CHARACTERIZATION OF ANTIBIOTIC RESISTANCE GENES FROM SALMONELLA ASSOCIATED WITH INVASIVE SALMONELLOSIS IN SELECTED HOSPITALS IN KATSINA STATE, NIGERIA

BY

KAMILU ISAH

DEPARTMENT OF MICROBIOLOGY,
FACULTY OF SCIENCE
AHMADU BELLO UNIVERSITY,
ZARIA NIGERIA

DECEMBER, 2016

CHARACTERIZATION OF ANTIBIOTIC RESISTANCE GENES FROM SALMONELLA ASSOCIATED WITH INVASIVE SALMONELLOSIS IN SELECTED HOSPITALS IN KATSINA STATE, NIGERIA

BY

KAMILU ISAH
(MSC/SCI/1802/2011-2012)

A DISSERTATION SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY, ZARIA
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF SCENCE DEGREE IN MICROBIOLOGY

DEPARTMENT OF MICROBIOLOGY,
FACULTY OF SCIENCE
AHMADU BELLO UNIVERSITY,
ZARIA NIGERIA

DECEMBER, 2016

DECLARATION

I hereby declared that the work in this Dissertationentitled ‘’Characterization of Antibiotic Resistance Genes from*Salmonella* Associated with Invasive Salmonellosis in Selected Hospitals in Katsina State Nigeria’’ has been carried out by me in the Department of Microbiology, under the supervision of Professor Olonitola O.S. and Professor Ado S.A. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this work has been presented for another degree or diploma at any institution.

KamiluIsah

______________________	________________	_______________
Name of student	Signature	Date

CERTIFICATION

This Dissertation entitled''CHARACTERIZATION OF ANTIBIOTICS RESISTANCE GENES FROM*SALMONELLA*ASSOCIATED WITH INVASIVE SALMONELLOSIS IN SELECTED HOSPITALS IN KATSINA STATE Nigeria'', by KAMILU ISAH (MSc/SCI/1802/2011 – 2012) meets the regulations governing the award of the Degree of Master of Science (Microbiology) of the Ahmadu Bello University, Zaria and is approved for its contribution to knowledge and literary presentation.

(Name) (Signature)

____

Chairman Supervisory Committee Date ___________________________

(Name) (Signature) ______________________

____

Member Supervisory Committee Date_____________________________

(Name) (Signature) ______________________

____

Member Supervisory Committee Date ___________________________

(Name) (Signature) ______________________

____

Head of Department Date ___________________________

(Name) (Signature) ______________________

____

Dean School of Post Graduate Studies Date ___________________________

DEDICATION

Dedicated to Almighty ALLAH, my beloved parents and my son (Shaheed).

ACKNOWLEDGEMENT

Praise be to God, the Lord of the world whose grace and affection gave me the opportunity towards completing my academic programme.

My profound gratitude goes to Prof.Olonitola O.S. and Prof. Ado S.A. for their supervisory attention, constant review and painstaking assistance despite their numerous academic commitments. May God through His mercy guide them all.

It is my pleasure to express my vote of thanks to my parents who sponsored my education. May God bless them Ameen.

My special thanks to entire staff of Department of Microbiology, M. Y.Y Pala of Avian Influenza Laboratory, MallamShitu and my wife HafsatRabeDaura.

ABSTRACT

The present research work was carried out to characterize the antibiotic resistance of *Salmonella* serovars associated with invasive salmonellosis in selected hospitals in Katsina State. A total of 300 blood samples was collected from the patients at three General hospitals [General hospital Katsina, Daura and Baure] and were processed according to standard microbiological technique for isolation and identification of*Salmonella typhi*and *Salmonella paratyphiA* from 14 samples,the result of serotyping (slide agglutination) of the isolates indicates that nine (9) serovars belonged to D group while five (5) of the serovars belonged to A group. The antibiotic susceptibility test that was carried out among the isolates against seven (7) antibiotics showed 88.9% resistance to ampicillin by *Salmonella entericaserovar*Typhi only, 100% resistance to penicillin G by both serovars and 100% sensitivity to ampicillin (by *Salmonella paratyphi A* only), Chloramphenicol, Gentamicin, Cephalex, Ofloxacin and Trimethoprim – sulfomethoxazole. The PCR analysis detected the presenceof *bla*$_{TEM\text{-}1}$gene in eight (8) serovars of *Salmonella entericaserovar*Typhi only.This study confirmed the presence of *Salmonella entericaserovar*Typhi and *Salmonella entericaserovar*Paratyphi A as the cause of invasive salmonellosis in the study, their susceptibility and resistance to antimicrobial agents. The study emphasized the use of antibiotics likeofloxacin, cephalex chloramphenicol and septrin for treating enteric fever.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

CHAPTER ONE

1.0 INTRODUCTION

Salmonellae are Gram negative, non-sporing, motile, and non-lactose fermenting rods. They belong to the phylum Proteobacteria, class Gamma-proteobacteria, order Enterobacteriales, family Enterobacteriaceae and genus; *Salmonella*. With the exception of *Salmonella Pullorun* and *Salmonella gallinarum*all *Salmonellae* are motile, also with the exception of *Salmonella typhi* all are non-capsulated. The genus was named in honor of Daniel Elmer Salmon an American Veterinary Pathologist (Graham 2009 ; FDA/CFSAN, 2009).

Salmonellae can be divided into two major groups for the purpose of understanding of the genus. Group one includes the members of the genus that involved the most invasive salmonellae which cause enteric fever/typhoid fever (Typhoidal Salmonellosis) and group two are the Non-typhoidal salmonellosis (NTS) (Graham 2009).

An infection (salmonellosis) is caused by ingesting salmonellae in food that has been contaminated with animal or human intestinal sources directly or indirectly (Redmond and Griffith,2003). Common sources of the infection are contaminated foods such as poultry meat and meat products, eggs and eggs products or water (Udeze*et al.*,2010). Once the bacteria are in the body, the incubation period is only about 8 to 48 hours(Willey *et al.*, 2008). The disease results from a true food borne infection because the bacteria multiply and invade the intestinal mucosa where they produce an enterotoxin that destroys the epithelial cells. Abdominal pain, cramps, diarrhea, nausea, vomiting, and fever are the most prominent symptoms, which usually persist for 2 – 5 days but can last for several weeks (Willey *et al.*,2008). During the acute phase of the infection as many as one billion salmonellae can be found per gram of faeces. Most adult patients recover, but the loss of fluids can cause problems for children and elders, blood and mucous may be present in faecal specimen (Al-jurayyan*et al.,* 2004). Salmonellae can be isolated from stool, food, blood, urine, bone narrow and duodenal aspirates. From blood the organisms can be detected in about 75-90% of patients during the first ten days of infection, and in about 30% of patients during the third week. From faeces, organisms can usually be isolated from about 40% to 50% of patients during the second week of infection and about 80% of patients during the third week (Cheesborough, 2002).

Salmonella pathogenicity depends on a variety of virulence factors that help the pathogens in adhesion and invasion mechanisms (Gebreyes*et al.,*2009). The *Salmonella* incorporates plasmid (spv) on which are borne virulence genes potentiate the systemic spread of the pathogen and in its replication in extracellular sites (Gebreyes*et al*., 2009: Hur*et al*., 2011). Other important virulence factors include the rck genes product which encodes an outer membrane protein and induces resistance to complement mediated killing (Fluit, 2005) and mediate adherence and invasion to cultured eukaryotic cell line(Ho*et al*., 2010). The sop proteins (Sop A – E) (Sop) and the heat labile *Salmonella*enteretoxin (stn) serve as effector proteins which are involved in the pathogenesis of *Salmonella* (Wallis and Galyov, 2000: Van Asten and Van Dijk, 2005).

The mgtc genes encodes mgtB^{2+} mg transporter and helps the pathogen to survive within the macrophages (Alix and Blanc Potard, 2007). Invasion genes (invA) exist in the majority of the *Salmonella* serovars and is related to intestinal mucosa invasion (Fluit 2005, Chuanchuen*et al*., 2010).

Treatment is with fluids and electrolytes replacement, use of effective antimicrobial therapies which reduce morbidity and mortality from typhoid fever (WHO, 2004). Without treatment, the disease may last for 3-4 weeks and case fatality rates may exceed 15% but with appropriate treatment, clinical symptoms fall drastically within few days and mortality rate reduced to 1% (WHO, 2004). Prevention depends on good food processing practices, proper refrigeration and adequate cooking of food (Griffith, 2003). However, the incidence of cases and death has been greatly increased by a combination of poor sanitation and hygiene, unavailability of vaccines and higher cost of effective antimicrobial chemotherapy. Besides, the effectiveness of antimicrobial therapy is also being challenged by the emergence of antibiotic resistance (Woodsford and Ellington 2007). In Nigeria the antibiotics most frequently available for the treatment of typhoid are chloramphenicol, ampicillin, and cotrimoxazole. With the emergence of plasmid-encoded chloramphenicol resistance (Mandal*et al.*, 2004,) ampicillin, although slightly less effective than chloramphenicol was used both for therapy and elimination of the carrier state (Cooke and Wain, 2004).

Antimicrobial resistance is a growing public health problem which causes increased morbidity and mortality among humans and animals. Antimicrobials are used for treatment of infected human or animals, to protect them from infectious diseases or to provide a faster growth rate (Turkyilmaz *et al.*, 2009). The routine use of antibiotics in medical and agricultural circles has resulted in the development of genetic mechanisms for the dissemination of antibiotic gene cassette, especially within and between species of gram negative (Tulkyilmaz*et al.*, 2009). *Salmonella* serovars have often accumulated antibiotic resistance gene by plasmid transfer or by transposons or integron – mediated mechanisms and have routinely harboured these resistance genes on plasmids (Tulkyilmaz*et al.,* 2009). Notably, class I integrons have been found in many multi resistance organisms and many gram negative species. Class I integrons contain a $5'$- conserved segments ($5'$ CS) that includes the integrase*intII* gene, the *attII* recombination site and pc promoter. It is followed by a variable region where one or more gene cassettes are located. This class of integron also contains a $3'$ conserved segment ($3'$ CS) that include the *sul1* which encode resistance to sulphonamides (Maria *et al.*, 2011).

1.1 Statements of the Research Problem

Typhoid fever otherwise known as enteric fever is a common world-wide illness, transmitted by the ingestion of food contaminated with faeces from an infected person. It remains a serious public health problem in many regions of the world especially in developing countries, where it became endemic. Approximately 21.5million cases occur per annum worldwide with 200,000 deaths in Asia, Africa and Latin America. About 80% of deaths due to *Salmonella* are found in Africa (Oyango*et al.*,2009).

In Nigeria Typhoid and Paratyphoid are endemic and present similar clinical signs and symptoms with other diseases like malaria, hepatitis, and jaundice, as a result the disease is wrongly treated (Olowe*et al.*,2007).

Indiscriminate antibiotic prescription for the treatment of typhoid fever, illegal selling of substandard drugs in the markets, resistance plasmid transfers from one strain of *Salmonella to* another or from close species are the major problems in the treatment of salmonellae (Hur*et al.*,2011).

1.2 Justification

Salmonellosis still remains a serious public health problem in many developing countries with significant morbidity and mortality, therefore, the understanding of the distribution and causative serovars is necessary in reducing the disease (Boyd *et al*., 2004).

Antimicrobial resistance is increasing in many parts of the world (Olowe, *et al.,* 2007). The knowledge of susceptibility pattern will provide the desired information for choosing the primary drugs that will minimize the development of resistance (Fluit, 2005).

Multi-antibiotics resistance *Salmonella enterica*is rarely reported from Africa (especially South and Central) (Mirza*et al.,* 2000). In relation to effective surveillance and the development of rational control strategies for this important human disease, the availability of detailed and accurate data related to molecular epidemiology of *Salmonella* species is important (Mirza*et al.,* 2000).
The characterization of antibiotics resistance genes in *Salmonella* would be very important for medical workers, public health and pharmaceutical industries in developing countries such as Nigeria where there are extremely limited data (Adeshina*et al.,* 2010).

Typhoid and paratyphoid remain an important but under estimated disease which has high mortality in developing countries. However, there are extremely limited data in Northern Nigeria (especially Zaria, Kano and Katsina) (Adeshina*et al.,* 2010). Hence the probable under estimation of the disease burden in the area.

1.3 Aim of the Study

The aim of this research was to characterized the antibiotics resistance of *Salmonella* species associated with invasive Salmonellosis in selected hospitals in Katsina State.

1.4 Objectives of the Study

1. To isolate and characterize the *Salmonella* serovars associated with invasive salmonellosisfrom patients attending selected hospitals in Katsina State

2. To determine the prevalence of *Salmonella* serovars associated with invasive salmonellosis in Katsina State.
3. To determine the antibiotics susceptibility of the *Salmonella* isolates to selected antibiotics.
4. To identify thebla_{TEM} genes in *Salmonella* serovars using PCR methods.

CHAPTER TWO

2.0 LITERATURE REVIEW

Salmonella are gram-negative, non-sporing, motile and non-lactose fermenting rods. They belong to the family Enterobacteriaceae and genus *Salmonella*.The genus was named in honour of Daniel Elmer Salmon an American veterinary pathologist (Graham 2009; FDA/CFSAN 2009). The Genus was divided into two species, *Salmonellabongori* and *Salmonella enterica*. The species *enterica* is itself comprised of six subspecies they are *S.enterica subsp. enterica, S.enterica subsp. Salamae, S.enterica subsp. Arizonae, S. entericasubp. diarizonae, S. enterica subsp. Indica, S. entericasubp. houtenae (*Porwallic*et al.*, 2003). Of these six subspecies only subspecies *enterica* is associated with the disease in warm – blooded animals. To date, there are over 2,300 serovars identified within subspecies *enterica*. However, only a small fraction of the thousands of described subspecies *enterica*serovars frequently caused disease in human and domestic animals. For better understanding of the genus, the serovars associated with the diseases are grouped into two, group one comprised those causing typhoidal salmonellosis and the second group involve those causing non-typhoidalsalmonellosis(Graham, 2009).

Typhoid fever is caused by *Salmonella typhi* a gram-negative bacterium.A very similar but often less severe disease is caused by *Salmonella paratyphi A, B or C* and is called paratyphoid fever. The two are collectively called Enteric fever. *Salmonella* typhiis widely distributed in the world but *Salmonella paratyphi B* is commonly found in Western Europe, *Salmonella paratyphi C* is found in far East and *Salmonella paratyphi A* is commonly found in Africa (Cooke and Gibson 1990). The organism is acquired by the ingestion of food or water contaminated faeces of an infected person. In earlier centuries the disease occurred in great epidemics. In the intestine, the incubation period is about 10-14 days. The bacteria colonize the intestine, penetrate, the epithelium, and spread to the lymphoid tissue, blood, liver and gall bladder. Symptoms include fever, headache, abdominal pain, anorexia and malaise which last several weeks. Bacteria then re-infect the gastrointestinal tract producing abdominal pain and diarrhoea. After approximately 3 months, most individuals stop shedding bacteria in their faeces. However, a few individuals continue to shed the organism in their faeces for extended periods but show no symptoms. In these carriers, the bacteria continue to grow in the gall bladder and reach the intestine through the bile duct.

2.1 Nomenclature of *Salmonella*

2.1.1 Kauffmann-white classification scheme;

Salmonella is named after an American Bacteriologist D. E. Salmon who first isolated *Salmonella choleraesuis*from porcine intestine in 1884 (Smith, 2001). *Salmonella* are a group of bacterial organisms with a high genetic similarity and differentiated by their serotyping results. The antigenic classification system of various *Salmonella* serotypes used today is a result collated from many years of studies on antibody interaction with surface antigen of *Salmonella* organism established by Kauffmann and White almost a century ago. All antigenic formula of recognized *Salmonella* serotypes are listed in a document called the Kauffmann-White scheme (Popoff, 2001). The World Health Organization Collaborating Centre for Reference and Research on *Salmonella* at the Pasteur Institute, Paris France (WHO collaborating Centre) is responsible for the updating of the scheme. Every year newly recognized serotypes are reported in the research in Microbiology by Popoff*et al.*(2001) and latest report published in 2004, there are a total of 2,541 serotypes in the genus *Salmonella* (Popoff 2004). The term ‘’serotype’’ and ‘’serovar’’ are both frequently used, but according to the rules of bacteoriological code (1990 Revision) established by the Judicial Commission of International Committee on the systematic of prokaryotes the serovar is preferred to the term serotype. Thus ‘’serovar’’ is used in the Kauffman-White Scheme.

2.1.2 Natural classification of *Salmonella*

This classification involves placing microorganism within hierarchical taxonomic levels. Microbes in each level or rank share a common set of specific features. *Salmonella* therefore, is classified by this scheme as follow:

Domain	Bacteria
Phylum	Proteobacteria
Class	γ –Proteobacteria
Order	Enterobacteriales
Family	Enterobacteriaceae
Genus	*Salmonella*
Species	*typhi, or paratyphi A, B or C.*

2.2 Pathogenesis

The aetiological agent of Enteric fever are*Salmonella typhi, and paratyphi A, B or C.* These *Salmonella* are members of a genus belonging to the Enterobacteriaceae family. Within the genus are more than 1,700 distinguishable serotypes. The agents of enteric fever can be distinguished from the large numbers of other in the genus in that they are human pathogens which do not normally affects animals, whereas the vast majority of *Salmonella* are animals’ parasites although they do not infect man. Another major practical distinction of enteric fevers agents is that the enteric fever organisms typically cause a general systemic infection whereas the others usually cause a simple food poisoning by their invasion of gastrointestinal tract, although they can on occasion cause systemic infection particularly in host with reduced resistance due to age, disease or other factors (Cooke, 1990).

During an acute infection *S.typhi* multiplies in mononuclear phagocytic cells before being released into blood stream. After ingestion in food or water, typhoid organism passes through the pylorus and reach the small intestine. They rapidly penetrate the mucosal epithelium via either micro-fold cells or enterocytes and arrive in the lamina propria, where they rapidly elicit an influx of macrophages that ingest the bacilli but do not generally kill them. Some bacilli remain within the macrophages of the small intestinal lymphoid tissue. Other typhoid bacilli are drained into mesenteric lymph nodes where there is further multiplication and ingestion by macrophages. It is believed that typhoid bacilli reach the bloodstream principally by lymph drainage from mesenteric nodes, after which they enter the thoracic duct and then the general circulation. As a result of this silent primary bacteraemia the pathogen reaches an intracellular haven within 24 hours after ingestion through the organs of reticuloendothelial system (spleen, liver, bone marrow etc.), where it resides during the incubation period usually, of 8-14 days (WHO, 2003). The incubation period in a particular individual defends on the quantity that is, it decreases as the quantity increases, and on host factors. Incubation period ranging from 3 days to more than 60 days have been reported. Clinical illness is accompanied by a fairly sustained but low level of secondary bacteraemia (WHO, 2003). The clinical presentation of typhoid fever varies from a mild low-grade fever, malaise and slight dry cough to a severe clinical picture with abdominal discomfort and multiple complications (WHO, 2003). Many factors influence the severity and overall clinical outcome of the infection. These include the duration of illness before the initiation of appropriate therapy, the choice of antimicrobial agent, age, the previous exposure or vaccination history, the virulence of the bacterial strain, the quantity of inoculum ingested and host factors (age, AIDS, or other immunosuppression). Patients who are infected with HIV are at significantly increased risk of clinical infection with *S. typhi and S. paratyphi (*WHO, 2003). Acute non-complicated disease is characterized by prolonged fever, constipation in adult or diarrhoea in children, headache, malaise and anorexia. Bronchitic cough is common in the early stage of the illness. During the period of fever up to 25% of patients show exanthema (rose spot), on chest, abdomen and back.

Complicated disease; acute typhoid fever may be severe. Depending on the clinical setting and quality of available medical care up to 10% of typhoid patients may develop serious complications since, the gut-associated lymphoid tissue exhibit prominent pathology, the presence of occult blood is a common finding in the stool of 10-20% of patients and up to 3% may have melena. Intestinal perforation has also been reported in up to 3% of hospitalized cases. Abdominal discomfort develops and increases. It is often restricted to the right quadrant but may diffuse. The symptoms and signs of intestinal perforation and peritonitis sometimes, follows, accompanied by a sudden rise in pulse rate, hypotension, marked abdominal tenderness, rebound tenderness and subsequent abdominal rigidity. A rising white blood cells count with a left shift and free air on abdominal radiographs are usually seen (WHO,2003).

Altered mental status in typhoid patients has been associated with a high case fatality rate. Such patients generally have delirium or obtundation, rarely with coma. Typhoid meningitis, encephalomyelitis, Guillain-Barre syndrome, cranial or peripheral neuritis and psychotic symptoms, although rare have been reported. Other serious complications documented with typhoid fever are haemorrhages, hepatitis, myocarditis, pneumonia, disseminated intravascular coagulation, thrombocytopenia and haemolytic uremic syndrome (WHO, 2003).

2.3 Diagnosis of Typhoid Fever

The definitive diagnosis of typhoid fever depends on isolation of *S.typhi* from blood, bone marrow stool or specific anatomical lesion. The presence of clinical symptoms characteristic of typhoid fever or the detection of a specific antibody response is suggestive of typhoid fever but not definitive. Blood culture is the mainstay of the diagnosis of this disease (WHO 2003).

2.3.1 Blood culture

A typical blood culture bottle contains 10ml or 45ml of tryptic soy broth or brain-heart infusion broth or thioglycollate broth. These are inoculated with 5ml of fresh blood and incubated at 37°C. Negative samples should be kept for at least seven days. Because *S.typhi*is not the only bacterial pathogen found in blood , subculturing is performed on days 1,2,3 and 7 on selective media such as McConkey agar. Isolates from sterilized parts such as blood, bone marrow or urine should be gram stained.

2.3.2 Stool or rectal swab culture

This involves inoculating 1g of stool into 10ml of selenite F broth and incubating at 37°C for 18-48 hours. Because selenite broth is very sensitive to heat the manufacturer's instructions should be carefully followed during preparation and overheating of the broth during sterilization should be avoided. Selenite broth inhibits the motility of *E. coli* found in stool but does not kill this bacterium (WHO, 2003). A culture of selenite broth on a selective agar is therefore, made from the surface of the broth without disturbing the sediment. The choice of agar media includes McConkey agar, Deoxycholate citrate agar, Xylose lysine deoxycholate agar and Hektoen enteric agar or Salmonella-Shigella agar. The plate is incubated at 37°C for 24 hours.

2.3.3 Colonial characteristics of *salmonella* species

Blood agar

On blood agar *S.typhi and S.paratyphi* usually produce non haemolytic white colonies.

MacConkey agar

On MacConkey agar, *Salmonellae* produce lactose non-fermenting smooth colonies.

Deoxycholate citrate agar

On DCA, *Salmonella* produce lactose non-fermenting colorless colony with black centres except *S.paratyphi A* whose colonies do not have black centres.

Bismuth sulfite agar

On Bismuth sulfite media *Salmonella* produce black colonies.

Xylose-lysine deoxycholate agar

On xylose-lysine deoxycholate agar, *Salmonella produce* transparent red colonies with black centres.

*Salmonella-Shigella*agar

On SS agar *Salmonella*produce lactose non- fermenting colonies with black centres except *S.paratyphiA* whose colonies do not have black centres.

Hektoen enteric agar

On hecktoen enteric agar, *Salmonella* produce transparent green colonies with black centres except *S. paratyphi A.*

2.3.4 Biochemical identification of Salmonella sp

Table 2.1 Some biochemical properties of *salmonella* sp

Isolates	TSI	Man	Suc	Glu	Lact	H_2S	Gas	Cit	Indole	Motility
Salmonella	R/Y	+	-	+	-	+	+	+/-	-	+

2.3.5 Serological identification of *salmonella* species

Salmonella can be characterized by their somatic (O) and flagella (H) antigens, the latter exist in some serotypes in phase 1 and 2. Some *Salmonellae* also have an envelope antigen called Vi (virulence). The *Salmonella* that cause typhoid fever and paratyphoid fever have the following antigenic compositions and belong to the serogroups indicated in table 2.2 (Murray *et al*., 1995).

Table 2.2 Serological group of some selected *Salmonella*

O group	Serovars	''O'' antigen	Phase 1 ''H'' antigen	Phase 2 ''H'' antigen	Vi antigen
A	*S.paratyphi A*	1,2, 12	A	Nil	Nil
B	*S.paratyphi B*	1, 4, 5, 12	b	1, 2	Nil
C	*S.paratyphi C*	6, 7	c	1, 5	Nil
D	*S.typhi*	9, 12	D	nil	Present

2.3.5.1 Slide agglutination test

The ''O'' antigen is usually determined by means of the slide agglutination test with group specific antiserum followed by agglutination with antiserum. Growth from non-selective media or Kligler iron agar can be used for the determination of ''O'' antigen. Strain of *S. typhi*and *S. paratyphi C* may possess Vi antigen that render the strain non- agglutinable in O antiserum. These cultures agglutinate in Vi antiserum. They will agglutinate however after destruction of the Vi antigen by boiling the culture for 10 minutes.

2.3.5.2 Felix- Widal test

This test measures agglutinating antibody level against O and H antigens. The levels are measured by using doubling dilutions of sera in a large test-tube. Usually O antibodies appear on days 6-7 and H antibodies on days 10-12 after the onset of the disease. The test is usually performed on an acute serum (at first contact with the patient). A convalescent serum should preferably also be collected so that paired titration can be performed (WHO, 2003).In practice, however, this is often difficult. At least 1ml of blood should be collected each time in order to have sufficient amount of serum. In exceptional circumstances the test can be performed on plasma without any adverse effect on the result.

The test has only moderate sensitivity and specificity. It can be negative in up to 30% of culture of proven cases of typhoid fever. This may be because of prior antibiotic therapy that has blunted the antibodies response. On the other hand *S.typhi* shares O and H antigens with other *Salmonella* serovars and has cross-reacting epitopes with other *Enterobacteriaceae,* and this can lead to false- positive result. Such results may also occur in other clinical conditions such as malaria, typhus, bacteraemia cause by other organism and cirrhosis. In areas of endemicity thereis often a low background level of antibodies in the normal population. Determining an appropriate cut-off for a positive result can be difficult since it varies between areas and between times in given areas (Clegg *et al.,* 1994).

2.3.5.3 Idltubex test

The Tubex test is simple and rapid (taking approximately two minutes). It exploits the simplicity and user friendliness of Widal and slide latex agglutination tests but uses the separation of colored particles in solution to improve resolution and sensitivity. Specificity is improved by means of an inhibition assay formed and by detecting antibodies to a single antigen in *S. typhi*only. The test pack includes, set of specially designed v-shaped tube that allow six samples per set to be examined simultaneously, reagent A comprising magnetic particles coated with *S. typhi*LPS, reagent B comprising blue coloured latex particles coated with a monoclonal antibodies specific for the O antigen. The reagents are stable for over a year at 4°C and for at least some weeks at ambient temperature (Clegg *et al,*1994).

A drop of test serum is mixed for 1 minute with a drop of reagent A in the tube. Two drops of reagent B are added and the contents are mixed thoroughly for 1-2 minutes. The set of tubes is then placed on magnet embedded stand, across which they are slid several times. The result, which can be read immediately or up to many hours later is based on the colour of the reaction mixture. A range of colours ranging from redness and blueness can be expected and a colour chart is provided for the purpose of scoring. Red indicates negativity while increasing blueness denotes positivity (Clegg *et al*,1994).

2.3.5.4 Typhoidot test

This test makes used of the 50kD antigen to detect specific IgM and IgG antibodies to *S.typhi* (Ismail *et al.,* 1991). It has undergone full-scale multinational clinical evaluation of its diagnostic value (Hatta*et al.,* 2002). This dot E1A test offers simplicity, speed, specificity, economy, early diagnosis, sensitivity 96% and high negative and positive predictive values. The detection of IgM reveals acute typhoid in the early phase of infection, while the detection of both IgG and IgM suggest acute typhoid in the middle phase of infection.

2.3.5.5 IgM dipstick test

The typhoid IgM dip stick assay is designed for the sero-diagnosis of typhoid fever through the detection of *S.typhi* specificIgM antibodies in serum or whole blood. The assay consists of a dipstick, lyophilized non enzymatic detection reagent, liquid to reconstitute the detection reagent, liquid to wet the test strip of dipstick before incubation with serum and detection reagent, and tubes. The components are stable for two years if stored in the temperature ranges 4-25°C in a dry place and protected from sun light.

The is based on the binding of *S.typhi* specific IgM antibodies to *S.typhi*LPS antigen and the staining of bound antibodies by an anti-human IgM antibody conjugated to colloidal dye particles. The white test strip of dipstick contains the antigen immobilized in a distinct line. The strip also has a control line with anti-human IgM antibodies. The is performed by incubation of the wetted test strip in a mixture of serum and detection reagent, the serum being diluted at 1:50 in the detection reagent. Whole blood may be tested at a 1:25 dilution in detection reagent.

The incubation period is 3 hours at room temperature. When incubation is completed the test strip is rinsed thoroughly with water and then allow to dry. The result is read by visual inspection of the test strip for staining of the antigen line occurs and graded 1+, 2+, 3+ or 4+ if there is weak, moderate, strong or very strong staining as indicated by comparism with a coloured reference strip. The control line should stain in all runs (WHO, 2003).

2.4 Treatment of Typhoid Fever

2.4.1 General Management

Supportive measures are important in the management of typhoid fever, such as oral or intravenous hydration, the use of antipyretics, and appropriate nutrition and blood transfusions if indicated. More than 90% of patients can be managed at home with antibiotics reliable care and close medical follow-up for complications or failure to respond to therapy (Pumjabi*et al.*, 1997). However, patients with persistent vomiting, severe diarrhoea and abdominal distension may require hospitalization and parenteral antibiotics therapy.

2.4.2 Antimicrobial Therapy

Efficacy, availability and cost are important criteria for the selection of first-line antibiotics to be used in developing countries. This section reviews the therapeutic guidelines for treatment of typhoid fever across all age groups. It should be noted, however, that therapeutic strategies for children e.g. the choice of antibiotics, the dosage of regimen and the duration of therapy may differ from those for adults.

The fluoroquinolones are widely regarded as optimal for treatment of typhoid fever in adults (Chinn *et al.,* 2000). They are relatively inexpensive, well tolerated and more rapidly and reliably effective than the former first-line drugs viz Chloramphenicol, ampicillin, amoxicillin, and trimethoprim-sulfamethoxazole. The majority of isolates are still sensitive. The fluoroquinolones attain excellent tissue penetration kill *S.typhi* in its intracellular stationary stage in monocytes/macrophages and achieve higher active drugs level in the gall bladder than other drugs. They produce a rapid therapeutic response i.e. clearance of fever and symptoms in 3-5 days and very low rates of post treatment carriage (Arnold *et al.,* 1993). Evidence from various settings in Asia indicates that the fluoroquinolones are equally effective in the treatment of typhoid fever in children.

However, the emergence of MDR strains has reduced the choice of antibiotics in many areas. There are two categories of drug resistance to antibiotics such as Chloramphenicol, ampicillin and trimethoprim –sulfamethoxazole (MDR strain) and resistance to fluoroquinolones drugs. Resistance to fluoroquinolones may be partial or total. The so called nalidixic acid resistant *S. typhi*(NARST) is marker of reduced susceptibility to fluoroquinolones compared to nalidixic acid sensitive strain.

Ciprofloxacin, ofloxacin, perfloxacin and fleroxacin have generally proved effective. In recent years however, there have been many reports of susceptibility and treatment failure for ciprofloxacin (Brown *et al.,* 1999). No evidence of toxicity and impact on growth has been described in children with typhoid who have received ciprofloxacin (Doherty *et al.,* 2000).

Chloramphenicol despite the risk of agranulocytosis in 1/10,000 patients is widely prescribed in developing countries for the treatment of typhoid fever (Islam *et al., 1993*). *S.typhi*from many areas of the world e.g. most countries in Africa and Asia remain sensitive to this drug and it is widely available in most primary care settings in developing countries for the treatment of pneumonia.

Ampicillin and amoxicillin are used at 50-100mg/kg/day orally, im or iv divided into three or four doses. No benefit has been reported from the addition of clavulanic acid to amoxicillin.

Trimethoprim-sulfamethoxazole can be used orally or i.v. in adult at a dose of 160mg TMP plus 800mg SMZ twice daily or in children at 4mg TMP/kg and 20mg SMZ/kg for 14 days (Thisyakorn and Mansuwan 1999). Of the third-generation cephalosporins, oral cefixime (15-20mg/kg/day for adult, 100-200mg twice daily) has been widely used in children in a variety of geographical settings and found to be satisfactory (Girgis*et al., 1995*). However, a trial of cefixime in MDR typhoid in Viet Nam indicated significantly higher treatment failure rates than with ofloxacin (Phoung*et al.*1999). Other agents e.g. cefodoxime have proved successful against typhoid fever (Bhutta and Khan 2000). There is clear need to identify improved strategies for treating MDR typhoid in children. Recent data on the use of azithromycin in children indicate that it may safely be given as an alternative agent for treatment of uncomplicated typhoid fever (Frenck*et al.,* 2000).

There are few data on the treatment of typhoid in pregnancy. The beta-lactam are considered safe (Seoud*et al., 1998).* There have been several case reports of the successful use of fluoroquinolones but these have not been generally recommended in pregnancy because of safety concerns (Koul*et al 1995*). Ampicillin is safe in pregnant or nursing women, as is cefriaxone in such women with severe MDR disease.

2.4.3 Management of Complications

Both outpatients and in inpatients with typhoid fever should be closely monitored for the development of complications. Timely intervention can prevent or reduce morbidity and mortality. The parenteral fluroquinolones are probably the antibiotics of choice for severe infection but there have been no randomized antibiotics trials (Dutta*et al., 1993).* In severe typhoid the fluoroquinolones are given for a minimum of 10 days. Typhoid fever patients with changes in mental status, characterized by delirium, obtundation and stupor, should be immediately evaluated for meningitis by examination of the cerebrospinal fluid. If the findings are normal and typhoid meningitis is suspected, adult and children should immediately be treated with high dose intravenous dexamethasone in addition to antimicrobials (Punjubi*et al 1990).* If dexamethosone is given in an initial dose of 3mg/kg by slow i.v. infusion over 30 minutes and if, after six hours, 1mg/kg is administered and subsequently repeated at six hourly intervals on seven further occasions, mortality can be reduced by some 90% in these high risk patients.

2.4.4 Management of Carriers

An individual is considered to be a chronic carrier if he or she is asymptomatic and continues to have positive stool or rectal swab cultures for *S. typhi*a year following recovery from acute illness. Overall some 5% of typhoid fever patients become chronic carriers. The rate of carriage is slightly higher among female patients, patients older than 50 years, and patients with cholelithiasis or Schistosomiasis. If cholelithiasis or schistosomiasis is present, the patient probably require cholecystectomy or antiparasitic medication in addition to antibiotic in order to achieve bacteriological cure. In order to eradicate *S. tyhi*carriage, amoxicillin or ampicillin (100/kg/day) plus probenecid g orally or 23mg /kg for children or TMP-SMZ (160-180mg twice daily) is administered for six weeks, about 60% of patients treated with either regimen can be expected to have negative cultures on follow-up. Clearance of up to 80% of chronic carriers can be achieved with the administration of 750mg of ciprofloxacin twice daily for 28 days or 400mg of norfloxacin. Other quinolones drugs may yield similar result (Ferreccio*et al.*, 1990).

Carriers should be excluded from any activities involving food preparation and serving as should convalescent patients and any person with possible symptoms of typhoid fever. Although it will be difficult for typhoid carriers in developing countries to follow this recommendation, food handlers should not resume their duties until they had three negative stool cultures at least one month apart.

2.5 Prevention of Typhoid Fever

The major routes of transmission of typhoid fever are through drinking water or eating of food contaminated with *S. typhi.* Prevention is based on ensuring access to safe water and by promoting safe food handling practices. Health education is paramount to raise public awareness and induce behaviour changes (WHO,2003).

2.5.1 Safe water

Typhoid fever is a water – borne disease and the main preventive measure is to ensure access to safe water. The water needs to be of good quality and must be sufficient to supply all the community with enough drinking water as well as for all other domestic purposes such as cooking and washing (WHO, 2003).

2.5.2 Food safety

Contaminated food is another important vehicle for typhoid fever transmission. Appropriate food handling and processing is paramount and the following basic hygiene measures must be implemented or reinforced during epidemics (Girgis*et al,*1995).

- Washing hands with soap before preparing or eating food.
- Avoiding raw food, shellfish, ice etc.
- Eating only cooked and still hot food or reheating it.
- During outbreak, food safety inspection must be reinforced in restaurants and for street food vendors' activities.

2.5.3 Sanitation

- Proper sanitation contributes to reducing the risk of transmission of all diarrheal pathogens including *S. typhi*.
- Appropriate facilities for human waste disposal must be available for all the community. In an emergency, pit latrines can be quickly built.
- Collection and treatment of sewage, especially during the rainy season, must be implemented.
- In areas where typhoid is known to be present, the use of human excreta as fertilizer must be discouraged (WHO, 2003).

2.5.4 Health education

Health education is paramount to raise public awareness on all the above mentioned prevention measures. Health education messages for the vulnerable communities need to be adapted to local conditions and translated into local language. In order to reach communities, all possible means of communication (e.g. media, school, women's group, religious group) must be applied. Community involvement is the cornerstone of behaviour change with regard to hygiene and for setting up and maintenance of the needed infrastructures.

2.5.5 Vaccination

Currently available vaccines; the old parenteral killed whole-cells vaccines were effective but produced strong side –effect because of LPS. Two safe and effective vaccines are now licenced and available. One is based on defined sub-unit antigen, the other on whole cells live attenuated bacteria.

1. The first of these vaccines is Vi vaccine containing Vi polysaccharide, is given in a dose subcutaneous (s.c.) or i.m. Protection begins seven days after injection, maximum protection being reached 28 days after injection when the highest antibody concentration is obtained. In field trials conducted in Nepal and South Africa where the disease is endemic and attack rates 900/10,000 the protective efficacy was 72% one and half years' after vaccination (Acharya*et al.*, 1990) and was still 55% three years after a single dose (Klugman*et al.*, 1996). The vaccine is approved for persons aged over two years. Re-vaccination is recommended every three years for travellers. The Vi vaccine is licensed in Australia and in more than 92 countries in Africa, the American, Asia and Europe. It is mainly used by travellers visiting areas at high risk of typhoid fever because of the presence of multidrug- resistance strains.

2. The second is the live oral vaccine T_y2ca available in enteric- coated capsule (Black *et al., 1990)* or liquid formulation. It should be taken in three doses two days apart on an empty stomach. It elicits protection as from 10-14 days Travelers should be re-vaccinated annually. The protective efficacy of enteric-coated capsule formulation seven years after the last dose is still 62% in areas where the disease is endemic.

Future vaccines;

- Vi–rEPA; a new Vi conjugate candidate vaccine bound to non-toxic recombinant *Pseudomonas aerugenosa*exotoxin A(rEPA) has enhanced immunogecity in adult and in children aged 5-14 years and has induced a booster response in children aged 2-4 years (Kossacka*et al.,* 1999). In a double –blind randomized field trial, 11091 Vietenamese children aged 2-5 years were given two injections of VirEPA separated by six weeks (Lin *et al.,* 2000). No serious side- reactions were observed. The efficacy after 27 months of active surveillance was 91.2%. passive surveillance in the 16 months since the study ended showed 88% efficacy.
- TAB vaccines; the TAB vaccine composed of inactivated *Salmonella* caused a strong side-effect. A new *S. paratyphiA* vaccine composed of the surface O – specific polysaccharide conjugate with tetanus toxoid was shown to be safe and immunogenic.

Other candidate vaccines; three live attenuated candidate vaccines are currently being evaluated. Each is administered as single oral dose.

- CVD 908- htrA is an *S. typhi*strain with a mutation deletion in the htrA gene (Tacket*et al., 2000)*. The second candidate
- vaccine is an *S. typhi*TY 2 strain with toiple mutation deletion in the oyacrp and cdt gene (Tackett *et al.,2000).*

- The third is a derivatives of an *S.typhi* Ty2 strain with a double mutation deletion in genes phop and pho Q (Hohmann*et al., 1999).*

2.6 Development of Antibiotics Resistance and Resistance Mechanisms

Antibiotic resistance occurs when a microbe acquires a genetic mutation making it resistant to the effect of one or more antimicrobial agents that were once effective. This genetic mutation may be acquired spontaneously or by genetic transfer. Bacteria can become resistant to antibiotics for which term antibiotic resistance is used. Microbes that are resistant to multiple antimicrobials are termed Multidrug resistant (MDR) or sometimes in the lay press superbugs. Bacteria often become resistant in several different ways. Unfortunately, a particular type of resistance mechanism is not confined to single class of drugs. Two bacteria may use different mechanisms to withstand the same chemotherapeutic agents. Furthermore, resistant mutants arise spontaneously and are then selected for in the presence of the drug. Pathogens often become resistant simply by preventing entrance of the drug. Many gram-negative bacteria are unaffected by penicillin G because it cannot penetrate the envelope's outer membrane (Willey *et al,* 2008*).* Genetic mutation that lead to changes in penicillin binding proteins also renders a cell resistant. A decrease in permeability can lead to sulfonamide resistance. *Mycobacteria* resist many drugs because of high content of mycolic acids in a complex lipid layer outside their peptidoglycan which is impermeable to most water soluble drugs (Willey *et al., 2008).*

A second resistance strategy is to pump the drug out of the cell after it has entered. Some pathogens have plasma membrane translocases, often called efflux pumps that expel drug. Because they are relatively nonspecific and can pump many different drugs, these transport proteins often are called multidrug resistance pumps. Many are drug/proton antiporters that is, proton enter the cell as drug leaves.

The third resistance strategy is by inactivating drugs through chemical modification. The best known example is the hydrolysis of the β - lactam ring of penicillin by the enzyme penicillinase. Drugs are also inactivated by the addition of chemical groups. For example, chloramphenicol contains two hydroxyl groups that can be acetylated in a reaction catalysed by the enzyme chloramphenicol acyltransferase with acetyl-CoA as a donor (Willey et al., *2008).* Aminoglycosides can be modified and inactivated in several ways. Acetyltransferase catalysed the acetylation of amino group. Some aminoglycosides modifying enzymes catalysed the addition to hydroxyl groups of either phosphates (phosphotransferases) or adenyl groups (adenyltransferase). Some antibiotics resistance genes and their resistance mechanisms found in *Salmonella* are as follows;

Table 2.3: Some Antibiotics Resistance Genes and Resistance Mechanisms

Gene	Resistance Mechanism
TetA	Efflux
tetB	Efflux
tetC	Efflux
tetG	Efflux
tetS	Ribosomal protection
***bla*TEM**	β – lactamase
***aad*B**	Aminoglycoside adenyltransferase
***aac*C**	Aminoglycoside acyltransferase
***cat*1**	Chloramphenicol acetyltransferase
***Cat*2**	Chloramphenicol acetyltransferase
***flo*R**	Efflux

2.7 Origin and Transmission of Antibiotic Resistance Genes

Genes for drug resistance may be present on bacterial chromosomes, plasmid, transposons and integrons (Willey *et al.2008,)*. Because they are often found on mobile genetic elements, they can freely exchange between bacteria. Spontaneous mutations in bacteria chromosome (although they do not occur very often) can make bacteria drug resistant. Usually such mutations result in a change in drug target, therefore, the antibiotic cannot bind and inhibit growth. Many mutants are probably destroyed by natural host resistance mechanisms. However, when a patient is being treated extensively with antibiotics, some resistant mutants may survive and flourish because of their competitive advantage over non-resistant strain. Frequently a bacterial pathogen is drug resistant because it has a plasmid bearing one or more resistance genes, such plasmid is called R-plasmid (resistance plasmid). Plasmid resistance genes often code for enzymes that destroyed or modify drugs. For example, the hydrolysis of penicillin or the acetylation of chloramphenical and aminoglycosides drugs. Plasmid associated genes have been implicated in resistance to the aminoglycoside, chloramphenicol, penicillins and cephalosporins, erythromycin, tetracyclines, sulfonamides and others. Once, a bacterial cell possesses an R-plasmid, the plasmid or its genes may be transferred to other cells quite rapidly through normal gene exchange processes such as conjugation, transduction and transformation. Because a single plasmid may carry genes for resistance to several drugs, a pathogen population can become resistant to several antibiotics simultaneously (Willey *et al, 2008).*

Antibiotic resistance genes can be located on genetic elements other than plasmid. Many composite transposons contain genes for antibiotic resistance, and some bear more than one resistance genes. They are found both in gram-negative and gram-positive bacteria. Some examples and their resistance markers are Tn5 (kanamycin, bleomycin, and streptomycin), Tn9 (chloramphenicol), Tn10 (tetracyclines), Tn21 (streptomycin, sulfonamides, spectinomycin), Tn551 (erythromycin) and Tn4001 (gentamicin, tobramycin, kanamycin). Resistance genes on composite transposons can move rapidly between plasmids and through a bacterial population. Often several resistance genes are carried together as gene cassettes in association with a genetic element known as integron (Willey *et al*, 2008).

2.8 Epidemiology of Typhoid Fever

Typhoid fever is more common in children and young adults than in older patients (Misra*et al., 1997)*. Worldwide, typhoid fever is most prevalent in impoverished areas that are overcrowded with poor access to sanitation. Non-epidemic incidence estimate suggest that South-central Asia, South-East Asia, and Southern Africa are regions with high incidence of *S.typhi* infection (more than 100case/100 000 persons/year) (Crump *et al.*, 2004). Other regions of Asia, Latin America, the Caribbean and Oceania have a median incidence of 10-100 cases/100, 000 persons/ year. These estimates, though are limited by lack of consistent reporting from all areas of the world and based on extrapolation of data across regions and age groups. As an example the incidence estimate within Africa are based on report from Egypt, Nigeria and South Africa only and may thus not be accurately defined (Crump *et al.,2004)*.

Because humans are the only reservoir for *S. typhi* a history of travel to settings in which sanitation is poor or contact with a known typhoid case or carrier is useful for identifying people at risk of infection outside of endemic areas, although a specific source or contact is identified in a minority of case.

Approximately 200-300 cases of *S. typhi* are reported in the United State each year. About 80% of these cases occur among travellers to countries where typhoid is endemic particularly countries in South Central Asia, Nigeria etc. In a study of 428 cases of typhoid fever reported among travellers from source rich countries through the multinational Geosentinel Surveillance Network between 2006 and 2011, 67% of case were acquired in South central Asia, 34% from India, 30% from Nigeria.

CHAPTER THREE

3.0 RESEARCH METHODOLOGY

3.1 Study Area

The study was conducted in Katsina State. Three General Hospitals were selected for this study, one from each of the three randomly selected Local Government Areas of the State; General Hospital, Baure was selected from Baure Local Government Area, General Hospital, Daura was selected from Daura Local Government Area and General Hospital, Katsina was selected from Katsina Local Government Area. Baure Local Government Area covered a total area of 707 km^2 and apopulation of 197,425 as far 2006 Census. Daura Local Government Area covered an area of 450 km^2 and a population of 78,283 while Katsina Local Government Area covered an area of 142km^2 and a population of 318,459. Majority of the people in these areas are Hausa and Fulani and were mainly Muslims. Farming is the main occupation and is mainly carried out by males. Both modern and olden buildings types are found in the area.

3.2 Study Population

The population used in this research work comprises the patients with some or all symptoms of Enteric fever (fever, diarrhoea, abdominal pain nausea and vomiting) from three (3) randomly selected General Hospitals within Katsina State. All together, the study population comprised three hundred (300) respondents who was selected randomly by the used of questionnaire.

3.3 The Study Design

Descriptive and cross sectional study were used. The targetpopulation was 300 peoples. Random sampling technique was employed to select the study samples

3.4 Ethical Consideration

Ethical approval was obtained from Research and Ethics Committee of Katsina State Ministry of Health.

3.5 Inclusion Criteria

People with some or all symptoms of enteric fever.

3.6 Exclusion Criteria

People without symptoms of typhoid fever

3.7 Sample Size Determination

The sample size was obtained using the following formula (Adkin 2006);

$n = Pq/(E/Z)^2$

Where P = Prevalence of previous study 14% = 0.14(Abdullahi 2010).

q = 100-P = 100-14=86.

E = Allowable error =5%.

Z = Standard normal distribution at 95% CI=1.96.

n = Number of sample to be collected.

$n = 14 \text{ x } 86/ (5/1.96)^2 = 1204/ (2.55)^2 = 1204/6.5 = 185.23$

However, for the purpose of obtaining precise results in the research work, 300 blood samples were collected from the above mentioned hospitals.

3.8 Collection of Sample

At least 5ml of blood samples was collected from patients with some or all clinical features of typhoid fever by qualified medical personnel and transferred into sterile, container containing 50ml of thioglycollate broth. Blood to broth ratio used was 1;10 (WHO, 2003)

3.9 Enrichment of Specimen for Isolation

Salmonella species were isolated using standard methods, 5 ml of blood collected from each patient were directly inoculated into 50ml of thioglycollate broth (LAB M) contained in a plain bottle. The tubes containing the sample were transported to Microbiology Laboratory of Ahmadu Bello University, Zaria and incubated at 37°C . The tubes were observed for turbidity daily for seven days. At the end of the incubation periods, the tubes with turbidity were noted and sub cultured on plates of DCA.

3.10 Isolation and Identification of *Salmonella* Spp Based on Cultural Characteristics

A loopful of the culture in thioglycollate broth was streaked onto Deoxycollate Citrate Agar (Oxoid U. K). Cultures were incubated over night at 37°C and colonies growing on the plate were further sub cultured onto Bismuth Sulfide Agar and incubated overnight at 37°C. Colonies growing on the Bismuth Sulfide Agar plates that exhibit morphological and biochemical properties similar to that of *Salmonella* spp were selected for Gram staining and biochemical tests

3.11 Microscopy

Smear was made by placing one drop of normal saline on a cleaned glass slide, using sterilized wire loop 1-2 colonies were removed from growth on Bismuth Sulfite Agar and mixed with the normal saline dried and heat fixed. The smear was stained with the crystal violet for one minute and then was washed with water for one minute. This is followed by treatment with iodine solution for one minute. The smear was decolorized with ethanol and counterstained with Safranin and was air dried before it was observed under 100X (oil immersion objective).

3.12 Identification of *Salmonella* Spp Based on Biochemical Characteristics

Suspected *Salmonella* colonies were subjected to the following biochemical tests.

3.12.1 Triple sugar iron agar (tsi);

Method;

At least one of each colony type of the well isolated colonies was selected using an inoculating straight wire. The centre of the colony was lightly touched and prepared TSI medium was inoculated by stabbing the butt and streaking the surface of the slants. The culture was then incubated at 37°C for 24hrs. The set up was observed for the presence of red slope and yellow butt, weak blackening along the stab line and bubbles or crack.

3.12.2 Motility test

Methods

Motility agar was inoculated with straight inoculating needle with growth from TSI making a single stab about 1-2cm down into the medium. The motility was examined after 24hrs at 37°C. Positive result was shown by diffuse, hazy growth that spread throughout the medium rendering it slightly opaque while negative result is indicated by growth confined to stab line.

3.13 Serological Identification of *Salmonnella Species*

3.13.1 Slide agglutination test;

Method;

Slides were divided into two sections using wax pencil.

A drop of physiological saline was placed on each section.

By using a sterile wire loop a portion of growth from the surface of TSI was removed, mixed and emulsified in each drop of physiological saline on the Slide. Milky suspension was created by careful mixing of the mixture.

A small drop of Vi antiserum was added to the first suspension. The second suspension served as control.

The suspension and antiserum were mixed and then the slide was tilted back and forth to observe the agglutination.

Steps 1-5 were used for identification of *Salmonella paratyphiA,* using O Group A antisera instead of vi antiserum.

3.14 Susceptibility Test of *Salmonella* Isolates

Antibioticssusceptibility tests were carried out on the *Salmonella* serotypes. Seven antibiotics were tested using Kirby- Bauer disc diffusion method (WHO, 2 010) on Mueller-Hinton agar (OxoidUK). Three colonies were inoculated onto a tube containing nutrient broth (Oxoid UK) and incubated at 37°C. standardization of inocula was performed. The turbidity of the inocula was adjusted to match that of 0.5 MacFarlandstandard. Within 15 minutes of preparing the adjusted inocula, a sterilized cotton swab was dipped into the inocula. The swab was rotated several times and pressed firmly on the inside wall of the tube above the fluid level to remove excess inocula from the swab. The surface of Mueller-Hinton agar plate of 16cm was streaked using the swab. Inoculation was completed by running the swab around the rim of the agar. Sterilized forcep was used to dispense the single disc onto the Mueller-Hinton agar surface. The discs were ensured to make complete contact with the agar surface by touching the top of the disc with forceps. The following discs were used for susceptibility testing; ofloxacin (5µg), co-trimoxazole (25µg), chloramphenicol (30µg), cephalex (30µg), gentamicin (10µg), ampicillin (10µg) and penicillin G (10µg). After 24 hours of incubation at

37°C, zones of inhibition were measured and recorded with the help of meter rule in millimetres (mm). The standard zones of inhibition interpretation chart by CLSI (2010) was used to interpret the sizes of inhibition. *Escherichia coli* ATCC 25922 was used as control.

3.15 PCR Detection of Antibiotics Resistance Genes in *Salmonella* Serovars

3.15.1 DNA Extraction

The DNA was extracted according to method described by Turk Yilmaz*et al*. (2009). A few colonies of *Salmonella* grown on selective agar was transferred to a 1.5ml tube containing 100µl sterilized lysis solution (1ml M Tris-HCl,22.5µl IGEPAL CA-630-Sigma,22.5µl Tween 20-Biorad 220µl protease K(10mg/ml),8,8µl DiH_2O). The tubes were vortexed and incubated for 10min at 60°C, 95°C for 10 minutes and was cooled at 4°C. The lysate was centrifuged for 5min, Phenol:Chloroform (1:1) treatment was followed with the clear supernatant. Plasmids DNA were precipitated with equal volume of chilled Isopropyl alcohol and DNA pellet dissolved in 100µl of TE buffer (diethylether). Molecular identification was done by PCR.

3.15.2 PCR amplification

PCR amplification was carried out using master-mix kit containing the Taq (*Thermos aquaticus*) DNA polymerase, PCR buffer,dNTP (dATP, dTTP, dGTP, dCTP) and $MgCl_2$. DNA template and primers were added to the mixture. The specific primers sets used in the PCR assay were designed by Turkyilmaz*et al.* (2009). The sequence of forward and reverse primers for *bla*Temgenes were CATTTCCGTGTCGCCCTTAT (F) and TCCATAGTTGCCTGACTCCC (R) amplification was performed in a thermal cycler. PCR product was separated by electrophoresis on 1.2% agarose gel stained with ethidium bromide. A 100bp ladder was used as molecular marker.

3.15.3 AgaroseGel Electrophoresis

A 1.2% agarose gel was used to separate the PCR product and it was prepared by combining 1.2 g agarose in ten times concentration of Tris acetate ethylene diaminetetraacetate (10ml 10× TAE) buffer and 135ml distilled water in a 250 ml beaker flask and heated in a microwave for 2 minutes until the agarose is dissolved. Then 5µl ethidium bromide (5.0mg/ml) was added to the dissolved agarose solution with swirling to mix. The gel was then poured onto a mini horizontal gel electrophoresis tank and the casting combs were inserted. It was then allowed to gel for 30 minutes. The casting combs were carefully removed after the gel has completely solidified. One times concentration (1×) TAE electrophoresis buffer was then added to the reservoir until the buffer just covered the agarose gel. Then 0.5µl of tracking dye (bromophenol blue) was added to 20µl of each samples with gentle mixing, 20µl of the sample was then loaded onto the wells of the gel, the mini horizontal electrophoresis gel set- up was then covered and the electrodes connected. Electrophoresis was carried out at 100 – 120 mA for 1hr. At the completion of the electrophoresis, the gel was removed from the buffer and gel was viewed under UV- light in the gel documentation unit. The band patterns of the DNA fragments were photographed with a Polaroid camera. A100 bp marker was used as lane marker.

CHAPTER FOUR

4.0 Results

A total of 300 blood samples were obtained for this study from three different hospitals. The distribution of the samples according to the hospitals were; General hospital, Baure 100 (33.3%), General hospital, Katsina 120 (40%) and General hospital, Daura 80 (26.7%) respectively (figure 1). Out of the 300 samples, only 14 samples had the evidence of growth characteristic of *Salmonella* and so considered positive. The remaining 286 had no evidence of growth and were considered negative.

The prevalence of *Salmonella* bacteraemia with regard to sample population was shown by Table 1. *Salmonella* was found to be higher among the people visiting General hospital, Baure 7 (7%) followed by General hospital, Daura 4 (5%) and General hospital, Katsina 3 (2.5%) and the difference is not significant ($P < 0.05$).

Table 2 shows the Biochemical and Serological characterization of *Salmonella* isolates. Out of the 14 isolates characterized, 9 isolates were *Salmonellatyphi* and 5 were *Salmonellaparatyphi*A

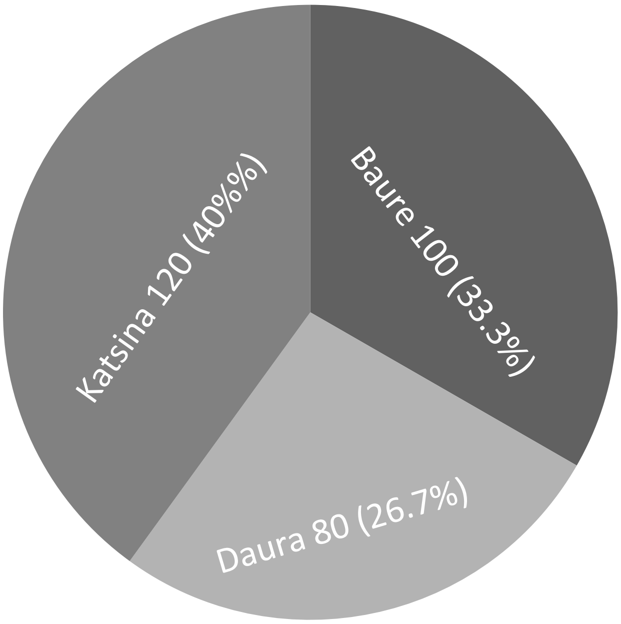


Fig 4.1: Distribution of Samples According to Hospitals in the study area

Table 4.1: Prevalence of *Salmonella*bacteraemia in the Study Area

Hospital	No. Examined	No. Positive	χ^2	P value
G.H Katsina	120	3(2.5%)		
G.H Baure	100	7(7%)	7.860	0.096
G.H Daura	80	4 (5%)		
Total	**300**	**14(5%)**		

KEY

GH = General Hospital

Table 4.2: Biochemical and Serological Characterisation of *Salmonella* Isolates

Isolates	Biochemical Test				Serological Test		
	TSI			Motility	Vi Antiserum	"O" Antiserum A	Comment
	Glu	H_2S	Gas				
1.	+	+w	-	+	+	-	*S.typhi*
2.	+	+w	-	+	+	-	*S.typhi*
3.	+	+w	-	+	+	-	*S.typhi*
4.	+	+w	-	+	+	-	*S.typhi*
5.	+	+w	-	+	+	-	*S.typhi*
6.	+	+w	-	+	+	-	*S.typhi*
7.	+	+w	-	+	+	-	*S.typhi*
8.	+	+w	-	+	+	-	*S.typhi*
9.	+	+w	-	+	+	-	*S.typhi*
10.	+	-	+	+	-	+	*S.paratyphi A*
11.	+	-	+	+	-	+	*S.paratyphi A*
12.	+	-	+	+	-	+	*S.paratyphi A*
13.	+	-	+	+	-	+	*S.paratyphi A*
14.	+	-	+	+	-	+	*S.paratyphi A*

KEY

- negative reaction. + = positive reaction. +w = weak positive reaction.

TSI = Triple sugar iron. H2S = Hydrogen Sulphide

Although, the relationship between the *Salmonella*bacteraemia and age groups was not significantly associated (P > 0.05), patients under age bracket of 1- 10 years were found to be more susceptible to typhoidal*Salmonella* bacteraemia with 6(9.7%) positive cases, followed in that order by 11 – 20 years 4(7.1%), 21 – 30 years 3(4.3%), 31 – 40 1(1.9%) and 41 – 50 0(0.0%) respectively (Table 3).

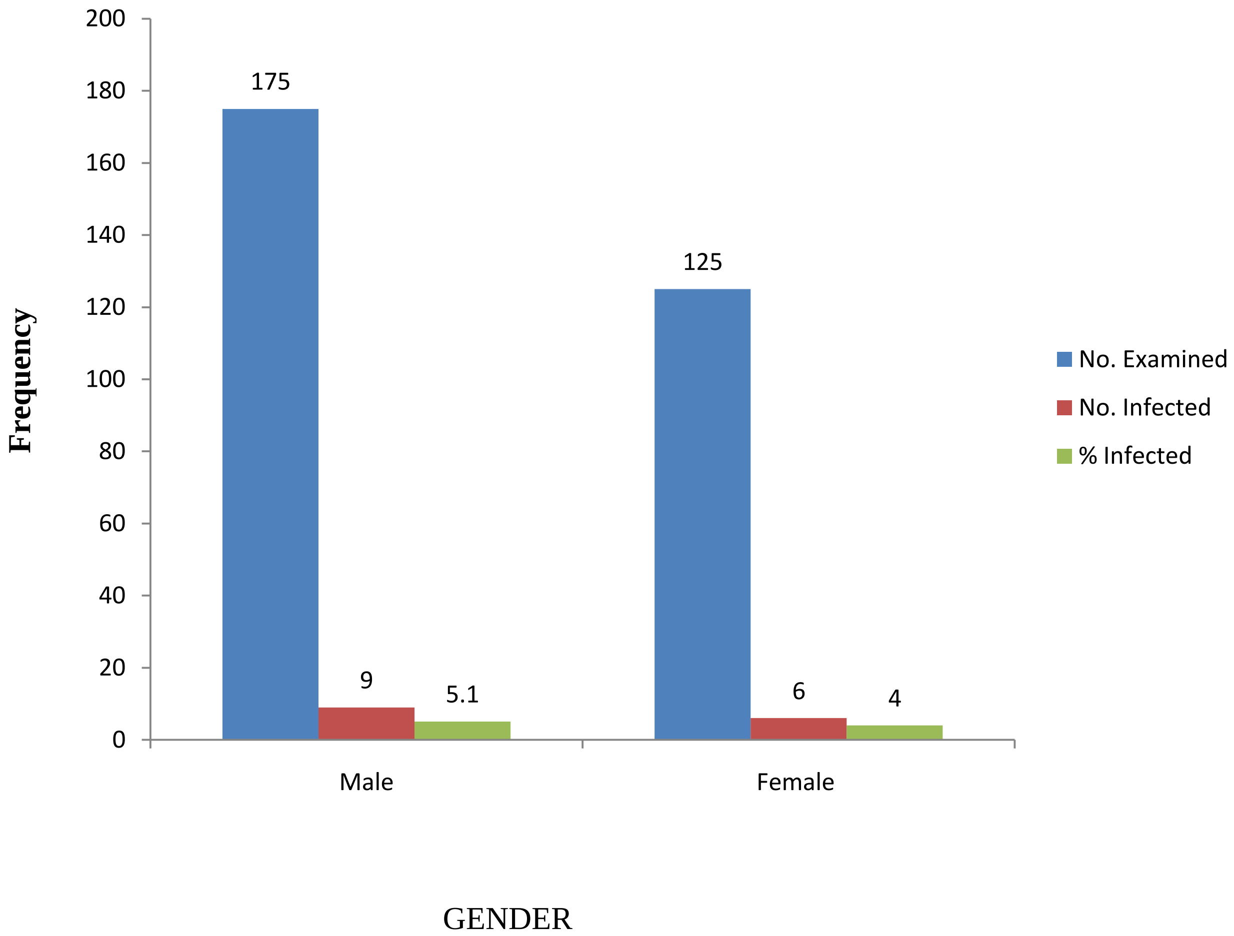
There was no significant association (χ^2 =0.039, p = 0.0844) between the *Salmonella bacteraemia* and gender status of the patients. The bacteraemia rate was higher in male than in their female counterpart (fig II).

There was no significant association (χ^2 =0.447, p =0.097) between the bacteraemia and the occupation status of the study subjects. The bacteraemia is higher among business subjects followed by farmers and civil servants (fig.III).

There was a statistically significant association (χ^2 = 10.276, p= 0.016) between the disease and the sources of drinking water of the study subjects (fig IV). The higher prevalence was seen among the people using bore-hole followed by the tap and no response.

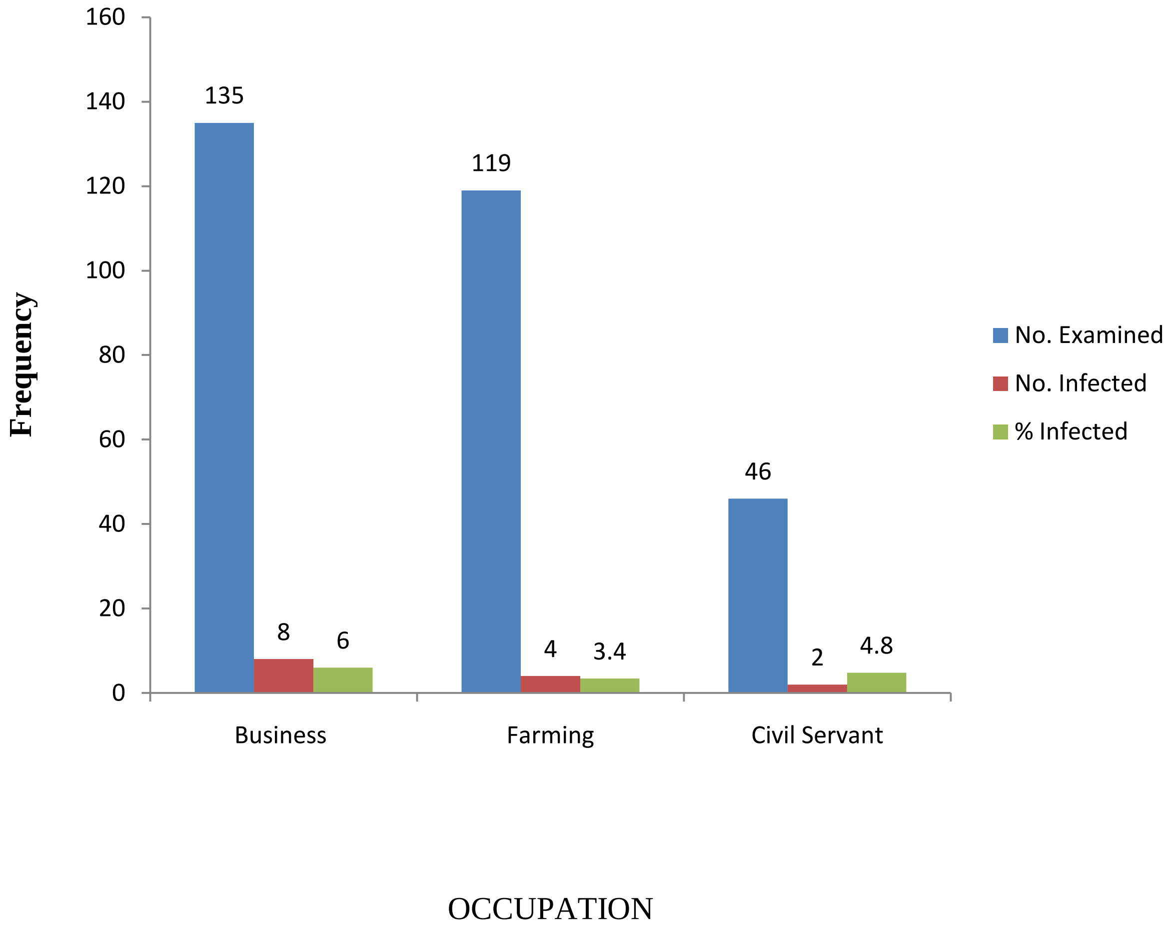
Table 4.3: The Prevalence of *Salmonella*Bacteraemiain Relation to Age Group of Patients Tested

Age Group	**No. Examined**	**No. Positive**	χ^2	**P**
1 – 10	62	6(9.7%)		
11 – 20	56	4(7.1%)	8.142	0.086
21 – 30	69	3(4.3%)		
31 – 40	53	1(1.9%)		
41 – 50	60	0(0.0%)		
Total	**300**	**14(5%)**		



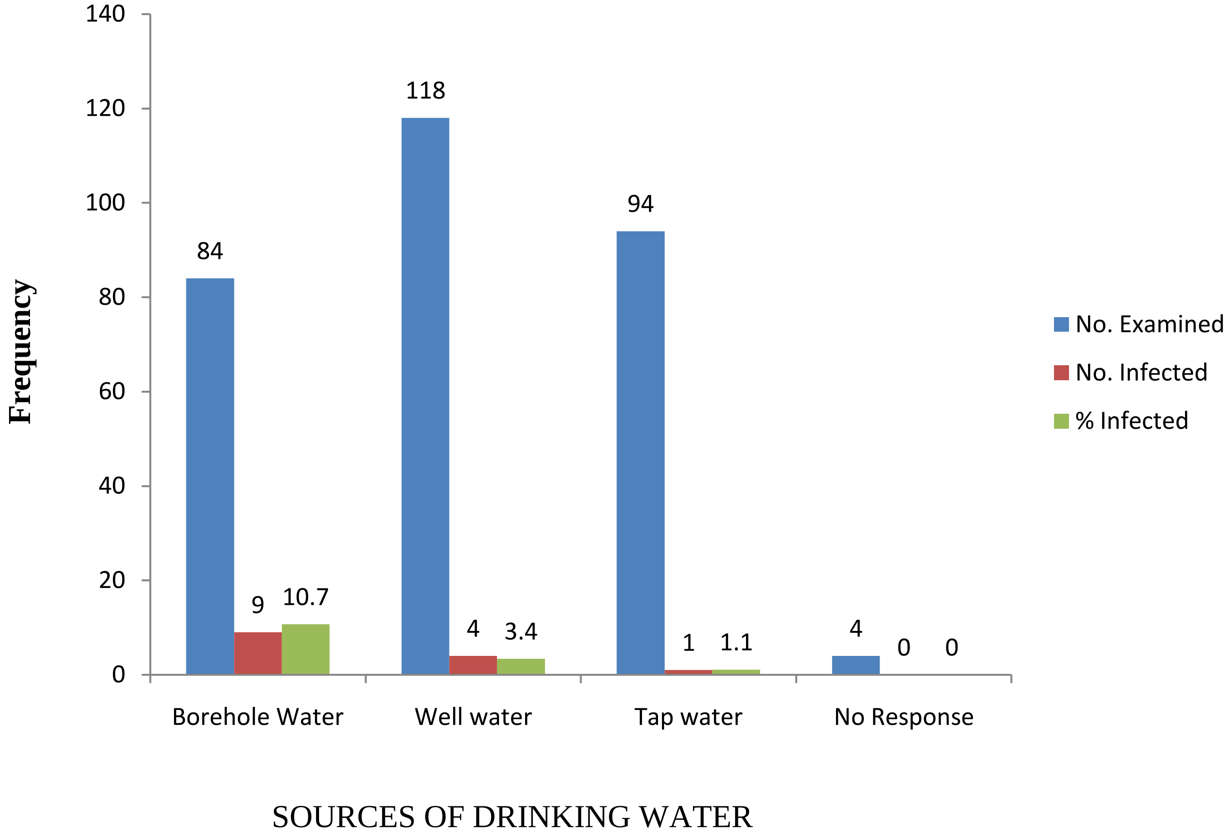
χ^2 = 0039, P = 0.0844

Figure II: **Prevalence of *Salmonella*bacteraemia with respect to gender of the subjects.**



χ^2 = 0.447 P-Value = 0.097

Figure III: Prevalence of *Salmonella*bacteraemia with regard to occupation



χ^2 = 10.276 P-Value = 0.016

Figure IV: Prevalence of *Salmonella*bacteraemia with respect to source of Drinking water

Out of 14 patients infected, higher rate of *Salmonella* infection was observed in patients with informal education 10(71.4%) followed in that order by the none education 3(21.4%), primary education 1(7.1%) and zero infection rates observed among the patients with secondary and tertiary education 0(0.0) and 0(0.0) respectively (Table 4);

There was a significant difference ($P < 0.05$) between the infection rate and the treatment status of the patients. (Table 5).The disease was found to be higher among untreated individuals than in treated individuals.

The prevalence of *Salmonella* infection by clinical manifestation Is shown in Table 6. Out of 14 patients with *Salmonella* infections recorded, patients that presented with fever had the highest incidence 8(5.8%), followed by those presented with diarrhea 6(4.1%), zero infection rate was recorded from the patients having vomiting, headache and gastroenteritis. There is no significant difference between the rate of infection and clinical manifestation ($P > 0.05$).

Table 7 shows the prevalence of *Salmonella* infection with respect to the immunization status of the studied subjects. The highest prevalence of *Salmonella* infection was found among the non – immunized subjects with 14(5.8%) prevalence rate and 0(0.0) prevalence rate was found among immunized subjects. There is no significant difference between the infection and immunization status of the patients ($P > 0.05$) (Table 7);

Table 4.4: Prevalence of *Salmonella*bacteraemia with Respect to Educational Status of the Subjects

Education	**No. Examined**	**No. Positive**	**χ^2**	**P – value**
Informal	207	10(5%)		
None	22	3(13.6%)		
Primary	9	1(11.1%)	7.866	0.097
Secondary	37	0(0.0%)		
Tertiary	25	0(0.0%)		
Total	**300**	**14(5%)**		

Table 4.5: Prevalence of *Salmonella*bacteraemia with Respect to Treatment Status of study subjects

Treatment Status (Antibiotics only)	No. Examined	No. Positive	χ^2	P – value
Yes	172	4(2.3%)	4.966	0.026
No	128	10(7.8%)		
Total	**300**	**14(5%)**		

Table 4.6: Prevalence of *Salmonella*bacteraemia with Respect to Clinical Symptoms of the Study Subjects

Clinical Symptoms	**No. Examined**	**No. Positive**	**χ^2**	**P – value**
Fever	139	8(5.8%)		
Diarrhea	147	6(4.1%)	1.206	0.877
Vomiting	7	0(0.0%)		
Headache	7	0(0.0%)		
Total	**300**	**14(5%))**		

Table 4.7: Prevalence of *Salmonella*bacteraemia with respect to Immunization Status of the study subjects

Immunization Status	No. Examined	No. Positive	χ^2	P – value
Yes	57	0(0.0%)	3.445	0.063
No	3	14(5.8%)		
Total	**300**	**14(5%)**		

The susceptibility of the isolates shows that all the *Salmonella* species were (100%) susceptible to trimethoprim - sulfamethoxazole, chramphenicol, ofloxacin, gentamicin and cephalex, but only 1 *Salmonella typhi is* susceptible to ampicillin while all the *Salmonella* species were resistant to penicillin G (Table 8);

In the table 9 below all the *Salmonella* serovars were resistance to penicillin G while 8 strains of *Salmonella typhi*were resistant to ampicillin.

Table 4.8: Antibiotic Susceptibility of *Salmonella* serovars

Drug (Antibiotic) /Disc potency	*Salmonella serovars*(%)			
	S typhi(n=9		*S paratyphi A* (n=5)	
	Sensitivity	**Resistant**	**Sensitivity**	**Resistant**
SXT (25)	9 (100)	0 (0.0)	5 (100)	0 (0.0)
CHL (30)	9 (100)	0 (0.0)	5 (100)	0 (0.0)
CL (30)	9 (100)	0 (0.0)	5 (100)	0 (0.0)
OFX (5)	9 (100)	0 (0.0)	5 (100)	0(0.0)
GN (10)	9 (100)	0 (0.0)	5 (100)	0 (0.0)
AMP (10)	1 (11.1)	8 (88.9)	5 (100)	0 (0.0)
P (10)	0 (0.0)	9 (100)	0 (00)	9 (100)

<u>KEY</u>

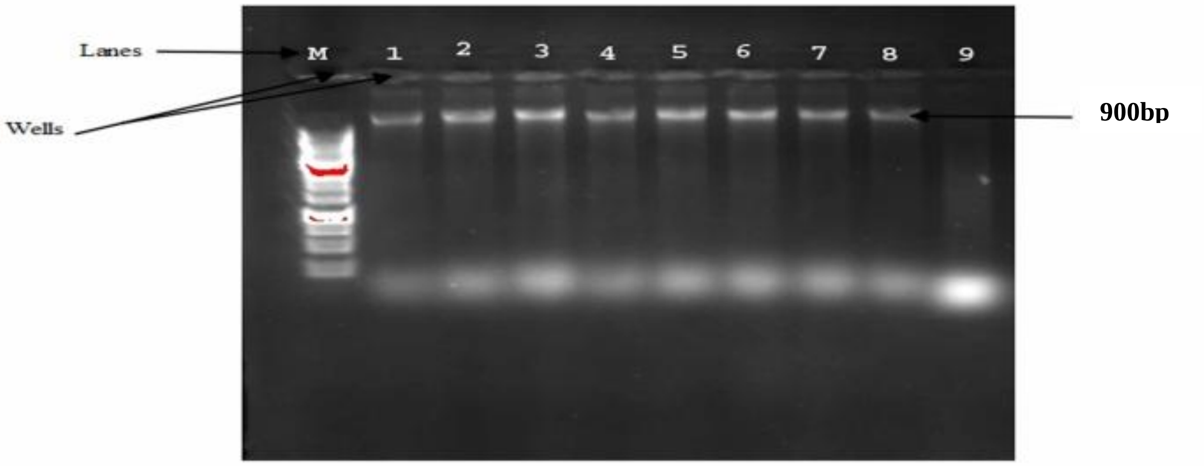
S X T = trimethoprim- sulfamethoxazole, CHL= chloramphenicol, CL= cephalex, OFX = ofloxacin, GN = gentamicin, AMP = ampicillin, P = penicillin G

Table 4.9: ***bla*$_{\mathbf{TEM}}$ resistance genes detected and their amplicon sizes**

Antibiotic resistance	Gene detected	Amplicon size (bp)
Ampicillin	*bla*Tem-1	800
Penicillin G	-	-

PLATE I: PCR amplication of *bla*$_{TEM}$ genes for *Salmonella typhi*serovars.

The PCR products were analyzed by 1.2% agarose gel electrophoresis and stained with ethidium bromide.



Lane M 100 bp ladder, lanes 1-9 pcr products of BlaTEM-1 gene of Salmoella typhi

PLATE I: **PCR amplicons of *bla*$_{\mathbf{TEM}}$ genes of *Salmonella typhi*serovar**

KEY

M = 100 bp ladder

Lane 1-8 = PCR product of bla$_{\mathrm{TEM}}$gene

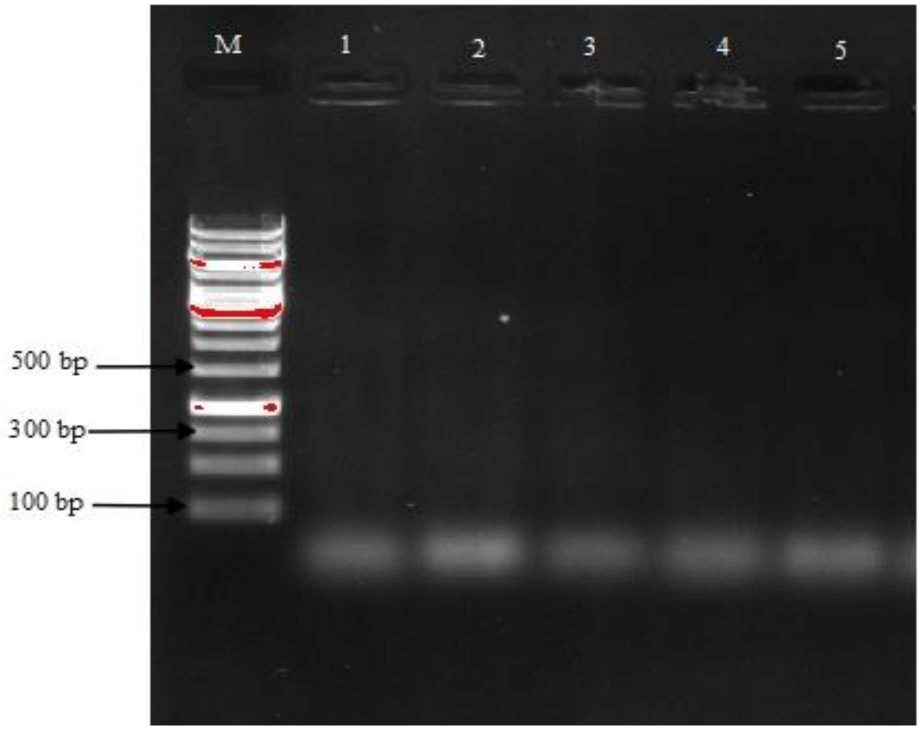


PLATE II: PCR amplicons of *bla*$_{TEM}$genes of *Salmonella paratyphi A*

Key

M = 100 bp marker

Lane 1- 5= negative pcr product of *bla*$_{TEM}$ genes

CHAPTER FIVE

5.0 DISCUSSION

In the study population a higher prevalence was recorded among people visiting General Hospital, Baure(7%) followed in that order by General Hospital Daura(5%) and General Hospital Katsina(2.5%) and the difference is not significant.This higher infection among the people visiting General hospital, Baure may be associate with poor personal hygiene, poor sanitation and illiteracy of the people but people visiting General hospital Katsina was least infected perhaps because they were mostly educated.

Biochemical and serological characterization of *Salmonella* in the study area revealed that only two species were present;*Salmonellaentericaserovar*Typhi and *Salmonella entericaserovar*Paratyphi A.

In our study a higher prevalence was recorded among the age group 1 – 10 years (9.7%) and 11 – 20 years (7.1%) and the lower prevalence of (0.0) was observed in the patients ≥ 40 years. However, the rate of bacteraemia was not statistically significant (P > 0.05). It was observed that the higher prevalence of (9.7%) and (7.1%) of *Salmonella*bacteraemia among the age group 1 -10 and 11 – 20 was probably due to the difference in the immune system between the children and the adults or elderly persons. (Abdullah *et al;* 2014). Similarly, children unlike adults could acquire the disease by drinking contaminated water during swimming in river which might have been contaminated by sewage (Abdullahi*et al;* 2014). Also the higher number of *Salmonella*bacteraemia recorded against age group 1 – 10 and 11 – 20 indicated that *Salmonella* manifests more in children than adults and elderly person.

This trend appears in agreement with the results of similar studies from Saudi Arabia (Kambal*et al;* 1996) and Hong Kong (Lin *et al;* 1991).

On the other hand, the adults ≥ 40 years understand the value of their health because they are mostly educated, wise and matured enough to protect and maintained their health unlike children. In addition, the elders do not usually predispose themselves to risk factors like children, (Abdullah *et al.*,2014). Furthermore, the presence of little or no infection in elders may be associated with the developed immunity which they had over the years (Udeze*et al.*, 2010).

The prevalence of *Salmonella* infection was higher (5.1%) in males than in their female counterpart (4%) although no significant difference exists between the disease and the gender ($P > 0.05$). It was observed that the higher prevalence of *Salmonella* infections in male could be connected with the nature of activities and attitudes such as farming, swimming, poor sanitation, poor personal hygiene, eating and drinking outdoor (Udeze*et al*., 2010).

Moreover, the low community awareness level of males about the disease and route of infection may increase the chance of transmission of the disease since individuals especially children would not care to protect themselves or take other cautions when exposure is inevitable.

The disease is less found in females because females are not fond of eating, drinking or defecating outdoors perhaps, because of the culture and religious teaching. Moreover, females are not usually exposed to the activities that are normally associated with the disease like poor sanitation, poor sewage disposal, swimming, farming, fishing or resting in a dirty environment as their male's counterpart (Udeze*et al.,* 2010).

Our study also indicated that the frequency of infection was higher among business individualswith (6%) followed by farmers (3.4%) and the least prevalence was found in Civil Servants (4.8%). The difference between the infection and the three occupations was not statistically significant ($P > 0.05$). This result is in agreement with the finding of (Iruka*et al*., 2007). Most of the business individuals and farmers were illiterates, and illiteracy is one of the risk factors associated with the disease, most of them interact with many people regardless of their personal hygiene during running their business, they by this mean acquired disease from their unhygienic customers through shaking hands with them or through eating what the so – called regular customer brought to them. However, the infection rate is also common among the farmers because majority of the farmers in the area where this study was conducted were poor and illiterate. They were not able to buy appropriate drugs for the treatment of *Salmonella* infection they therefore rely on orthodox medicines that are readily available, cheaper and sometimes free.

On the other hand, civil servants were least infected probably because majority of them were educated they might have acquired knowledge on what *Salmonella* associated infection is all about through newspaper or internet. Also civil servants tend to practice personal hygiene, disposed sewage and refuse properly hence live in less contaminated environment than the farmers and business individuals, this reduces their chances of being infected by the disease.

This study revealed that *Salmonella* infection prevalence was higher among patients using bore - hole water as their source of drinking water followed in that order by the patients using well water, the patients using tap water and the patients with no response. In addition, the difference was statistically significant ($P < 0.05$). This observation agrees with earlier studies by (Shamsuddeen*et al.* 2010). This higher prevalence among people using bore hole as their source of water could be attributed to the fact that in the study area, borehole water was the main sources of drinking water and most of the residents buy the water from vendors who use carts with multiple Jerry cans, the vendors their carts and Jerry cans are unhygienic they contaminate the water before selling it to the residents (Shamsuddeen*et al.* 2010).

However patients using well water were infected than peopleusing tap water, probably because, the wells in the study area were constructed without cover, and in the area there was no good waste disposal system, therefore, domestic wastes were indiscriminately disposed of in the surrounding, well water is therefore polluted especially during the rainy season when the rain washed debris and litter garbage into the wells used as domestic source of water (Zailani*et al*; 2004).

On the other hand, tap water is pure because it is usually filtered and chlorinated to reduced microbial load, and it is perhaps boiled by the people (who are usually educated) before use.

In our study subjects the rate of *Salmonella* infection was higher (5%) among patients with informal education, followed by those without education (13.6%), primary education (11.1%), secondary education (0.0) and tertiary education (0.0) respectively. The relationship between the infection and the educational status of these groups was found to be statistically non – significant (P > 0.05). This finding agrees with the finding of Udeze, *et al.* (2010). This higher prevalence among people with informal education was probably been due to the low knowledge and misconception especially of *Salmonella* infection. About 80% of them were not aware of the *Salmonella* infections (Abdullahi, *et al.*, 2014).

However, patients having primary, secondary and tertiary education were least infected because they probably had the opportunity to acquire good knowledge on personal hygiene, sanitation, refuse and sewage disposal from their teacher during their school.

The rate of *Salmonella* infection was statistically higher among patients not on treatment (7.8%) than in those that are on treatment (2.3%). The higher rate of *Salmonella* infection among patients not on treatment could be connected with their illiteracy that always hindered them from going to hospital for treatment hence, did not take any drug to stop the multiplication of the bacteria in their blood.

However, the low recovery of *Salmonella* in patients on treatment may be connected with the drug they took coupled with the refusal to withdraw from drug intake at least three days prior to blood culture. These affect the recovery of *Salmonella* in their blood specimens (Arunava, *et al*., 2012).

Most of the patients presented during the study were the patients with either fever or diarrhea. This was in agreement with the study conducted in the area by Abdullahi, *et al.* (2014) and another separate study conducted by Malla, *et al.* (2006) in Kathmandu who reported 100% of fever in patients infected by *Salmonella.* The fever in the study patients could result probably from the immunological response of the body to the bacterial Lipopolysaccharide (LPS) which is an endotoxin capable of producing systemic effect including fever and diarrhea.

The rate of *Salmonella* infection was higher among unimmunized subjects (5.8 %) than immunized subjects (0.0).This finding is in agreement with the result of the research conducted by Arunava, *et al.,* (2012). The higher prevalence among immunized subjects could be associated with the presence of antibodies against *Salmonella* in the sera of immunized subjects.

Antibiotic Susceptibilities of *Salmonella* Isolates

The susceptibilities to selected antibiotics used in the study have been observed. High susceptibility of *Salmonella*serovars was observed to fluoroquinolones (ofloxacin), 100 % of the serovars were susceptible to ofloxacin. This finding is similar to a report by compo, *et al.* (1990) and Abdullahi, *et al.* (2014). The high susceptibility of the serotypes to ofloxacin recorded in this study may be connected with relatively high cost of ofloxacin and other fluoroquinolones and therefore are not used indiscriminately by the people. cephalex however, was one of the most effective cephalosporin as it was seen to give an excellent antimicrobial susceptibility of a 100 %. It is an antibiotic that is quite expensive and not easily affordable and therefore cannot be use randomly.

In this study chloramphenicol showed a very good activity of 100 % against both *Salmonella typhi and S. paratyphiA*. The other former first line drugs such as trimethoprim-sulfamethoxazole also showed an excellent activity (table 8). Re-emergence of susceptibility to chloramphenicol and other former first – line drugs in previously resistant areas have been reported in the studies done earlier (Harish and Menezes, 2011). The decreased use of these former first – line antibiotics in treating *Salmonella* and other infections could be the likely reason for this high susceptibilities of the *Salmonella* to these drugs.

Antibiotic Resistance Pattern

Resistance was encountered with ampicillin and penicillin G at high level. High level of resistance to ampicillin has been well documented in Zaria (Adeshina, *et al.*,2010), in Osogbo, (Olowe, *et al.,* 2007) and in Katsina (Abdullahi*et al.,* 2014) .

Our study also encountered high resistance to penecillin G even though, resistance to penicillin G was not well documented we have attempted to investigate a molecular basis on whether the resistance is due to penicillase or otherwise.Our study further investigated the presence of some antibiotic resistance genes in the *Salmonella* species which showed phenotypic resistance expression using some selected primers in PCR method. The phenotypic resistance observed by disk diffusion method was seen to be genetically confirmed by the presence of Bla_{TEM}-1 genes detected in eight strains of *Salmonella typhi* and is probably responsible for ampicillin resistance observed. The bla_{TEM}-1 genes was 900bp.

In the case of penicillin G the genes responsible for its resistance were not detected. Regarding the strains that showed phenotypic resistance to penicillin G but did not have genes, it is possible this resistance is due to the fact that penicillin G couldn’t penetrate the envelope’s outer membrane or genetic mutation had occurred in penicillin binding protein which can render a cell resistant (Willey *et al., 2008*).

CHAPTER SIX

6.0 Summary

The study revealed the presence of*Salmonella*serovars associated with invasive salmonellosis, determined their prevalence rate, their susceptibility or resistance to some selected antibiotics and attempted the detection the presence of antibiotic resistance genes. There was a higher prevalence rate of *Salmonella* (67.3%) over *Salmonella paratyphiA* (35. 7%).The genes conferring resistance to ampicillin have been detected in the blood samples of the study subjects, but genes conferring resistance to penicillin G were not detected. *Salmonella* infection was observed to be common among; the age – group 1-10 years, male, business people, individuals using bore-hole as a source of drinking water, those with informal education, as well as who have not been immunized. Susceptibility was extremely higher to chloramphenicol, cephalexofloxacin and gentamicin while ampicillin and penicillin G suffered extreme resistance by both serovars.

6.1 Conclusion

In the study area *Salmonella typhi*and *Salmonella paratyphi A* were isolated and characterized indicating that they are the serovars associated with invasive salmonellosis in the study area .The prevalence of *Salmonella*serovars is not very high in the study area but it cannot be overlooked because only the people who visited the selected hospitals were used for the research, therefore, the rate recorded may not represent the true picture of *Salmonella* infection in the area. The re-emergence of susceptibility of *Salmonella*serovars to chloramphenicol and septrin observed in this study indicates that septrin and chloramphenicol could still be the drug of choice for treating salmonellosis. The detection of bla_{TEM} resistance genes implies that these genes are responsible for the phenotypic resistance observed during our sensitivity test and confirmed the presence of *Salmonella* serovars in the study area.

6.2 Recommendation

1. Conventional antibiotics (septrin and chloramphenicol) should be re-considered for treating enteric fever.
2. Further research especially molecular research on resistance mechanism of *Salmonella* species against penicillin G need to be conducted.
3. Potable pipe - borne water, education, Immunization should be provided to the people living in the areas
4. General hygiene and environmental sanitation must be practiced as they play a key role in the reducing the spread of disease.
5. Authorities should come to the aid of the people by providing modern bathroom toiletfacilities and bore holes to prevent frequent human contact with the infections.

6. Further research work need to be conducted in the entire area to ascertain the true prevalence rate.

REFERENCES

Abdullahi, B. Abdulfata K.Wartu, J.R., Mzungu, I., Muhammad H.I.D. and Abdulsalam, A.O. (2014) Antibiotics susceptibility pattern and characterization of clinical *Salmonella* serotypes in Katsina State. African *Journal of Microbiology research 8:915 – 921.*

Abdullahi, M. (2010) Incidence and antimicrobial susceptibility pattern of *Salmonella* species in children attending some Hospital in Kano State Nigeria.*Bayero Journal of Pure and Applied Science* 3(1);200-205.

Adkins H.J., and Santiago L.T.(2006). Increased Recovery of Enteric Pathogens by use of blood , stool and rectal swab specimens. *Journal of Clinical Microbiology* 25;58-59.

Alix E, and Blanc- Potard A.B. (2007) MgtC; A key player in intramacrophages survivals. Trends Microbiology 15;252-718

Acharya, V.I., Lowe, C.U. Thapa R, Gurubocharya, V.L., Shreshtha, M.B., Codoz,M.,Schulz D., Armand J., Bryla, DA, Trollfors, B, Cramtom T, Schgneerson, R, and Robbins JB, (1990) Prevention of typhoid fever in Nepal with the vi capsular polysaccharide of *Salmonella*typhi A, preliminary report. *New England Journal of Medicine 317:1101-4.*

Adeshina, G.O., Osuagwu, N.O.,Ebere, C.L., and Ehinmindu J. O. (2010) Plasmid DNA analysis and conjugative study of antibiotics resistance *Salmonella typhi*and *paratyphi*to ten selected antibiotics in Zaria-Nigeria. *Africa Journal of Pharmacyand pharmacology* 4(4);151-157.

Aljurayya, A.M., Al-Rasheede, N.A.M, Mohammad, N., Al-Nasser, M.M,Mugarin, A.T and Abdullahi, S.A (2004) Childhood bacterial Diarrhoae in regional Hospital in Saudi Arabia.*Journal of Tropical Medicine Hygiene 97:87-90.*

Arnold, K., Hong, C.S., andNelwar, R., (1993) Randomized comparative study of Fleroxacin and chloramphenicol in typhoid fever. *The American Journal of Medicine 94:195 – 200.*

Arunava, D.S.,SeenivasanSreehari, Umochandranshalini, A., Ganeshkumar and Mugudeshwaran, K. (2012) Molecular characterization of *SalmonellaentericaserovarTyphi* Isolated from TyphoidalHumans.*Malaysian Journal of Microbiology 8:148 – 155.*

Black, RE., Levine, M.M., Ferreccid, C., Clement, M..L, Lanata, C., Rooney, J., Germanier, R.,(1990) Efficacy of one or two doses of Ty 21 of *Salmonella typhi* Vaccine in enteric coated capsules in a controlled filed trial vaccine 8;81 – 84.

Brown, JC, Shanahan PM, Jesudason MU, Thomson CJ, and Aymes (1999) Mutations responsible for reduced susceptibility of 4-9 Quinolones Clinical Isolated of Multi-Resistant *Salmonella* typhiin india. *The Journal of Antimicrobial Chemotherapy 36;891 – 900.*

Cheesborough, M. (2002) District Practice in Tropical Countries part II. Low price edition. Cambridge University PressPP.182—18

Chinh NT, Parry CM, LY, NT, (2000) A randomized controlled comparison of azithromycin and of loxacin for multidrug – resistant and nalidixic acid resistant enteric fever.*Antimicrobial Agents agenst and chemotherapy* 44;185.

Chuanchuen, R., Ajariyakhajorn. K., Koowatananukal, C., (2010) antimicrobial resistance and virulent genes in *Salmonella enterica* isolates from dairy cows. Food borne pathogens 7: 63 – 69.

Compos LC, Reis EM, Reberio, Dias JC (1990) in vitro susceptibility of *Salmonella* to ciprofloxacin and perfloxacin compared with three other Antibiotics Chemotherapy 36 (I);29-32.

Cooke, EM, and Gibson, G.L. (1990) Generalized Bacterial infection. Essential Clinical microbiology 10;137-153.

Cooke, F, and Wain J, (2004) The emergence of antibiotic resistance in typhoid fever *Trav. Med. Infect. Dis*. 2: 67 – 74.

Crump, JA, Luby SP, and Mintz ED, (2004) The global burden of typhoid fever. Bulletin World Health organization 82(5); 346.

Clegg A, Passey M., Omena M.K. Karigifa K., andSueve N. (1994) Re-evaluation of the Widal agglutination test in response to the changing pattern of typhoid fever in the highlands of Papua New Guinea. Acta Tropical 57(4);255-63

Clinical and Laboratory Standards Institute (2010) *Performance Standards for Antimicrobial Susceptibility Testing; Twentieth Informational Supplement (June 2010 update).*

Dutta, P, Rasaily, R, andSaha, MR, (1993) Ciprofloxacin for treatment of sereve typhoid fever in children *Antimicrobial Agents and Chemotherapy* 37;1197-9.

FDA/CFSAN (2009) Food safety A-Z Guide *Salmonella*.FDA-centre for food safety and Applied Nutrition, Retrieved 2009-02-14 (dead link).

Ferreccio, C, Levine, MM, Manterola A, Rodrigurz G. Rivara I, (1990) Efficacy of Ciprofloxacin in the treatment of typhoid carries. *The Journal of infectious Diseases* 157; 1235-9.

Fluit, AC, (2005) Towards more virulent and antibiotics resistance *Salmonella*? FEMS *Immunol Med. Microbial*, 43: 1 – 11.

Frenck, RW, Nakhla I, and Sultan Y, (2000) Azithromycin versus ceftriazone for the treatment of uncomplicated typhoid fever in children the *Journal of infectious Diseases 31'1134-8.*

Gebreyes, WA, Thakur S, Dorr P, (2009) Occurrence of spvA virulence genes and clinical significance for multi – drugs resistance *Salmonella*serovars 47: 777 – 780.

Girgis NI,Sutan Y, Hammad O, FaridZ;(1995) Comparism of the efficacy safety and cost of cefixime, ceftriaxone and aztreonam in the Treatment of multidrug – resistant *Salmonella typhi*septicaemia in children. *The padiatric infectious Diseases Journal 14;603-5.*

Gutuzzo, E, Frisancho O, Sanchez J, Liendo G, Carillo C, Black RE, Marris JG, (1991) Association between the acquired Immunodeficiency syndrome and infection with *Salmonella typhi* or *Salmonella paratyphi* in an endemic typhoid area. *Achives of Internal Medicine* 151;381-2.

Graham S.M., (2009). Non typhoidal*Salmonella* a management challenge for children with acquired invasive disease in Tropical African countries. Lancet, 372;267-269

Harish B.N., andMenezes, G.A, (2011) Antimicrobial resistance in typhoidal*Salmonella*.*Indian Journal of Medical Microbiology* 29;223-9.

Hatta, M., Goris, MGA, Hearkens, GC, (2002) Simple dipstick assay for the detection of *Salmonellatyphi* specific immunoglobulins response in typhoid fever. *The American Journal of Tropical Medicine and Hygiene* 66;416-21.

Hohmann EL, Oletta CA, Killeen KP, (1996) Phop/pho Q deleted *Salmonella*typhi (Ty 800) is a safe and immunogenic single dose typhoid fever Vaccine in Volunteers. *The Journal of Infectious Diseases* 173;1408-14.

Hur, J, Kim, JH, Park JH (2011) Molecular and virulence characteristics of multi – drugs resistance *Salmonella enteritidis* strains isolated from poultry . *Journal of Veterinary Medicine* 4;189: 306 – 311.

Ho D.K., Jarva H., and Meri S., (2010) Complement factor H factor binds to outer membrane protein Rck of *Salmonella* . Immunology 185;1763-1769.

Islam A, Butter T, Kabir I, andAlam, N.H., (1993) Treatment of typhoid fever with ceftrixone for 5 days or chlorampenicol for 14 days a randomized controlled trial *Antimicrobial agents and chemotherapy* 37;157-2.

Iruka N., Okeke O., Abodarin A., Denis K., Byarugaba K.K., Ojo S., and Japheth A., (2007).

Growing Problem of Multidrug-resistant Enteric Pathogens in Africa. Emerging

Emerging Infectious Diseases.www.cdc.govleid, 13(11) ; 1641-1644

Ismail A., Kadar S.A., OngKk.h., (1991). Dot Enzyme Immunosorbent Assay for the

Serodiagnosisof Typhoid Fever. *The South East Asian Journal of Tropical Medicine and Public Health 22 (4) ;563-6*

Klugman, K, Hendrik J. and Le and N. (1996) Immunogenicity efficacy and Serological correlate of protection of *Salmonella typhi* vi capsular polysaccharide Vaccine three years after immunization Vaccine 14;435-8.

Kossaka Z, lin F, HO, V, Thuy N, (1999) Sacety and immunogenicity of Vi,, conjugate Vaccines for typoid fever in adults teenagers and 2-4 years old children in viet Nam. *infection and immunity* 67;5806.

Koul, P.A., Wani J.I. and Wahid A, (1995) Ciprofloxacin for multi resistant enteric fever in pregnancy*lancet* 346;648.

Lin F.Y., HO, VA and Khiem H.B., (2001) the efficacy of *Salmonella* typhiVi, conjugate Vaccine in two to five years old children. *New England Journal of Medicine 344;1263-9.*

Malla, S, Kansaakar, P. and Serichantalery, (2005) *Journal of Nepal Medical Association 44;18-22.*

Mandal, S, Deb, M.M, and Pol NK (2004) Plasmid encoding multi – drugs resistance of *Salmonella typhi* and some enteric bacteria in and around kalkata, India. *A preliminary study J. Health Allied Science* 3: 4.

Mirza, S., Kariuku, S. Mamun K.Z., Beeching N.G. and Hart C.A. (2000) Analysis of plasmid and chromosomal DNA of multi-drugs resistance *Salmonella enterica*serovarTyphi from Asia .*J. Clin. Microbiol*. 38;449-1452.

Maria T., Yolanda S., Emilia C., Reatiz R., Marta G., Esther U., Carmen T., (2011). Genetic Characterization of the Mechanisms of Resistance to Amoxicillin / clavulanate and third- generation Cephalosporins in *Salmonella enterica*from three Spanish Hospitals. Research article; *International Microbiology* 14;173-181

Olowe, O.A.Okanlawon, B.M., Olowe, R.A, AdedosuO.T.andOlayemiA.B. (2007). Multi-drugs resistance pattern of *Salmonella*typhimurium infections in Osugbo, South Western Nigeria.*Journal of American Science* 3 (4).

OyongoD.Ghebremedhim, B. and Waindi N.E. (2009) Phenotypic and genotypicanalysisclinical isolates*Salmonella* serovarsTyphimurium in Western Kenya: 3 (9): 685-694.

Phoung C.X.I., Kneen R., Anh, NT, andLuat T.D. (1999) A comparative study of loxalin and cefexime for treatement of typhoid fever. *The pediatric infections Disease Journal 18;245-8.*

Popoff, MY, and Le Minor L, (2001) Antigenic formulas of *Salmonella*Serovars seven edition Paris; Pasteur institute.

Porwallic S, Frye J, Florea LD, andBlackmer F, (2003) A non redundant microarray of genes for two related bacteria Nucleic acid 31;1889.

Punjabi NH, Hoffman SL,and Edman DC, (1997) Treatment of severe typhoid fever in children with high dose dexamethosone. *The pediatric infectious Disease Journal 7;598-600.*

Redmond, EC and Griffith, C.J (2003) Consumer Food handling in the home .A result of food safety studies. J. food project 2003. 130-61

Seoud, M, Seade, G, Uwaydah M, and Azoury R. (1998) Typhoid fever in pregnancy obstetrics and Gynecology 346;307-8.\

Shamsuddeeni, U. Muktar M.D., Bukar A., Usman A.D., Kabir M.H., and Abdulmalik, S.A. (2010) Bacteriological quality of water used for ice making in some part of Kano Metropolis, Nigeria. *Bayero Journal of pure and Applied science 3(1);199-201.*

Smith, T., (2001) The cholera group of bacteria U.S. Bur Animal and Bull 6;6-40.

Tacket, C.O.,Sztein, MB,Losonky GA, Leving MM, (2000) Safety and immune response in humans oral *Salmonella typhi* Vaccine strains deleted intr A and aro C, aro D, infection and immunity 65;452-6.

Thisyakorn, U. andMansuwan, P. (1999) Comparative efficacy of mecullinam/Amoxycilin and trimethoprim-sulfomethoxazole for treatment of typhoid fever in children.*The pediatric infections Journal* 11;979-80.

Turkyilmaz S., Hazimoglu S., and Bozdogan B., (2009) *Antimicrobial Susceptibility and Resistance Genes in Salmonella EntericaserovarEnteritidis Isolated from Turkeys. Israel Journal of Veterinary Medicines 64(3);175-200.*

Udeze, A.O., Abdulrahaman, I.O. Okonko and Anibijiwon I,I (2010) Sero prevalence of *Salmonella* typhiand *Salmonella* paratyphi among the first year students of university of Ilorin Nigeria. *Middle East Journal of scientific research 6(3);257-262.*

Van,Asten A.J. and Van Dijk, J.E. (2005) distribution of classic virulent factor among *Salmonella* species. FEMS immunol Med. Microbial 44: 251 – 259.

Wallis, T.S and.Galyov, E.E. (2000) monocular basis of *Salmonella* induced enteritis. Mol microbial. 36: 997 – 1005.

World Health Organization (WHO) (2010) Laboratory protocol; susceptibility testing of Enterobacteriaceae using disk diffusion WHO Global Food borne infection Network.

World Health Organization (WHO) (2004) *Manual for Laboratory Investigations of Acute Enteric Infectious.Worid Health Organization, CDO/83*

World Health Organization (WHO) (2003). Background document; The diagnosis, treatment and prevention of typhoid fever 1- 31.

Willey, M, Linda S. and Christopher J. (2008) Prescott, Harley and Kleins Microbiology Seventh edition MC, Graw-Hill; 34;835-870.

Zailani S.B., Abdul A.A., and Abdullahi B., (2004) Effect of socioeconomic status, age and sex antibody titre profile to *Salmonella typhi* and *Salmonella paratyphi* in Ile-ife.

APPENDICES

APPENDIX I

MINISTRY OF HEALTH
KATSINA STATE

Tel" Hon. Commissioner 065-434537 (DL)
434518(DL)

Permanent Secretary 065-34554

State Secretariat Complex,
I. B. B. Way Dandagoro
P.M.B. 2075, Katsina

Our Ref: MOH/ADM/SUB/1167/2/67

Date: 18/08/2014

Your Ref:

KATSINA HREC FULL ETHICAL CLEARANCE CERTIFICATE

Re: **"MOLECULAR CHARACTERIZATION OF ANTIBIOTICS RESISTANCE GENES IN *SALMONELLA* SEROVARS ASSOCIATED WITH INVASIVE SALMONELLOSIS IN SELECTED HOSPITALS IN KATSINA STATE"**

Katsina HREC assigned number -MOH/ADM/SUB/1167/II/67
Name of Principal Investigator - Kamilu Isah
Address of Principal Investigator -Department of Microbiology, ABU Zaria
Date of Receipt of valid Application -18/08/2014
Date of ERC meeting and approval -18/08/2015

This is to inform you that the research described in the submitted protocol, the consent forms, and other participants information materials have been reviewed and given full approval by the Katsina State Health Research Ethics Committee and accordingly by the Honorable Commissioner of Health.
Please note; this approval dates from 18th August 2014 to 18th August 2015
No participant recruited into this research may be conducted outside these dates.

All informed consent forms in this study must carry the Katsina HREC assigned number and the duration of the Katsina HREC approval for the study.

The HREC expects that you submit your application as well as an annual report for ethical clearance renewal 3 months prior to the expiration of the study dates. This is to enable you obtain renewal of your approval and avoid interruption of your research.

If there is a delay in starting the research, please inform the Katsina HREC so that starting dates can be adjusted accordingly.

No changes are permitted to the research without prior approval by Katsina HREC except in circumstances outlined in the national code for health research ethics. http://www.nhrec.net

Katsina HREC reserves the right to conduct compliance assessment to your research site without prior notification.

Dr Abduljalil U Abdullahi
Director Public Health
Chairman, Katsina HREC

"Home of Heritage and Hospitality"