These were the Xylose Lysine Deoxycholate agar and Mac Conkey agar (small colonies with dark centres and pink colonies indicating lactose fermenters respectively) were picked. These were then sub-cultured in nutrient agar for 18-24 hours. Conventional serotyping of *Salmonella* species was done using Kauffman and le minor method (Kauffman and le minor, 1929). A sterile loop was used to remove the upper portion of growth from a nutrient agar media. The growth was then emulsified in three drops of physiological saline and mixed well with an applicator stick. A small drop of antiserum O group D was added to the suspension and a drop of VI antiserum to the second suspension. The third suspension was used as a control for auto agglutination. The suspensions were mixed with antiserum then mixed thoroughly for 30 seconds and observed. A positive result was indicated by clumping or agglutination after 30 seconds to one minute and the positive samples were set aside for confirmative identification.

Selected positive isolates after serotyping were confirmed through the recommended biochemical tests. The selected isolate was picked using a sterile loop and carefully

stabbed into the centre of the Triple Sugar Iron slant (TSI) agar down to the butt and streaked on the slant. The tube was then incubated at 37^{o}C for 24 hours. TSI Agar was used for the determination of carbohydrate (sugar) fermentation. Hydrogen sulphide and gas production was detected by the presence of gas and a visible colour change (from red to yellow) of the pH indicator phenol red. The production of hydrogen sulphide was indicated by the presence of a precipitate that blackened the medium at different levels (Cheesbrough, 2000). *Salmonella* ATCC 25822 was used as a positive control.

Indole, Methyl-red, Voges-Proskauer and citrate test (IMViC) was performed alongside TSI. The isolate were tested for Indole by inoculating in peptone water and incubating overnight at 37^{0} C. Following incubation, a few drops of Kovac's reagent was added. Formation of a pink to red coloured ring at the top was taken as a positive result. *Escherichia coli* ATCC 25922 were used as a positive control while *Klebsiella pneumonia* was used as a negative control.

In the Methyl Red test, the isolates were inoculated into glucose phosphate broth, which contained glucose and a phosphate buffer and incubated at 37^{0} C for 48 hours. The pH of the medium was tested by the addition of 5 drops of methyl red (MR) reagent. Development of a red colour was taken as a positive result. *Escherichia coli* ATCC 25922 were used as a negative control while *Klebsiella pneumonia* was used as a positive control.

Simmon citrate test samples were inoculated on a medium containing sodium citrate and a pH indicator bromothymol blue. Utilization of citrate resulted in change of medium's colour from green to blue. Selected colonies were picked with a straight wire and inoculated into the butt and slope of Simmon's citrate agar and incubated

overnight at 37^0 C. The organisms with the ability to utilize citrate changed the medium's colour from green to blue. *Salmonella* ATCC 25822 was used as a positive control while *Klebsiella pneumonia* was used as a negative control.

Urea Agar was used to separate *Salmonella* from other enterobacteria. The selected isolate were picked using a sterile loop and planted in to urea media. The tubes were then incubated at 37^0 C for 24 hours. *P. mirabilis* ATCC 12453 was used as a positive control for the test while *E. coli* ATCC 25922 was used as the negative control.

3.7.3 Antimicrobial Susceptibility testing

The Kirby-Bauer disc diffusion method (Bauer *et al*., 1966) was used to test susceptibility of the isolated *Salmonella* to antibiotics under test. The antimicrobial sensitivity was done on Mueller-Hinton (MH) agar (Oxoid) according to the recommendations reported by the Clinical Laboratory Standards Institute (CLSI). The organisms were tested for their susceptibility to commonly used antimicrobials on disks containing; Amox-Clav (10 mg), Oflofloxaxin (20mg), Ceftriaxone (30mg), Doxycycline (30 mg), Chloramphenicol (30 mg), Sulphamethoxazole/Trimethoprin SXT (30:2 mg), Gentamicin (10 mg), Streptomycin (10 mg), Nalidixic acid (30 mg) Ciprofloxacin (5 mg) and Ampicillin. The inoculums of each *Salmonella* isolate were adjusted to a concentration of about 10^6cfu/ml by comparing its turbidity to that of Barium chloride 0.5 McFarland standard. Adjusted bacterial inoculate were delivered onto the plates with a micropipette inoculator and spreading done by the use of a flamed and cooled glass rod in a safety hood. Inoculated agar plates were incubated at 37^0 C for 18 hours.

3.8 Ethical consideration

The study was reviewed and approved by the Institutional Research Ethics Committee, Moi University (IREC) and Moi Teaching and referral hospital. Consenting participants were given consent forms to sign after being explained to the purpose of the study and its benefits. Laboratory numbers were used to conceal the identity of the participant and to maintain confidentiality of the results.

3.9 Data Management

The collected data was entered in excel spread sheets where data could be retrieved easily and reliably and the data's safety, accuracy and integrity was ensured. Data was backed up in CD-ROMs, flash disks, computer's hard drive as well as in another computer to ensure its safety in case of the systems' breakdown of the main computer.

3.10 Statistical Analysis

The number of samples with *Salmonella* isolates was estimated as a percentage proportion of the sampled asymptomatic food handlers. The mean and standard error for all the replicates for the zones of inhibition were calculated and presented in tables and graphs.

The threshold for statistical significance was $P \leq 0.05$ at 95% confidence interval. Data was analysed using Statistical Analysis System (SAS), SAS was used to calculate the means and Least Significant Difference (LSD) which were used to compare the means in relation to the variation of data with the zones of inhibition measurement of antibiotics response by the isolates being the main factor and the isolate type being the interaction. Analysis of variance (ANOVA) (SAS) was used to analyse the result, using the inhibition zones measured in mini meters (mm) and

serotype isolated as the main factors. The latter being the dependent variable and the serotype as the independent variable.

CHAPTER FOUR

4.0 RESULTS

4.1 *Salmonella* species isolated from the asymptomatic food handlers

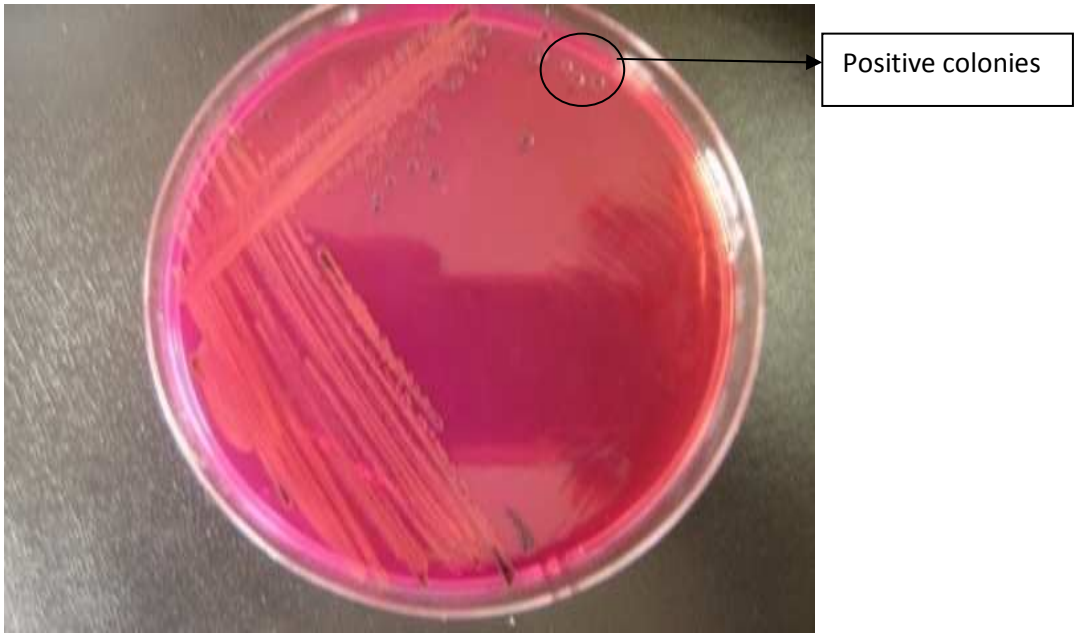
Among the 400 samples collected, 8 (2%) turned out to be positive for *Salmonella* while 392 (98%) turned out to be negative for *Salmonella.* Initially, after the primary isolation 14 samples were picked indicating positive *Salmonella* samples but after serotyping, 8 samples reacted positive for *Salmonella*. There were five different serotype isolates in the asymptomatic food handlers (Plate 4.1). Plates with positive results are shown in (Plate 4.1 – 4.4); XLD and Mac Conkey were used in this study. Though they produce almost similar results, Mac Conkey was used for confirmation of positive XLD plates. Biochemical test reactions that were observed for *Salmonella* positive samples are indicated in (Figures 4.1 and 4.2).

Table 4.1: *Salmonella* serotypes isolated from asymptomatic food handlers

O antigen group	**Isolate serotype**	**Number of isolates**
A	*Salmonella* ser. Para*typhi* A	2
B	*Salmonella* ser. Typhimurium	1
B	*Salmonella* ser. Para*typhi* B	2
C_1	*Salmonella* ser. Typhisuis	1
D	*Salmonella* ser. Enteritidis	2
Total		**8**

From a sample of 400 samples, 8 isolates were *Salmonella*. *S.* Para*typhi* A, *S.* Para*typhi* B and *S.* Enteritidis had the highest rates of isolation at 2 each while the remaining *S.* Typhimurium *and S.* Enteritidis had one isolate each. Serotyping of the

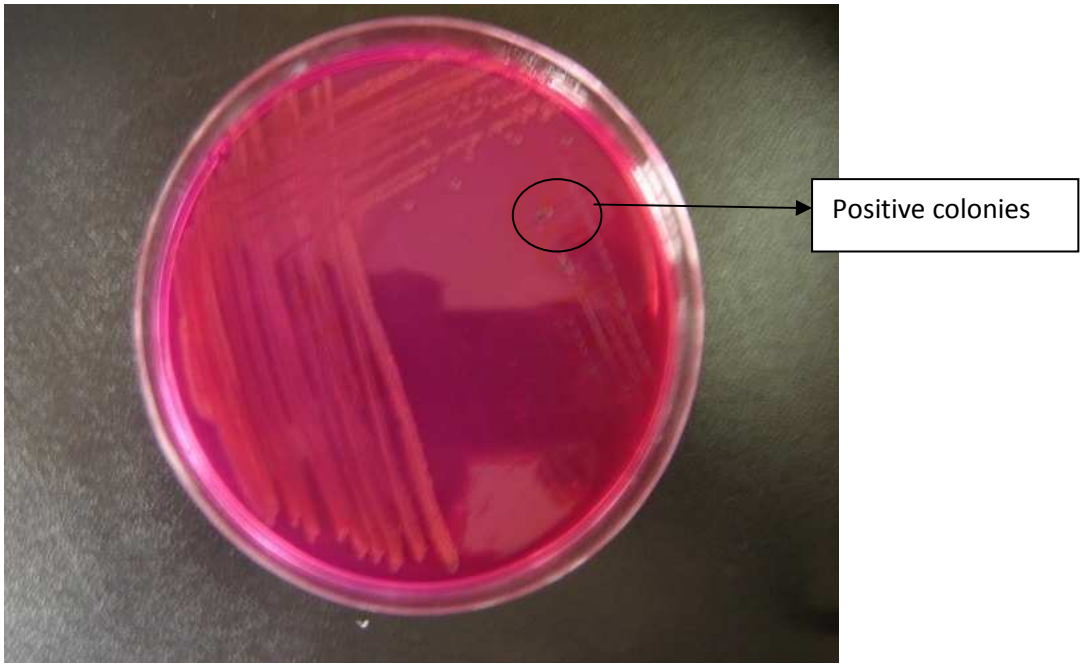
O antigen was used in the identification of *Salmonella* in this study. The isolates were grouped into four O (somatic) antigen groupings as shown in the first column of Table 4.1.



(Source: Author, 2013)

Plate: 4.1: *S.* Enteritidis culture on XLD

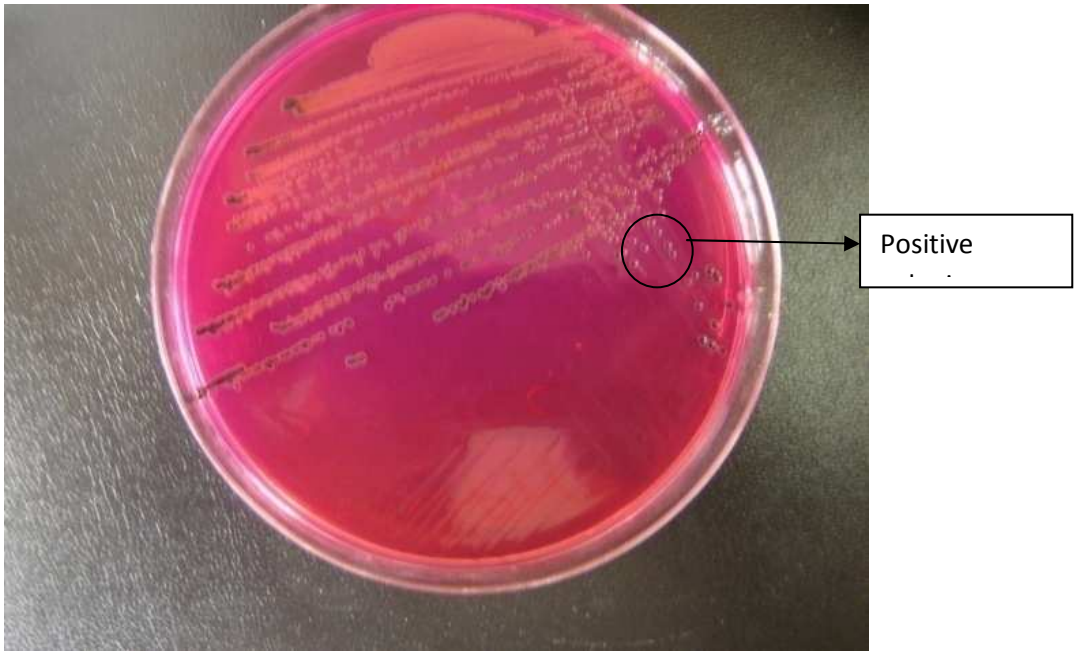
Observation of small, pink colonies with or without black centres on XLD media plate as those above, indicated presence of *Salmonella* on the plated sample.



(Source: Author, 2013)

Plate 4.2: *S*. Typhimurium isolate on XLD

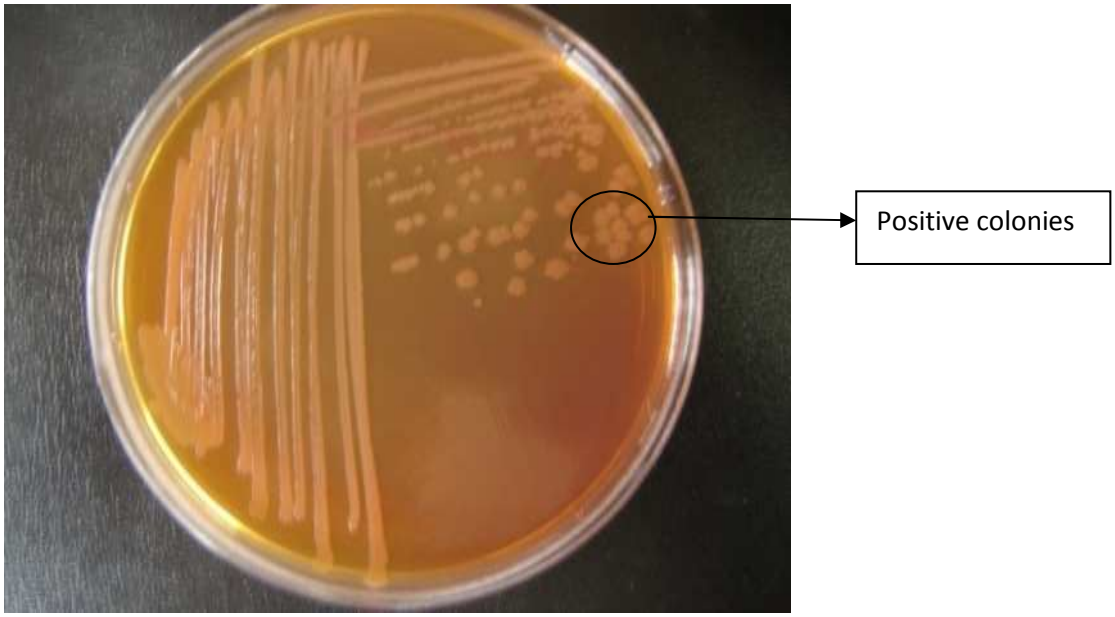
Observation of small, pink colonies with or without black centres on XLD media plate as those above, indicated presence of *Salmonella* on the plated sample.



(Source: Author, 2013)

Plate 4.3: *S*. Para*typhi* B culture on XLD

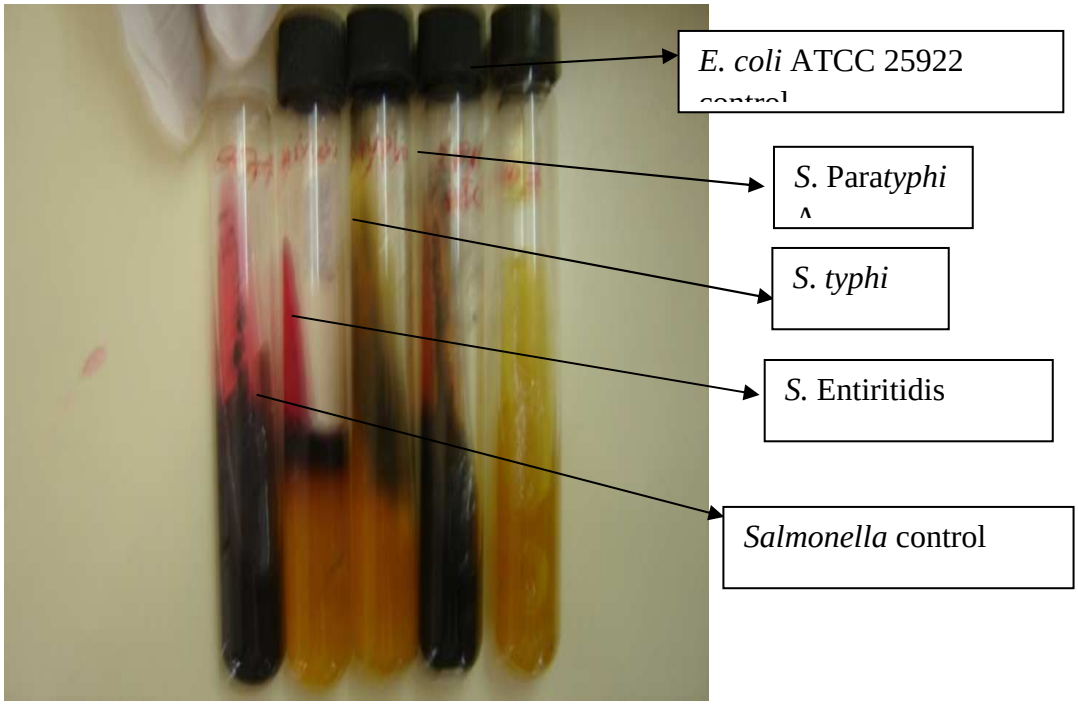
Observation of small, pink colonies with or without black centres on XLD media plate as those above, indicated presence of *Salmonella* on the plated sample.



(Source: Author, 2013)

Plate 4.4: *S*. Enteritidis on Mac Conkey agar

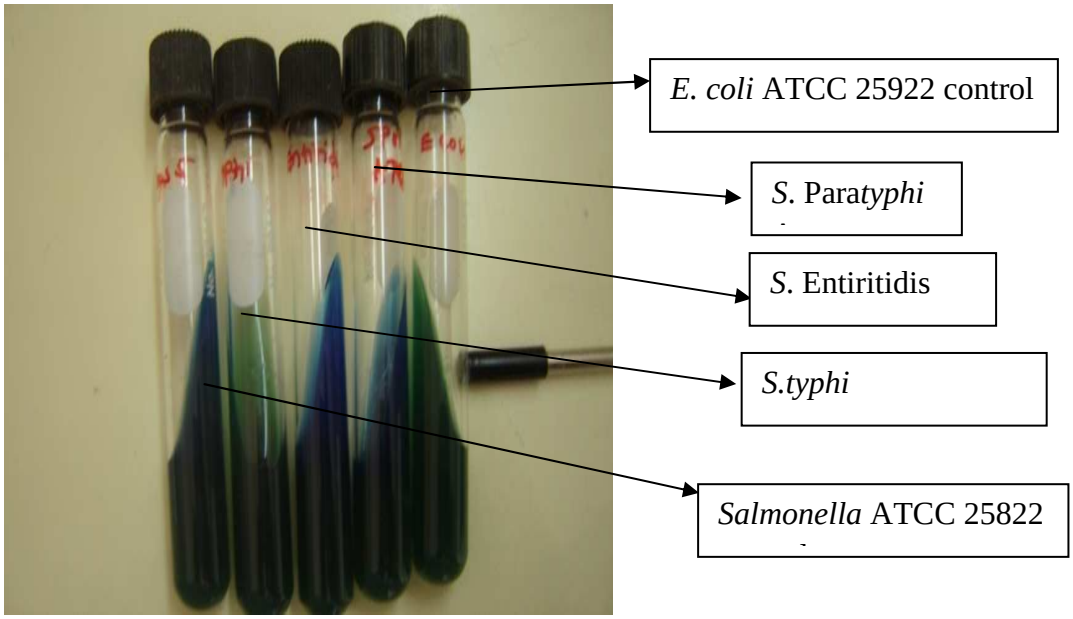
Media colour change from red to yellow is also an indication of an enteric bacterium as above.



(Source: Author, 2013)

Figure 4.1: Triple Sugar Iron ag4ar slants used for the identification of *Salmonella* indicating the reaction of the different *Salmonella* isolates from samples.

The different types of colour changes in the slant and butt of a TSI agar slant indicates different results on presence and absences of *Salmonella* species. Black colour indicates presence of H_2S producing *Salmonella* species.



(Source: Author, 2013)

Figure 4.2: Citrate utilization test reaction results of the isolates from the asymptomatic food handlers.

Utilization of citrate as an alternative sugar source by isolate is indicated by the change of media colour from green to blue. Different species of enterobacteria give a varied outcome. *Salmonella* bacteria are citrate positive hence this test separates and identifies them from the enterobacteriace.

4.2 Antibiotic sensitivity reactions by the isolates

4.2.1 Susceptibility of the isolates to the test drugs

The antibiotics used in this research are considered the most common or readily available for the population in the Kenyan market. The Disk susceptibility data were interpreted according to criteria set by the Clinical and Laboratory Standards Institute (CLSI, 2010). Classified as either sensitive (S), intermediate (I) or resistant (R) (Table 2). The inhibition zones results from the different antibiotics and isolates were

compared (Table 2) and analyzed to determine the efficiency of the drug against the isolated *Salmonella* species. The number of occurrence of the S, R and I in the isolated *Salmonella* were counted, and their percentage (%) occurrences calculated to determine the overall reaction of the isolates to the drugs under test. This is demonstrated in (Table 4.3) where 73.0% isolates reactions were sensitive, 1.6% was intermediate, and 25.4% were resistant to the drugs being tested. Multi-drug resistance (MDR) was defined as resistance to at least two of the antimicrobials tested (Table 4.5 and Table 4.6). Each MDR profile had 12.5% prevalence, and in total isolates had a MDR of 37.5% (Table 4.5). The results in (Figure 4.4) were demonstrated by plotting the estimated marginal means of minimum inhibitory concentrations of the serotypes. The largest zone was noted in *S.* Typhimurium and the least in *S.* Para*typhi* A, meaning *S.* Typhimurium demonstrated sensitivity to most of the antibiotics being tested, unlike *S.* Para*typhi* A.

Table 4.2: Antimicrobial susceptibility pattern of *Salmonella* isolates from food handlers

Antibiotic	Sensitive	Intermediate	Resistant	Total
Amox-Clav	3 (37.5%)	1 (12.5%)	4 (50%)	8
Oflofloxacin	8 (100%)	0	0	8
Ceftriaxone	6 (75%)	0	2 (25%)	8
Nalidixic acid	7 (87.5%)	1 (12.5%)	0	8
Doxycycline	6 (75%)	1 (12.5%)	1 (12.5%)	8
Ciproflaxin	8 (100%)	0	0	8
Gentamicin	8 (100%)	0	0	8
Streptomycin	6 (75%)	0	2 (25%)	8
Chloramphenical	6 (75%)	0	2 (25%)	8
Sulphamethoxazole-Trimethoprin	6 (75%)	0	2 (25%)	8
Ampicillin	5 (62.5%)	0	3 (37.5%)	8
Total	69 (78.4%)		16(18.2%)	88

Table (Table 4.2) indicates the number of isolates a drug was observed to be sensitive, intermediate and resistant to. Example; of all *Salmonella* isolates, 4 were sensitive, 1 was intermediate and 3 were resistant to Amox-Clav. In total 69 S, 3 I and 16 R results were observed. Measurement was taken in mini meters (mm).

Table 4.3: Replication of the S, I and R observed in the *Salmonella* isolates

		Count [a]	Percent [c]
Sensitivity	Intermediate	3	3.4
	Sensitive	69	78.4
	Resistance	16	18.2
Overall		88	100.0

a. Count: number of times of occurrence of a specific reaction

b. Percentage: % occurrence of a specific reaction in relation to total outcome

4.3 Variations in inhibition zones of the different serotypes to test antibiotics

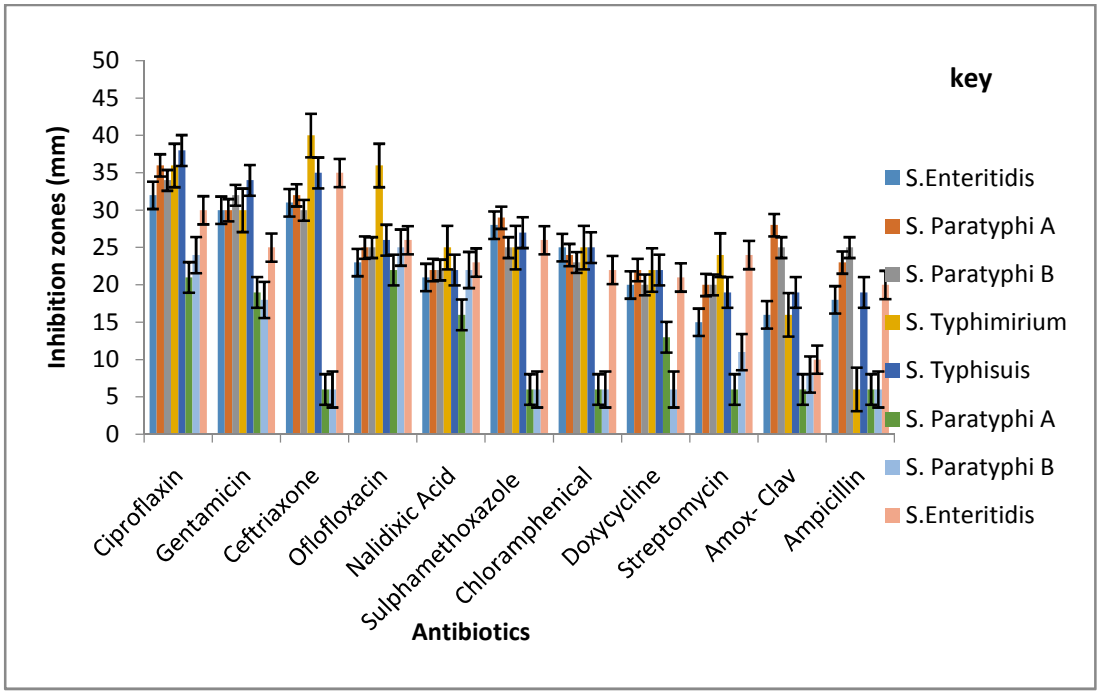


Figure 4.3: Average inhibition zone sizes of different antibiotics to isolated *Salmonella*

A significant variation in inhibition zone sizes of *salmonella* under the commonly used drugs was observed $F=19.48$ while $p \leq 0.05$, thus significant difference in their effectiveness. Ciproflaxacin (31.375) had the largest mean inhibition zones while Amox-Clav (16.00) and Ampicillin (15.375) had the smallest recorded mean inhibition zones.

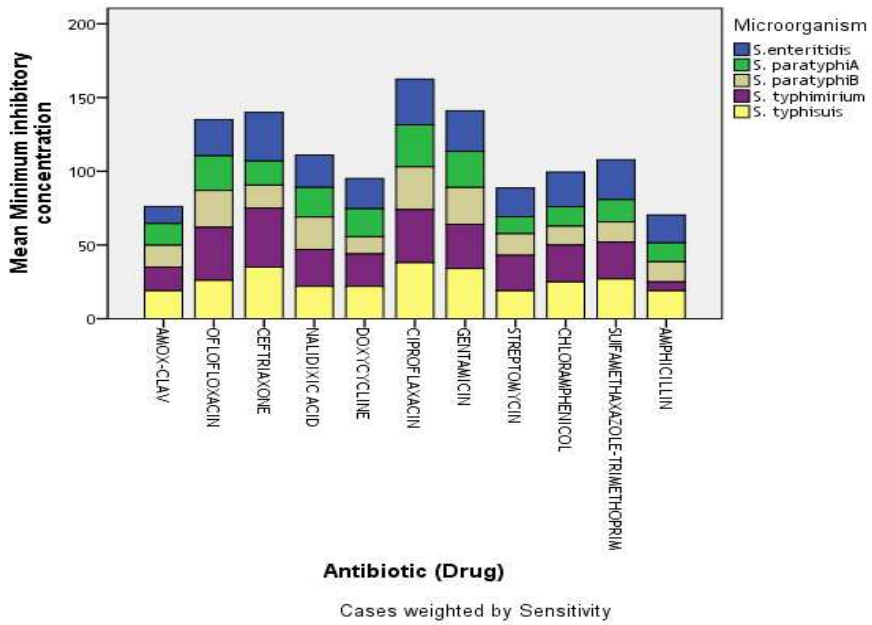


Figure 4.4: Reactions of *Salmonella* isolates against the selected antibiotic drugs under test.

Figure 4.4 shows comparison of the average zones of inhibition to different drugs by the isolates, *S.* Typhimurium had the largest average zone of inhibition size under all the used

4.4 Observed variation in sizes of inhibition zones under different drugs

Table 4.4: Comparison of antibiotic efficacy through their means

Antibiotic	n	Mean*
Ciprofloxacin	8	31.375[a]
Gentamicin	8	27.250[a]
Ceftriaxone	8	26.875[ab]
Ofloflaxin	8	26.000[bc]
Nalidixic acid	8	21.625[cd]
Sulfamethoxazole-Trimethoprim	8	21.500[cd]
Chloramphenical	8	19.500[de]
Doxycycline	8	18.250[de]
Streptomycin	8	17.375[de]
Amox-Clav	8	16.000[e]
Ampicillin	8	15.375[e]

*Means with the same letter are not significantly different

4.5 Multi-drug resistant profiles in the isolates

Table 4.5: Multi-drug resistance (MDR) profiles identified among isolated *Salmonella*

	Profile	Isolate	Percentage
	AMC, AMP	*S.* Typhimurium	12.5
	AMC, AMP, SXT, CTR, S, C	*S.* Para*typhi* A	12.5
	AMC, AMP, SXT, CTR, S,C, D	*S.* Para*typhi* B	12.5

Key

AMP	Amox-Clav
AMC	Ampicillin
CTR	Ceftriaxone
D	Doxycycline
S	Streptomycin
C	Chloramphenical
	Sulphamethoxazole
SXT	Trimethoprin

Table 4.5 above indicates the frequency and percentages to which different isolated strains reacted to several groups of antibiotics under test suggesting multidrug resistance (resistance in more than one antibiotic by an isolate).

Table 4.6: Antimicrobial resistance profiles of isolated *Salmonella*

	Profile	Isolate
	AMC	*S.* Enteritidis
	AMC, AMP	*S.* Typhimurium
	AMC, AMP, SXT, CTR, S, C	*S.* Para*typhi* A
	AMC, AMP, SXT, CTR, S,C, D	*S.* Para*typhi* B

Key

AMP	Amox-Clav
AMC	Ampicillin
CTR	Ceftriaxone
D	Doxycycline
S	Streptomycin
C	Chloramphenical
	Sulphamethoxazole
SXT	Trimethoprin

Table 4.6 indicates the antimicrobials groupings that resistance by several isolates was observed, their frequencies of occurrences and percentages

Multi-drug resistance (MDR) was observed in 3 of the 8 *Salmonella* isolates which represented 37.5 % of all isolates tested. A total of 4 antimicrobial resistance profiles were observed in this test based on the types of antibiotics and isolate. The most frequent antibiotic that the isolate demonstrated resistance to was Ampicillin and Amoxicillin-Clavulanic acid with 3 isolates showing this type of resistance. Among the *Salmonella* isolated, only three of the isolates were sensitive to all the drugs tested representing 37.5% of the total isolates, which is slightly higher compared to a similar research by Kariuki, Revathi, Mwituria, Muyodi & Hart, (2006), with 23.4% isolates from asymptomatic food handlers sensitive to all the tested antibiotics this could be due to the geographical difference and types of antibiotics that were used in these two research.

4.6 Prevalence of *Salmonella* spp. among the asymptomatic food handlers

Table 4.7: Prevalence of *Salmonella* isolates

Serotypes	Frequency	Percentage
Salmonella Para*typhi* A	2	0.5
Salmonella Para*typhi* B	2	0.5
Salmonella Typhisuis	1	0.25
Salmonella Typhimurium	1	0.25
Salmonella Enteritidis	2	0.5

According to the Table 4.7 above, among the asymptomatic food handlers tested, a prevalence of 2% was observed.

CHAPTER FIVE

5.0 DISCUSSION

5.1 *Salmonella* species isolated from the asymptomatic food handlers

Most of the human pathogenic *Salmonella* belong to the *S. enterica* ser. Enterica subspecies such as *S.* Typhi, *S.* Enteritidis, *S.* Para*typhi*, *S.* Typhimurium and *S.* Choleraesus. *Salmonella* serotype Typhi is the etiological agent for typhoid fever. It is estimated to cause millions of infections and thousands of deaths worldwide each year (WHO, 200). A similar syndrome to typhoid fever is usually caused by paratyphoid serotypes such as *S.* Para*typhi* A, *S.* Para*typhi* B and *S.* Para*typhi* C, though they are known to be isolated less frequently compared to *S.* Typhi, among those expressing the symptoms of *Salmonella* infection.

In the present study, feacal samples were taken from persons who were presumed healthy and working in the food industry handling food for consumption by their customers. From the 400 samples taken, 8(2%) turned out to be positive for *Salmonella* (NTS) of the total results from asymptomatic persons. An asymptomatic carrier is an individual who has recovered from the symptoms of the disease but continues to carry the bacteria and may serve as a cause of infection for others, such as the case of “typhoid Mary” (Shanson, 1989; Atlas, 1995). This carrier state occurs in about 3% of all individuals treated and recovered from typhoid and paratyphoid fever (Asperilla, Smego & Scott 1990).

Salmonella serotypes Typhimurium and Enteritidis are most common causes of disease in both human and swine. Other *Salmonella* serotypes are human host specific such as *S.* Typhi. Though in the above study, species that are known to be isolated from other animals such as *S.* Typhisuis was among the isolates. *Salmonella*

Typhisuis is normally isolated from pigs thus the name suis, and this can be linked to human infection from handling infected pork or consumption of contaminated pork.

Some of the serotypes isolated in this study are known to cause serious infections in man. Isolates such as *S.* Para*typhi* A and *S.* Typhimurium cause severe gastro intestinal infections. Infections such as, paratyphoid /enteric fever/salmonellosis and paratyphoid fever/*Salmonella* toxic food borne infection respectively (WHO, 2005). Serovar Para*typhi* A is reported as the second most prevalent cause of typhoid, responsible for one third of cases or more in Southern and Eastern Asia (McClelland *et al*., 2004). *Salmonella* Para*typhi* A and *S.* Typhi cause similar illness, with relapsing fever. *Salmonella* Para*typhi* A generally causes a milder disease but has been reported to be particularly virulent in a number of outbreaks (McClelland *et al*., 2004). Despite the fact that *S.* Typhisuis and *S.* Enteritidis do not cause severe infections as the ones mentioned above, they cause stomach flu leaving one feeling sickly and unable to perform daily chores as long as the sickness lasts. Such infections among the young children and the older persons with weak immune systems can be fatal (Gomez and Cleary, 1998).

5.2 Antibiotic sensitivity reactions by the isolates

5.2.1 Susceptibility of the isolates to the test drugs

Most isolates were observed to be sensitive to the drugs under test, with 69 sensitive reactions observed. The most efficient drugs observed were Oflofloxacin, Ciproflaxin and Gentamicin. These were sensitive in all the *Salmonella* serotype isolates. This agrees with the finding by Kariuki *et al*., (2004) that all the NTS isolates were found to be susceptible to Ciproflaxin and Ceftriaxone. In this study, resistance was observed in Ceftriaxone. *S.* Para*typhi* A (IF 207) and *S.* Paratyphi B (UF 03) were

observed to be resistant to Ceftriaxone, this is in agreement with (Kariuki *et al*., 2004, Verma *et al*., 2010). This indicates that Ciproflaxin has maintained its efficacy to NTS and is an effective choice of remedy unlike Ceftriaxone.

5.2.2 Variations in inhibition zones of the different serotypes to test antibiotics

Intermediate resistance of 3.4% was observed in Amoxi-Clav by *S.* Enteritidis, Doxycycline and Nalidixic acid by *S.* Para*typhi* A, indicating the presence of antibiotic resistance among *Salmonella* isolates from the asymptomatic food workers. This was observed to indicate reduced potency of the antibiotic drugs to the isolates.

Among the Salmonella isolates, 4 showed resistances to Amox-Clav, 3 to Ampicillin, 2 to Sulfamethaxazole-Trimethoprim, Chloramphenicol, Streptomycin, Ceftriaxone each and for Doxycycline one isolate was observed. This resistance pattern is identical to the Ampicillin, Chloramphenical, Streptomycin, Sulfonamide and Tetracycline (ACSSuT) and AmpC (resistant to at least Ampicillin, Chloramphenicol, Streptomycin, Sulfonamides, Tetracycline, Amoxicillin/Clavulanic acid, and Ceftiofur, and with decreased susceptibility to Ceftriaxone) (Kariuki *et al*., 2004, Greene *et al*., 2008) resistance type, except that in this research Tetracycline and Ceftiofur were not among the antibiotics tested.

5.2.3 Observed variation in sizes of inhibition zones under different drugs

Elevated levels of antibiotic resistance by the *Salmonella* isolates were observed in Ampicillin and Amoxicillin-Clavulanic acid; and these are among the readily available still in use antibiotics in a majority of the developing countries (Kariuki *et al*., 2004) including Kenya. This corresponds to an outcome in a study by Kariuki *et al.,* (2004) and Verma *et al.*, (2010) who noted increased resistance by *Salmonella* isolates to Ampicillin. There was observed variation in the response to the different

antibiotics used in the research by the isolates. *S. Typhimurium* isolate had the highest average zones of inhibition while *S.* Para*typhi* A had the lowest. These indicated possible reduction in sensitivity to the drugs under test by *S.* Para*typhi* A isolates. Isolates of *S.* Typhimurium were sensitive to most antibiotics used in this study. A significant variation in inhibition zone sizes of *Salmonella* under the commonly used drugs was observed $F=19.48$, $P \leq 0.05$, thus significant difference in their effectiveness. Ciproflaxacin (31.375) had the largest mean inhibition zones while Amox-Clav (16.00) and Ampicillin (15.375) had the smallest recorded mean inhibition zones. Significant differences were observed in the efficiency of the antibiotics to the *Salmonella* isolates. These variations were grouped into five categories of similar significance in efficiency through comparison of the antibiotic average means of inhibition zones. Ciproflacin, Gentamicin and Ceftriaxone were observed to be more susceptible among the isolates with average means of 26.875-31.375 compared to the rest of antibiotics tested while Amox-Clav and Ampicillin with average means of 15.375 and 16.00 respectively were the least susceptible.

5.2.5 Prevalence of *Salmonella* spp. among the asymptomatic food handlers

Of the 400 samples obtained in the above study, 8 samples were found to be *Salmonella* positive. This represents 2% of the stool samples obtained from the asymptomatic food handlers, with a distribution of *S.* Para*typhi* A (0.5%), *S.* Para*typhi* B (0.5%), *S.* Typhimurium (0.25%), *S.* Typhisuis (0.25%) and *S.* Enteritidis(0.5%), these results contrast with a research by Murakami *et al.*, (2007), who found that of the 331,644 faecal samples from asymptomatic food handlers, a prevalence of 106 (0.032%), with *S.* serovar Infantis being the dominant serovar accounting for 48.1% of total isolates, followed by *S.* serovar Corvallis and *S.* serovar Enteritidis. In that study, *S.* serovar Infantis *and S.* serovar Corvallis were observed as not being

dominant among symptomatic patients. Though this agrees with a study by Medus *et al.,* (2006) that showed a similar prevalence of *Salmonella* among the food handlers, 110 out of 4,976 which represent (2.2%) confirmed *Salmonella* cases reported were identified as food workers.

The occurrence of *S.* Enteritidis in this study was observed to be higher among the asymptomatic food handlers. This concurs with other research on food handlers (Suhana *et al.*, 2010, Murakami *et al.*, 2007). Similar results were also observed by Kariuki *et al.*, (2005) who noted that prior to 1997, *S.* Typhimurium predominated (prevalence of 75%) among cases of NTS bacteraemia in Kenya, while *S.* Enteritidis made up only 4.8% of the cases. However, the study indicated that more recently, isolations of *S.* Enteritidis cultures have progressively increased to an occurrence of 40%. This may be attributed to changing lifestyles with more people rearing chickens for eggs as a supply of protein.

In the current study, total *Salmonella* prevalence was relatively low among the food handlers at 2%, indicating relatively low frequency by asymptomatic food handlers, to possibly passing the infection to their customers. Though this does not rule out the possibility of infection of consumers by asymptomatic food handlers, Smith *et al.*, (2010), found that more than half (62.2%) of the food handlers washed their hands with water alone prior to eating as 27.7% did not wash their hands at all times prior to food preparation. This possibility can predispose consumers of food prepared by asymptomatic food handlers to *Salmonella*, if food being prepared is contaminated by faecal material from the unwashed hands of the asymptomatic food handler.

All the serotypes had a prevalence of less than 1%. An asymptomatic food handler found co-infected with more than one *Salmonella* serotype was not observed in this

study. The most prevalent O antigen group in this study was B (*S.* Para*typhi* B and *S.* Typhimurium), accounting for 37.5% of the total *Salmonella* isolates isolated in this study. A total of 4 different groups were isolated in the study (*S.* Para*typhi* A in group A, *S.* Typhisuis in group C_1 and *S.* Enteritidis in group D).

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATION

6.1 Conclusion

1. Drug and MDR serotype are present among the isolates from the asymptomatic food workers. This indicates the presence of risk in the spread of *Salmonella*, especially MDR *Salmonella* in most urban populations who consume food prepared by the asymptomatic food handlers from the food outlets.

2. There is presence of *Salmonella* serotypes among the collected samples of the asymptomatic food handlers in Westland's Nairobi. These have the potential to be passed to consumers. They included *S*. Para*typhi* A and B, *S*. Enteritidis, *S*. Typhimurium.

6.2 Recommendation

1. Screening of food handlers is made mandatory by the public health officials and appropriate advice and treatment be given to the carriers and those diagnosed as carriers be given paid sick off to encourage openness in the approach of the treatment of the disease by the institutions in which they work for until they are proven safe by the public health officials to handle food for consumer consumption.

2. Education of the food handlers on proper work ethics by the public works ministry.

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APPENDICES

Appendix 1: Informed consent form

Analysis of *Salmonella* spp. isolated from faecal samples of asymptomatic food handlers for prevalence and antibiotic resistance patterns in Westland Nairobi, Kenya

Study no:

Description

I am carrying a study on the carriage of *Salmonella* among the food handlers who do not show any sign of *Salmonella* infection. *Salmonella* is a bacterium that causes intestinal infection where some people can be carrying the bacteria without showing any signs; such individual can spread the bacteria to others through handling of food. I would like to ask for your help in my research by answering to a few questions and allow me use your stool and blood samples collected for routine food handler's examination as required by ministry of public health and sanitation.

Procedure

If you agree to participate in this study I will ask you a few questions relating to yourself and to use your stool samples collected from you for the purposes of your food handler's routine examination.

Risks and Benefits

There is no known effect of collecting stool and blood samples. And all your information will be kept in confidence. Individuals found infected with *Salmonella* will get result for their diagnosis which drugs to use prescribed by a qualified clinician.

Subject's rights: If you have read this form and have decided to participate in this study, please understand your participation is voluntary and you have the right to withdraw your consent or discontinue participation at any time without penalty. You have the right to refuse to answer particular questions. Your individual privacy will be maintained in all published and written data resulting from the study.

If you have questions about your rights as a study participant, or are dissatisfied at any time with any aspect of this study, you may contact - anonymously, if you wish MU – IREC (institutional research and ethics committee PO Box 4606 Eldoret or Tel no. 33471/2/3 For emergency contact ZeddyYegon on 0725211001

I have read and understood clearly what the research entails and I voluntary give consent to participate.

Name of participant……

Signature…….Date……
……

Appendix 2: Sample collection sites

Code	Sample size	Type	Number of positive samples
1	6	Restaurant	0
2	61	Restaurant	0
3	20	Restaurant	3
4	11	Restaurant	0
5	5	Restaurant	0
6	27	Restaurant	0
7	13	Restaurant	1
8	28	Restaurant	0
9	9	Restaurant	0
10	15	Restaurant	1
11	13	Restaurant	0
12	33	Restaurant	0
13	11	Restaurant	0
14	6	Restaurant	0
15	7	Restaurant	0
16	11	Restaurant	0
17	2	Restaurant	1
18	43	Restaurant	2
19	13	Restaurant	0
20	42	Restaurant	0
Total	**400**		**8**

Appendix 3: Antibiotics used in the study and their potency

Antibiotic	**Symbol**	**Potency**
Ampicillin	AMP	10 µL
Amoxicillinclavulanic acid	AMC	20:10 µL
Ciprofloxacin	CIP	30 µL
Sulfamethaxazole-Trimethoprim	SXT	25 µL
Nalidixic acid	NA	30 µL
Doxycycline	D	30 µL
Ceftriaxone	CTR	30 µL
Oflofloxacin	OFX	5 µL
Streptomycin	S	10 µL
Chloramphenicol	C	30 µL
Gentamicin	GM	10 µL