ISOLATION AND MOLECULAR DETECTION OF ANTIBIOTIC RESISTANT SALMONELLA SPECIES FROM SOME POULTRY FARMS IN SOUTHERN PART OF KATSINA STATE, NIGERIA

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JUNE, 2017

DECLARATION

I declare that the work in this dissertation titled **"Isolation and Molecular Detection of Antibiotic Resistant *Salmonella* Species from Some Poultry Farms in Southern Part of Katsina State, Nigeria"** was carried out by me in the Department of Microbiology, under the supervision of Prof. O.S. Olonitola and Prof. (Mrs.) M. Aminu- Mukhtar.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

Bashir, Muideen	Signature	Date

CERTIFICATION

This dissertation, entitled "**Isolation and Molecular Detection of Antibiotic Resistant *Salmonella* Species from Some Poultry Farms in Southern Part of Katsina State, Nigeria**", by MuideenBASHIR meets the regulations governing the award of Master of Science Degree of the Ahmadu Bello University, Zaria, and is approved for its contribution to knowledge and literary presentation.

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LIST OF ABBREVIATION

NLF: Non lactose fermenting

PCR: Polymerase chain reaction

XLD: Xylose lysine deoxycholate

TSI: Triple sugar iron agar

K: Alkaline

A: Acid

G: Gas

H_2S: Hydrogen sulphide

MR: Methyl Red

VP: Vogesproskauer

GRM: Gram reaction and morphology

U: Urease

C: Citrate

I: Indole

DJ: Danja poultry farm

S_2: Sabuwa poultry farm 2

B1: Bakori poultry 1

S_1: Sabuwa poultry farm 1

FD: Faecal dropping

C: Cloacal swab

LYS: Lysine

ORN: Ornithine

GLU: Glutammine

MANN: Mannose

XYL: Xylose

IND: Indole

ID: Identification

WHO: World Health Organization

EFSA: European Food Security Agency

χ^2: Chi- square

DF: Degree of freedom

P- VALUE: Probability value

%: Percentage

TET A: Tetracycline resistance gene

SUL1: Sulphonamide resistance gene

BLA-CMY-2: Beta lactamase resistance gene

DHFR: Dihydrofolatereductase

CAT1: Chloramphenicol acetyl tranferases

<: Less than

OR: Odds ratio

CI: Confidence interval

CDC: Centre for disease control

DHPS: Dihydropteratesynthetase

NTS: Non Typhi*Salmonella*

HIV: Human immune virus

RDNA: Ribosomal deoxyribonucleic acid

RRNA: Ribosomal ribonucleic acid

NIAID: National institute of allergy and infectious disease

OIE: International office of epizootics

CIDRAP: Centre for infectious disease research and policy

µm: Micrometre

ABSTRACT

Meat and poultry products are major vehicles for transmitting *Salmonella* species to human. The study was aimedat isolating and molecularly determining the antibiotic resistance genes of *Salmonella* in poultry farms in Southern part of Katsina State Nigeria. A purposive sampling technique was employed in the selection of the study area. Three hundred and twenty samples were collected from broiler birds using simple random sampling consisting each of one hundred and sixty cloacal swabs and faecal droppings from four Local Government Areas in southern part of Katsina state, Nigeria. Samples were pre- enriched in buffered peptone water and selectively enriched in selenite cysteine broth. Isolation and identification was done by inoculating the enriched samples onto xylose lysine deoxycholate agar. Confirmation of the presumptive *Salmonella* colonies was carried out using different biochemical test and Microgen GNA- A identification system. Antibiotic susceptibility testing was performed using standard guidelines according to CLSI (2012). Polymerase chain reaction was used to detect the presence of antibiotic resistance genes in the *Salmonella* to tetracycline, beta lactams, sulphonamides, trimethoprim and chloramphenicol.Risks factors related to contamination of the farm and drug resistance to*Salmonella in* the poultry farmswas determined using a structured questionnaire.*Salmonella*Arizonae was detected from the study with a prevalence of 0.94% from the 320 samples. Statistically, significant difference ($p<0.05$) was observed between isolation from the different samples collected and from the different farms. All the isolates were 100% resistant to cefuroxime, amoxicillin- clavulanic acid, ciprofloxacin, chloramphenicol, tetracycline, and sulfomethoxazole-trimethoprim. The *Salmonella*Arizonae possesses resistance genes to tetracycline (66.7%), beta- lactams(100%), sulphonamides (66.7%),trimethroprim (100%), and chloramphenicol (66.7%). Knowledge of salmonellosis (OR13.00; CI 95% 334.50-505.25), leaving dead chicken in the farm (OR1.44; CI 95% 0.04-56.14), sweeping of feed on the ground in the farm (OR3.86; CI 95% 0.12-126.74), contamination of food during transport and storage (OR5.00; CI 95% 0.07-366.35) were found to be associated with*Salmonella* contamination in the poultry farms. Also, addition of antibiotics to the feed (OR0.73; CI 95% 0.02-25.09), faeces in water (OR0.20; CI 95% 0.003-14.65), washing of hands after handling the birds (OR0.20; CI 95% 0.003-14.65), were found to be protective against *Salmonella* contamination. Furthermore, addition of antibiotics to the

feed daily(OR2.60; CI 95% 0.04-170.39), overuse or misuse of drugs (OR13.60; CI 95% 0.02-26.91), sensitivity testing before drug prescription(OR0.26; CI 95% 0.04-170.39), taking of drugs according to prescription (OR13.60; CI 95% 0.02-26.91), practicing ofself-medication(OR2.33; CI 95% 0.67-76.67) and buying of drugs in local shop(OR13.00; CI 95% 334.50-505.25) were found to be associated with drug resistant *Salmonella*Arizonae.The study reveals the presence of multi- drug resistant*Salmonella*Arizonae in the poultry farms in southern part of Katsina State.

CHAPTER ONE

1.0INTRODUCTION

Poultry and poultry meat products are considered some of the main carriers of Salmonella species and represent a significant share of the attributed sources of salmonellosis in humans. The widespread occurrence of *Salmonella* in natural environment and the intensive husbandry practice used in the meat, fish and shellfish industries have been a significant problem in public health (Kaniz*et al* ., 2014).

*Salmonella*infections in humans and animals have been recognized as a major public health problem (Akinyemi*et al.*, 2007). *Salmonella*, a primary inhabitant of the gastrointestinal tract, is recognized as one of the most common cause of food borne infections worldwide, resulting in millions of infections and significant human death annually (Elaine*et al.*, 2011).*Salmonella*species are responsible for an estimated 93.8 million cases of food borne diseases in humans and an average of 155,000 deaths annually worldwide (Elaine *et al.*, 2011).

*Salmonella*is non-lactose fermenting (NLFs), Gram negative, rod shaped organism, ranging from 0.7 to 1.5 and 2 to 5 μm in size. *Salmonella*is oxidase negative, catalase positive, indole and VogesProskauer (VP) negative, methyl red and Simmons citrate positive, H_2S producing and urease negative. Some of these characteristics are used for the biochemical confirmation of *Salmonella*(WHO, 2003; Kaniz*et al.*, 2014).

*Salmonella*belongs to the family *Enterobacteriaceae* (Guthrie, 1991). The genus *Salmonella* contains two species; *S. enterica*and *S. bongori*, which was formerly subspecies V. Six subspecies are differentiated within *S. enterica*based on their biochemical

characteristics and genomic structures, Roman numerals and names are used for the designation of these six subspecies as follows: I, *S. enterica*subsp. *enterica*; II, *S. enterica*subsp. *salamae*; IIIa, *S. enterica*subsp. *arizonae*; IIIb, *S. enterica*subsp. *diarizonae*; IV, *S. enterica* subsp. *houtenae*, and VI, *S. enterica*subsp. *Indica* (Brenner *et al*., 2000). With regard to food safety *S. enterica* subsp. *enterica*is the sub-species of most concern because the strains withinthese serogroups are responsible for 99% of *Salmonella*infections in humans (Brenner *et al.,* 2000; Bell and Kyriakides, 2002). Food borne illnesses are often caused by non-typhoid *Salmonella*species. This group includes over 2,500 serotypes that are found in the gastrointestinal tracts of birds, mammals, reptiles and insects (Molbak*et al.*, 2006).

Infections caused by *Salmonella*serotypes can produce enteric fever, gastroenteritis, and bacteremia or septicaemic conditions (Guthrie, 1991;Monteville and Matthew, 2008). The typhoidal*Salmonella*(*S.typh*i and *Paratyphi*) are responsible for causing enteric fever (Guthrie, 1991). The period of incubation for this infection ranges from 8 to 28 days and the common symptoms include fever, diarrhea, abdominal pain, headache (Monteville and Matthews, 2008). The antibiotics of choice for treatment of enteric fever are chloramphenicol, ampicillin or trimethoprim-sulfamethoxazole (Montevilleand Matthews, 2008). The non-typhoidal Salmonella strains are responsible for causing gastroenteritis, due to the consumption of contaminated food item such as poultry products. Symptoms appear 8 to 72 hours after ingestion, and non-bloody diarrhoea and abdominal pain regress within 5 days. The treatment is based more on fluid and electrolyte replacement than on antibiotic

use. Infections caused by non-typhoid *Salmonella*serotypes can also evolve into systematic infections followed by chronic conditions (Monteville and Matthews, 2008).

However, following the wide use of antibacterial by human by the end of 1940s, reports on the emergence of resistant strain was observed in most bacterial species, and against all drugs available (Magistrali*et al* ., 2008). The indiscriminate use of antimicrobial agents in any environment creates selective pressures that favour the emergence of antibiotic-resistant pathogens. The routine practice of antibiotic use in domestic animals as a means of preventing and treating diseases, as well as promoting growth, is an important factor in the emergence of antibiotic-resistant bacteria that are consequently transferred to humans in the course of the food chain (Tellefson*et al.,* 1997;Wiite, 1998; Akond*et al.,* 2012).In recent times, a significant increase in the occurrence of antimicrobial drug resistance in *Salmonella*strains is of great concern in both developed and developing countries (Threlfall, 2002;Akond et *al.,* 2012;Abdullahi*et al.,* 2014).

The bacterial resistance against antimicrobial agents is known to be driven by the interplay of several mechanical and epidemiologic factors including the chromosomal defects, random mutation, plasmid acquisition, and by the transfer of drug resistance genes by the activities of integron or transposon (Ochiai*et al.,* 1959; Bennet, 2008; Canton, 2008; Hung and Kaufman, 2010). In different parts of the world, multidrug resistant strains of *Salmonella*are ubiquitous in poultry and poultry environments (Alambedji*et al.,* 2006;Okoli*et al.,* 2006). There are reports of high prevalence of antibiotic resistant *Salmonella*isolates in Nigeria (Agada*et al.,* 2014).

Like in many other developing countries, policies on good hygienic practices in handling of raw poultry and poultry products and epidemiological study of antimicrobial resistance are either lacking or are in their infancy in Nigeria. In addition, the lack of rigorous controls on antimicrobial usage, particularly in farm animal production system increases the risk of pathogenic microbes harboring an array of resistance genes. Therefore, this study focuses on the determination of the level of *Salmonella*contamination in the poultry environments and their resistance against different antibiotics in some poultry farms in southern parts of Katsina state, Nigeria.

1.1Statement of the Research Problem

Meat and poultry products are recognized as major vehicles for transmitting *Salmonella*species to human with 40 % of the clinical cases attributable to the consumption of egg and other poultry products (Chashni*et al* ., 2009;Ruban*et al* ., 2010).

The worldwide annual estimation of the incidence of non- typhoidal salmonellosis is 1.3 billion and annual death is estimated to be three million cases (Kariuki*et al.*, 2006; Hussein *et al.*, 2010). In Africa, it is estimated that 2.5 million cases of non-typhoidal gastroenteritis and 4100 deaths are recorded per year (Majowicz*et al*., 2010). In Nigeria, the prevalence rate of salmonellosis due to consumption of contaminated poultry product is 18.8%, the infection accounts for an estimated 155,000 death annually (Ifeanyi*et al*., 2014). *Salmonella* species account for 24% and 59.8% of bacteremia in North western Nigeria (Obaro*et al*., 2015).Abdullahi*et al*. (2014) reported a prevalence of 43.5% of non- typhoidal salmonellosis in Katsina State.

*Salmonella*is acommon cause of morbidity and mortality in poultry birds (Baumler*et al.,* 1997;Prakash*et al.,* 2005) and a potential cause of such human infectious diseases as salmonellosis, enteric fever and bacteremia (Hussein *et al.,* 2010;Akbermehr, 2014).

Salmonella species have been found to resist most of the commonly used antibiotics probably due to horizontal acquisition of resistance genes from such organism as enterococci and *Escherichia coli* (Rianatou*et al.,* 2006).

Similarly, wide usage of antibiotics in the diet of chicken has led to the emergence of drug-resistant bacteria which could be transferred to humans (Rianatou*et al*., 2006).

1.2 Justification of the Study

Most cases of salmonellosis in humans are the consequence of consuming contaminated poultry products (Akond et *al.,* 2012). Contaminated poultry products are among the most important sources for food-borne infection or outbreak than from any other animal or food products (Braden, 2006;Linam and Gerber 2007;Kabir, 2010; Habtamu*etal*., 2011).

Prompt and accurate detection of *Salmonella*spp.in contaminated food provides an opportunity to prevent the contaminated food from entering the food chain (WHO, 2010).For this reason, sensitive and specific laboratory methods for the isolation, identification and serotyping of *Salmonella* are essential elements for monitoring and control programmes (WHO, 2010).

Similarly, identification of*Salmonella* serotypes provides information about the severity of the disease, the source of contamination and the resistance patterns (Molbak*et al.*,

2006).Because of its ubiquitous characteristics and zoonotic nature, *Salmonella*can be used as a good indicator organism for surveillance studies on microbial resistance to antibiotics. The investigation of antibiotics susceptibility in *Salmonella* is essential for the proper understanding of the epidemiology of emerging multi-drug resistance in *Salmonella*serovers (Usera*et al.,* 2002).Since most antibiotics are expensive there is therefore a need to find a suitable antibiotic for treating *Salmonella* infection in poultry (Kohinur*et al.*, 2010).

1.3Aim

The aim of this research was to isolate and use molecular techniques to detectantibioticresistant*Salmonella* species isolated from birds in some poultry farms in southern parts of Katsina state, Nigeria.

1.4Objectives of the Study

The objectives of this study were to:

i. Isolate and identify*Salmonella* species from poultry droppings and cloacal swab using cultural and biochemical methods.
ii. Determine the susceptibility patterns of the *salmonella* isolates to selected antibiotics.
iii. Detect some antibiotic resistance genes in *Salmonella*isolates using polymerase chain reaction.
iv. Determine risk factors for *Salmonella* colonization of the farms andits drug resistance using a structured questionnaire.

1.5 Research Questions

The study hopes to provide answers to the stated research questions.

i. What is the prevalence of *Salmonella* in the poultry farms studied?

ii. What is the susceptibility pattern of the *Salmonella* to the selected antibiotics?

iii. What types of antibiotic resistance genes are responsible for resistance to the antibiotics in the *Salmonella*?

iv. What are the risk factors associated with *Salmonella* infection in the farms and its drug resistance in the poultry farms?

CHAPTER TWO

2.0 LITERATUREREVIEW

2.1 *Salmonella*

Salmonella is usually spread by the faecal-oral route. *Salmonella* is commonly found in the gastrointestinal tracts of animals, and has been commonly associated with foods such as raw meat, poultry, eggs, and dairy products (Akpabio, 2015). Poultry and other poultry products are believed to be the main vehicle of transmission of *Salmonella* (Texas Dept. of Health, 1998; EFSA, 2013; Kaniz*et al*., 2014). The commercial poultry industry has a common turnover rate of forty two days in a broiler setting which can lead to the contamination of sites such as litter, air, or feed (Humphrey, 2000). Birds are also raised in close proximity with other birds, which increases the likelihood of horizontal transfer. *Salmonella* is recognized as one of the most common cause of food borne infection worldwide, resulting in millions of infections and significant human death annually (Elaine *et al*., 2011). *Salmonella* species are responsible for an estimated 93.8 million cases of food borne disease in humans and an average of 155,000 deaths annually worldwide (Elaine *et al*.,2011).

2.2Taxonomy of *Salmonella*

Scientifically *Salmonella* classification is described under Domain: Bacteria, Phylum: Protobacteria, Class: GammaProtobacteria, Order: Enterobacteriales, Family: Enterobacteriaceae, Genus *Salmonella* and Species: *Salmonella enterica*and *Salmonella bongori*(Hafez, 2005).

Sixsubspecies are differentiated within *S. enterica*based on their biochemical characteristics and genomic structure, a Roman numeral and a name are used for the designation of these six subspecies as follows: I, *S. enterica*subsp. *enterica*; II, *S. enterica*subsp. *salamae*; IIIa,*S. enterica*subsp. *arizonae*; IIIb, *S. enterica*subsp. *diarizonae*; IV, *S. enterica*subsp. *houtenae*, and VI, *S. enterica*subsp. *indica* (Brenner *etal*.,2000).The 2 *Salmonella* species, *S. enterica*and *Salmonella bongori*, were further separated by 16S rDNA sequence analysis and found to be closely related to the *Escherichia coli* and *Shigella*complex by both 16S and 23S rDNA analyses (Papoff and Le minor, 2005).16S rRNA sequence analysis indicates that *Salmonella* belong to the Gammaproteobacteria (Papoff and Le minor, 2005).

By newer convention, names are retained only for subspecies *enterica*serovars, and these names are no longer italicized. The first letter is a capital letter "*S*" followed by the serovar names of subspecies *enterica*(e.g. Typhimurium or Montevideo). At the first citation of the serotype the genus name is given followed by the word "serotype" or the abbreviation "ser," followed by the serotype name. The antigenic formular are also used to name *Salmonella* serotypes. This designation includes: (i) subspecies designation (subspecies I through VI), (ii) O (somatic) antigens followed by a colon, (iii) H (flagellar) antigens (phase 1) followed by a colon, and (iv) H antigens (phase 2, if present) i.e. *Salmonella* serotype IV 45: g, z51:- (Brenner *et al.*, 2000). With regard to food safety *S. enterica*subsp. *enterica*is the subspecies of most concern because the strains withinthese serogroups are known to cause 99% of *Salmonella* infections in humans (Brenner*et al.*, 2000; Bell and Kyriakides, 2002).

2.3Features of *Salmonella*

Salmonella are straight rods usually motile with peritrichous flagella , facultative anaerobe, ferment glucose usually with production of gas (Except *S.* Typhiand*S.*Dublin), do not ferment lactose, sucrose, salicin and urea, reduce nitrate to nitrite and most are phototropic (Johnson *etal*., 2007). *Salmonella* multiply optimally at a temperature of 35 to 37°C, pH of 6.5-7.5 and water activity between 0.84-0.94. Salmonellae do not grow well at low temperatures (Sorqvist, 2003). However, salmonellae are hardy and not always killed by freezing (Cosby *etal*., 2015). Most salmonellae survive well in acidic foods (pH $\leq$ 4.6, (FDA, 2011) and resist dehydration. *Salmonella* grows readily on blood agar, MacConkey agar, Bismuth Sulphite agar or Deoxycholate agar. They are chemo-organotrophic, obtaining their energy from oxidation and reduction reactions using organic sources.

Most *Salmonella* species produce H_2S, which can readily be detected by growing them on media containing ferrous sulphate such as triple sugar iron (TSI) where the *Salmonella* is able to produce H_2S from thiosulphate. The bacteria are sensitive to heat and will not survive at temperature above 70°C so it is sensitive to pasteurization, but resistant to drying even for years especially in dried faeces, dust and other materials such as feed and certain food (Radostitis*et al*., 2007). The usual habitat of different *Salmonella* species and subspecies is the intestines of both cold and warm blooded animals. The bacteria can also be found throughout the natural environment. Sources of the organism include water, soil, factory surfaces, kitchen surfaces, animal faeces, raw meats, raw poultry and raw sea foods. *Salmonella* can live for number of weeks in water and some years in soil if there are favorable conditions such as temperature, pH and humidity (Grimont and Weill, 2007).The

Diagnostic Services of Monitoba (DSM) suggested the number of *Salmonella* serotypes that cause diseases in human and animals are now approaching 3000 and new serotypes are being identified continuously (DSM, 2009).

2.4 InfectionsDue to*Salmonella*

The ability of *Salmonella* species to cause human infection involves attachment and colonization of intestinal columnar epithelial cells and specializedmicrofoldcell overlying the Peyer's patches (Ponka*et al*., 1995). Symptoms of salmonellosis include diarrhoea, abdominal pain, nausea, and vomiting lasting 1 to 7 days. The illness is generally self-limiting in healthy adults with a mortality rate of <1% (Andrews *et al*.,2001). In severe cases, infection may progress to septiceamia and death, unless the person is promptly treated with the appropriate antimicrobials. Presently fluoroquinolones, macrolides, and third-generation cephalosporins are the drug of choice (Klotchko and Wallace, 2011; Tessari*etal*., 2011). Individuals who are immuno-compromised, children, infants, and theelderly are most likely to require antimicrobial treatment. Infections with antimicrobial-resistant strains may compromise treatment outcomes thus resulting in increased morbidity and mortality (WHO, 2011a). In rare instances, some individuals can develop chronic conditions including reactive arthritis, Reiter's syndrome, and ankylosing spondylitis (NIAID, 2011). The infective dose for salmonellosis in adult humans is estimated to be in the range of 10^4 to 10^6 cells or higher, but can be as low as 10^1 to 10^2 cells in highly susceptible individuals. Symptoms observed may be different due to variation in the dose ofinoculation, mechanisms ofpathogenicity, virulence factors, age and immune response of the host (Andino and Hanning, 2015). Other than gastroenteritis, *Salmonella* may also

cause extra intestinal infection like meningitis, osteomyelitis, pneumonia, colecystitis, peritonitis, pyelonephritis, endocarditis, pericarditis, vasculitis and chronicconditions like aseptic arthritis and Reiter's syndrome (Andino and Hanning, 2015).

2.5 Pathogenesis of *Salmonella*Species

Almost all strains of *Salmonella* are pathogenic as they have the ability to invade, replicate and survive in human host cells, resulting in potentially fatal disease. *Salmonella* displays a remarkable characteristic during its invasion of non-phagocytic human host cells (Hansen-Weste*et al*., 2002).Whereby, it actually induces its own phagocytosis in order to gain access to the host cell. When the bacteria enter the digestive tract *via* contaminated water or food, they tend to penetrate the epithelial cells lining the intestinal wall. *Salmonella* pathogenicity islands (SPIs) encode for type III secretion systems, multi-channel proteins that allow *Salmonella* to inject its effectors across the intestinal epithelial cell membrane into the cytoplasm (Takaya *et al*., 2003).

Following the engulfment of *Salmonella* into the host cell, the bacterium is encased in a membrane compartment called a vacuole, which is composed of the host cell membrane. Under normal circumstances, the presence of the bacterial foreign body would activate the host cell immune response, resulting in the fusion of the lysosomes and the secretion of digesting enzymes to degrade the intracellular bacteria. However, *Salmonella* uses the type III secretion system to inject other effector proteins into the vacuole, causing the alteration of the compartment structure. The remodelled vacuole blocks the fusion of the lysosomes and this permits the intracellular survival and replication of the bacteria within the host

cells. The capability of the bacteria to survive within macrophages allows them to be carried in the reticuloendothelial system (RES) (Monack*et al*., 2004).

Salmonella causes a wide range of human diseases such as enteric fever, gastroenteritis and bacteremia (Monteville and Mathew, 2008).

2.5.1Salmonellosis

Salmonellosis is a disease caused by members of the genus *Salmonella* (Nietfeld and Kennedy, 1999). It is an important cause of diarrheal illness in humans, responsible for approximately 1.4 million illnesses and 600 deaths annually in the United States (Brenner *et al.*, 2000). Much of what is known about the epidemiology of salmonellosis came from outbreak investigations. These investigations have determined that most human infections result from the ingestion of foods of animal origin that are contaminated with *Salmonella* species (Mead *et al.*, 1999). *Salmonella* of animal origin causes an intestinal infection, characterized by sudden onset of fever, myalgia, cephalagia, abdominal cramps, nausea and vomiting. There is increased risk of exposure among those who work in abattoirs, poultry processing plants and those in contact with animals and their products.

2.5.2Enteric Fever

The infection is caused by *S.*Typhi and it occurs most frequently in children and young adults (3-19) years. Symptoms of enteric fever include persistent high fever with low pulse rate, severe headache, enlargement of the spleen, nausea, and apathy or mental confusion. The organisms multiply in reticulo endothelial cells. Invasion of the intestine causes inflammation and perforation, epistaxis, intestinal hemorrhages,toxaemia and renal failure

may occur in untreated late typhoid. A rash on the trunk may be seen on light coloured skin. In uncomplicated typhoid, the total white cell count is normal or low with a relative lymphocytosis. There may be also anaemia. A sudden increase in white cell count may occur with intestinal perforation. Infection with *S*.Typhi can also cause osteomyelitis and typhoid arthritis particularly in those with sickle cell disease and thalassaemia(Cheesbrough, 2006).

2.5.3Enterocolitis

This can be caused by many *Salmonella*serovars. In developing countries, *S*.Typhimurium and *S*.Enteritidisare common causes. Infection is by ingesting Salmonellae in food that has become contaminated from animal or human intestinal sources, directly or indirectly. Common sources of infection are poultry, meat and meat products, eggs and egg product. Diarrhoea, vomiting, fever and abdominal pain occur 12-36 hours after eating contaminated food. In acute infections, blood and mucous are present in faecal specimens. Infants and the elderly, persons in poor health, those with ulcerative colitis, malignancy and immunosuppressed persons are at greater risk of developing serious disease (Cheesbrough, 2006).

2.5.4Bacteraemia

Non-typhi salmonellae (NTS) particularly S.Typhimurium and S.Enteritidis are common causes of bacteraemia and septicaemia in young children in developing countries. In Africa and elsewhere, NTS bacteraemia is also common in those co infected with HIV. Others at high risk are the elderly in poor health, those with malignancy, sickle cell disease and chronic schistosomiasis(Cheesbrough, 2006).

2.6Salmonella Detection

There are four steps for the recovery of*Salmonella* cells from a food matrix. First is the pre-enrichment, where buffered peptone water or lactose broth can be used. This is followed by enrichment in selective broth, such as Rappaport- Vassiliadis (RV) broth, Selenite Cysteine Broth (SC), or tetrathionate broth (TT). Finally the subsequent isolation is done on selective media such as xylose lysine deoxycholate(XLD), Brillant green agar, Bismuth sulphite agar or Hektoen agar (HA) and Salmonella Shigella agar as reported by Molbak*etal*. (2006).

2.6.1Pre-enrichment Media

The number of salmonellae in faeces from asymptomatic animals, environmental samples, animal feed and food are usually low, and it is therefore necessary to use pre-enrichmentmedia, such as buffered peptone water oruniversal pre-enrichment broth, to assist isolation. This may allow the small numbers of salmonellae, which may otherwise be killed by the toxic effect of selective enrichment media, to multiply, or it may help to resuscitate salmonellae that have been sub-lethally damaged, e.g. by freezing, heating, and exposure to biocides, organic acids, bacteriocins, phage or desiccation. Pre-enrichment may not be the best method for isolating less vigorous *Salmonella* strains, such as the host-adapted strains, from faeces because of overgrowth by competing organisms during non-selective pre-enrichment(OIE,2010).

2.6.2Enrichment Media

Enrichment media are liquid or semi-solid media that contain additives that selectively permit salmonellae to grow while inhibiting the growth of other bacteria. Some, however, are relatively toxic to certain serovars of *Salmonella,* e.g. selenite inhibits *S. Choleraesuis*,

and brilliant green is toxic to many strains of *S.* Dublin. Elevated temperatures have also been used to increase the selectivity of enrichment medium, and a temperature of 43°C is used in some laboratories, although this may be inhibitory with some media, e.g. tetrathionate and Rappaport–Vassiliadis at 43°C inhibit temperature-sensitive strains, especially *S.* Dublin and 41.5°C is now recommended for incubation of Rappaport–Vassiliadis broth-based media (Voogt*et al.*, 2001). The formulation of the medium, which may vary between suppliers, temperature and duration of incubation, andthe volume of the samples used to inoculate the medium, may all serve to influence the isolation rate, and these variables should always be taken into account. Examples of selective enrichment media are sodium tetrathionate, as in Müller–Kaufman broth, selenite F, selenite cysteine, brilliant green broth and Rappaport–Vassiliadis broths, or semi-solid Rappaport–Vassiliadis medium. In some cases it may be advantageous to use more than one selective broth or both pre-enrichment and direct selective enrichment/direct plating, although often the benefit does not justify the extra cost. Additives such as Ferrioxamine E can be included to selective media to enhance isolation of *Salmonella* from iron or nutrient-limited samples such as eggs, water or soil (Reissbrodt, 1995) or antibiotics such as novobiocin suppress most Gram-positive organisms or other Gram-negative bacteria such as *Proteus*. Specific antibiotics can be added to enhance the isolation of antimicrobial resistant *Salmonella* strains (OIE, 2010).

2.6.3 Selective Plating Media

These are solid, selective agars that permit differential growth to varying degrees. They have the characteristics of inhibiting the growth of bacteria other than *Salmonella* and give

information on some of the principal differential biochemical characteristics – usually non-lactose fermentation and hydrogen sulphide (H_2S) production. Salmonellae form characteristic colonies on such media that are usually distinguishable from the colonies of other bacteria on the plate, with the possible exceptions of *Proteus, Pseudomonas, Citrobacter*and *Hafnia*. Lactose-fermenting salmonellae may occasionally be isolated and H_2S production may be variable. Examples of selective media are brilliant green agar, xylose lysine deoxycholate agar, deoxycholate/citrate agar, and bismuth sulphiteagar.A wide range of chromogenic agars such as Rambach agar, are now available. Many of these may aid differentiation of suspected colonies, but must be validated for the sample matrices, culture systems and serovar range used as sensitivity can be poor in some circumstances (OIE, 2010).

2.6.4Nucleic Acid Recognition Method

Although the conventional identification and characterization process is still the gold standard, most laboratories are adapting molecular techniques for rapid identification and characterization. Several techniques such as the polymerase chain reaction (PCR) are currently complementing the conventional methods for rapid identification of *Salmonella* in food (Tatavarthy, 2005). The advantages of using DNA based assays for detection of a pathogen are many. Nucleic acid assays provide information about the virulence of the pathogen as well its relatedness to other organisms. They offer information about the genome of the organisms and are very useful for epidemiological investigations (Belgrader*etal*., 1999).

2.6.5 Molecular Detection of Antimicrobial Resistance Genes

Molecular methods that enable the detection of antimicrobial resistance determinant are critical surveillance tools that are necessary to aid in curbing the spread of antibiotic resistance (Leski*et al.,* 2013). Molecular techniques such as PCR and DNA sequencing are employed in identifying genes and genetic assemblages responsible for antibiotic resistance, multidrug resistance, monitoring the spread of resistance determinants, and elucidating the genetic elements responsible for the dissemination of resistance determinants.

Polymerase chain reaction is a technique use to make several copies of specific DNA region so that it can be analysed or used for sequencing. The sequence of the bases in DNA is determined using DNA sequencing either for medical or research uses. PCR involves cycles of heating the sample for denaturing, annealing of the primers and the elongation of the primers by the thermostable DNA polymerase. In theory, each round of amplification gives a doubling of the number of DNA target molecules, but the process is seldom 100% efficient because of the presence of inhibitors, and in later rounds of amplification DNA polymerase may become limited (Saiki*et al*., 1988)

DNA sequencing provides an ability to read DNA sequences. Furthermore, DNA arrays and DNA chips are employed in the screening of DNA sequence. They are based on the principle of hybridization where a DNA sample is rendered single stranded and allowed to combine with a single stranded probe (Fluit*et al*., 2001).

2.7 Transmission of *Salmonella*

The primary reservoir of salmonellae is the intestinal tract of humans and animals, particularly in poultry (Cosby *etal*., 2015). The organisms are excreted in feaces from which they may be transmitted by insects and other creatures to various places such as to water, soil and kitchen surfaces. Eggs, poultry and raw meat products are the most important food vehicles of *Salmonella* infection in human (Akpabio, 2015). *Salmonella* infection is usually acquired through direct or indirect contact with colonized animals, as well as by the oral route, mainly by ingesting contaminated food or drink (De Oliveira, 2004; Bauer-Garland *etal*., 2006; Lammerding, 2006; Chai and Mahon 2011). Any food product is a potential source of human infection. *Salmonella* can be transmitted directly from human to human or from animal to human without the presence of contaminated food or water, but this is not a common mode of transmission.

The Center for disease Control and Prevention (CDC) estimates that 76 million people suffer food borne illnesses each year in the United States, accounting for 325,000 hospitalizations and more than 5000 death (Vo*et al*., 2014). The WHO (2015) Food borne Disease Burden Epidemiology Reference Group (FERG) reported an estimated 582 million cases of 22 different food borne enteric diseases, among them *Salmonella* were the main enteric disease agent responsible for most deaths in 2010. Infections with *Salmonella* alone accounted for one billion dollarsyearly, in direct and indirect medical costs (NIAIDFact Sheet, 2005). Since *Salmonella*has several opportunities to be introduced to the host, there is an increased risk of *Salmonella* reaching the consumer.

Salmonellae can be transmitted vertically to the progeny of infected breeder flocks and horizontally within and between flocks (Gast, 1997). Vertical transmission of paratyphoid salmonellae to the progeny of infected breeder flocks can result from the production of eggs contaminated by salmonellae in the content or on the surface. During oviposition eggshells are often contaminated with paratyphoid salmonellae by faecal contamination. The penetration of salmonellae into and through the shell and shell membranes can result in direct transmission of infection to the developing embryo or can lead to exposure of the chick to infectious Salmonellae when the shell structure is disrupted during hatching.

Horizontal transmission can occur by direct bird to bird contact, ingestion of contaminated faecesor litter, contaminated water, farm handlers (through their clothing, hand, shoes), farm equipment, and a variety of other sources (Nakamura *etal*.,1997). Snoeyenbos*et al.* (1969) reported that, horizontal transmission could occur when unexposed day old chicks were raised together with infected day old chicks. Similarly, Hurst and Ward (2001) reported that rodents serve as a means of introducing *Salmonella* into poultry facilities. Birds could acquire *Salmonella* when they feed on the droppings of mice in the farm. Likewise, the faeces of mice or snakes can contaminate the water and the feeds of the birds, which could serve as a means of transmission to the birds. Garber *et al*. (2003) suggested that rodents should be included in the transmission of *Salmonella* in poultry farms. Barbour *etal*.(1983) in his study confirmed the role of mice as a means of dissemination of *Salmonella* when he isolated *Salmonella* serovars S. Concord, S. Kentucky, S. Mbandaka, S. Montevideo and S. Molade from mice caught from broiler poultry farm. Contaminated

poultry house environments are identified as one of the major implication of paratyphoid Salmonellae. Since the chicken itself is a reservoir of *Salmonella*, control measures need to start from the hatchery.

2.7.1 Prevalence of Salmonellae in Poultry Samples in Nigeria

Salmonellosis is a public health problem of major importance. It is a leading cause of food poisoning in humans and an important cause of various diseases of livestock resulting in high morbidity and mortality. In a cross sectional study of 150 intensively reared and 150 backyard chickens conducted by Obi and Ike (2015) in Nsukka environs, 412 birds representing a prevalence of 4% were positive for *Salmonella* with 1.3% and 6.7% in intensively reared and backyard reared birds from cloacal swab sample. The prevalence of*Salmonella*was significantly higher ($P < 0.05$) in backyard chickens than in intensively reared chickens. Similarly, Salihu*et al*. (2014) reported an overall prevalence rate of 2.4% of S. Gallinnariumfrom the cloacal swab of birds in Nassarawa State, Nigeria. Fashae*et al*. (2010) in a study to determine the prevalence and antibiotic resistance of *Salmonella* serovars from humans and chickens in Ibadan, Nigeria, in 2004-2007 recorded a prevalence of 11% from chicken fecal samples out of the 641 fecal samples that were collected from poultry farms in Ibadan. In a study by Salihu*et al*. (2014) to determine the distribution of *S*.GallinarumIsolates from Free Range Chickens in Nasarawa State, Nigeria, a prevalence rate of 2.5% was determined out of the 1970 samples examined by cloacal swab.

2.8 Risk Factors for the Transmission of *Salmonella* in Poultry

The physiology of*Salmonella* has contributed to the difficulty in controlling environmental contamination and transmission of the organism. Salmonellae are bacteria with several potential vehicles, vectors, and reservoirs within a poultry flock.

2.8.1Hardiness of *Salmonella*

The environmental persistence of *Salmonella* is a significant factor in the epidemiology of *Salmonella* in poultry by creating opportunities for horizontal transmission of infection within and between flocks. *Salmonella* Heidelberg can be isolated from contaminated litter after 7 months of holding at room temperature (Snoeyenbos*et al*., 1999). Richardson and Weiss *et al*. (2000) reportedthat*S.*Typhimurium can survive for 6 months in feed and 18 months in litter stored at 25^{o}C. In addition *Salmonella* can survive for 28 days on the surface of refrigerated vegetables (Snoeyenbos*et al*., 1999).

2.8.2Feed as a Source of *Salmonella*

Poultry feed and feed ingredients are the most common source of *Salmonella* contamination and consequently serve as an indirect means of infection to people consuming poultry meats and poultry products. Feeds are contaminated either from feed mills or on farm during feed formulation, feeding or handling and subsequently spread to poultry mostly through ingestion. *Salmonella* have the ability to survive under prolong periods in dry conditions like feeds and may be recycled in all production stages in commercial feed preparation (Whyte *et al*., 2003). Several factors most especially ingredients used in preparing poultry feeds have been implicated to be the major source of contamination (Bale *et al*., 2002; Maciorowski*et al*., 2004; Okoli*et al*., 2006).

In Nigeria, Okoli*et al*. (2006) reported an incidence of 22.2% *Salmonella* from different feed samples in Imo State; this was attributed to hygiene practice in the farm. Okonkwo*et al*. (2010)also reported 15% isolation rate of *Salmonella* species from poultry feed in Calabar, Cross River State. He observed that the main route of contamination was the feed that attracted animal and insect vectors that are *Salmonella* carriers and serve as a vehicle for introducing *Salmonella* into poultry feeds.

2.8.3 Poultry as a Source of Salmonellosis

Among livestock production systems, *Salmonella is* more frequently isolated from poultry (chicken, turkey, duck, geese and pheasants) than from other animals (OIE, 2008). CDC (2013) reported that public health problems associated with salmonellosis were of poultry origin.

Poultry and eggs are considered the most important reservoirs from which *Salmonella* is passed into the food chain and ultimately transmitted to humans (Howard *et al*., 2012). The levels of these pathogen in poultry can vary depending on country, production system and the specific control measures in place. Poultry can carry some *Salmonella*serovars without any outwards signs or symptoms of disease. *Salmonella* can contaminate eggs on the shell or internally, but the egg shells are much more frequently contaminated than the white or the yolk. Furthermore, egg surface contamination is associated with many different serotypes, while infection of the white or yolk is primarily associated with *S. enteritidis*(Herikstad*et al*., 2002). Contaminated poultry and poultry-derived products, including meat and eggs are a major source of food borne salmonellosis (Barrow *et al*.,

2012; Barua*et al.,* 2013). *Salmonella* is able to remain viable in frozen products as well as foods stored at high temperatures for long periods, due to their markedabilityto persist in a wide range of varying environmental conditions (Balamurugan, 2010).

Salmonella can enter the food chain at any point. Contamination can occur at several stages in the slaughter process of poultry, like faeces during evisceration, surfaces on the production line or cross contamination from contaminated products. Particular contamination hot spots in the poultry slaughter process include defeathering, evisceration and cutting, while chilling in a water bath enhances cross-contamination (Northcutt *et al.*, 2003). The principal site of multiplication of these bacteria is the digestive tract, particularly the caecum (Beal *et al*., 2006), which may result in widespread contamination of the environment due to bacterial excretion through faeces (Kabir, 2010). *Salmonella* can be introduced to a flock via environmental sources such as feed, water, soil, bedding, litter material, faecal matter, rodents or contact with other poultry.

As *Salmonella* colonizes the gastrointestinal tract, the organisms are excreted in faeces from which they may be transmitted by insects and other animals to a large number of places and are generally found in contaminated water with faeces. Humans and animals that consume polluted water may shed the bacteria through faecal matter continuing the cycle of contamination.All the fractions of poultry production can be affected by *Salmonella* organisms, like hatchery, incubators, breeding facilities, commercial raising operations of layers and broilers, feed preparation units and factories, transportation systems,

commercialization facilities and slaughterhouses (Rodriguez *et al*., 2006; Hoelzer*et al*., 2011).

2.9 Public Health Significance of Salmonellosis

With the increase in poultry meat and egg consumption, the dynamics of animal production and consumer exposure have changed leading to new challenges in limiting poultry borne zoonosis like salmonellosis. This has significant implication in Nigeria where poultry industry is among the fastest growing sector. Globalization, commercialization and distribution have made it possible for a contaminated foodstuff to affect the health of people in several countries at the same time. The identification of only one contaminated food ingredient may lead to the discarding of entire lot, causing economic losses to the production sector and restrictions for international trade. The loss in animal production and public health issues associated with salmonellosis has a substantial impact on the economy of several countries (Hoelzer*et al*., 2011; Mather *et al*., 2013).

Salmonella is mostly transmitted to humans, through contaminated food and water. In hospitals, person to person transmission may also happen. Among veterinarians and farm workers, transmission by contact with infected animals is possible. Cross contamination of poultry can occur in slaughter houses as well as during preparation of poultry products (Olsen*etal*., 2003). *Salmonella* can also leads to severe condition like sepsis and death especially in infants and immuno compromised adults (Tessari*et al.*, 2012).

Several cases of food borne salmonellosis that originated from poultry products have been reported globally (Calvert *et al*., 2007; Zielicka-Hardy *et al*., 2012; Jakociune*et al*., 2014). Isolation of *Salmonella* from chicken has also been reported (Dallal*et al*., 2014). The predominant serovars of *Salmonella*, having public health importance are mainly *S*. Enteritidisand *S*. Typhimurium (Jinu*et al*., 2014), but changes in the environment and poultry raising practices coupled with increased international trading of poultry and its products have led to changes in predominance of different *Salmonella* serotypes. Recent concern in public health point of view is antibiotic resistant serotypes.

2.10 Definition of Antibiotics and Antimicrobial Resistance

Antimicrobial agents can be defined as chemicals, drugs or substances that can reduce or eliminate the growth of a micro-organism (CDC, 2008a). Antibiotic resistance is defined as the ability of bacteria to stop the inhibitory (bacteriostatic) or killing (bacteriocidal) effects of antibiotics to which it is previously sensitive and susceptible (Lee *et al.*, 2009). An organism can be resistant intrinsically to an antibiotic agent due to changes in the genetic constituent and it can also be susceptible naturally (Forbes *et al.*, 2002). The antimicrobials are characterized by their specificity of targeting entities or organisms; an example is antiviral agent, antibacterial drugs and antifungal (CDC, 2008a).

Over the years, there was increasing concern about resistance that was reported in many pathogens, spreading rapidly in different geographical regions of the world (Byarugaba, 2005). This could be as a result of selective pressures of antimicrobial use, changes in microbial characteristics, and technological changes that enhanced the modern development

and transmission of drug-resistant microbes. At the same time, antimicrobial resistance is a natural occurring phenomenon, and it is often enhanced as a result of infectious etiological agents' adaptation and exposure to antimicrobials used in humans and veterinary medicine or widespread use in agricultural farm and disinfectants at household levels (Walsh, 2000). However, the most difficult aspect of antimicrobial resistance is the multidrug resistance of pathogens, which limits the choice of antimicrobials in the therapy of the clinical disease.

2.10.1Antibiotic Resistant *Salmonella*

Antibiotics as growth promoters are of utmost concern because its usage at sub therapeutic levels, stimulate survival of resistant bacteria in the ecosystem (Al-Ferdous*et al*., 2013). The WHO observed an alarming rate increment of resistant *Salmonella* strains due to the abusive use of antibiotics in intensive animal raising (WHO, 2014). Different findings showed the consequences of antibiotic resistance on human health. These consequences can be divided into two categories: first, the infections that may not have occurred and secondly, the high incidence of treatment failures and increase in the severity of disease (WHO, 2005). Moreover, resistance can spread from non-human sources to human by various routes such as animal, water and contaminated foods. However, contact with *Salmonella* carrier animal is the most important pathway in transmission of resistance to humans.

Resistance to combinations of many classes of antimicrobial agents in *Salmonella* has led to the re-emergence of multidrug resistant *Salmonella*(MDR) strains that may pose a risk to humans (White *et al*., 2001; O'Brient, 2002). According to CDC's National Antimicrobial

Resistance Monitoring System (NARMS), the serovars with greater resistance to antimicrobials are *S.* Typhimurium, *S.* Enteritidis, *S.* Newport, *S.* Heidelberg and *S.* Dublin. In terms of multidrug resistancethe most prevalent serovars of epidemiological importanceare *S.* Typhimurium, *S.* Heidelberg, *S.* Dublinand *S.* Paratyphi(Andino and Hanning, 2015). The horizontal transmissionof virulence genes in multidrug resistant *Salmonella* strains can increase virulence and invasiveness and a resultanthigh mortality rates (Han *et al.*, 2011).

2.10.2Global Trends in Resistance Pattern

Antibiotics resistance is one of the biggest challenges facing global public health. Although antimicrobial drugs have saved many lives and eased the suffering of many millions.Poverty, ignorance, poor sanitation, hunger and malnutrition, inadequate access to drugs, poor and inadequate health care systems, civil, environmental conflicts and bad governance (Byarugaba, 2004) misdiagnosis, counterfeit drugs and lack of education in developing countries have tremendously limited the benefits of these drugs in controlling infectious diseases worldwide (Walia, 2006).

2.10.3Antibiotic Resistance Pattern of *Salmonella* in Nigeria

Considering the role of chickens and its products in the human food chain in Nigeria; and the close interaction between poultry and man, antibiotic resistant organisms may pose dangers to humans through the food chain or zoonotic infection and precipitate a similar pattern of resistance in man (Anyanwu*et al*., 2010).

Antibiotics have been used successfully in human and veterinary medicine in the past sixty years to turn many of the life threatening bacterial infections into treatable conditions.

However, antibiotic resistance has become an important health and food safety issue with the emergence of many drug-resistant species of microbial pathogens in human (Maurer, 2004). The use of several antibiotics for therapeutic or prophylactic administration, especially in the poultry operations is particularly worrisome in view of the potential to extend such drug into the human food chain or the possibility of reducing the efficacy of such drugs sometimes administered by non-qualified personnel (Boonmar*et al*., 1998; Van den Bogaard*et al*., 2001; Van den Bogaard*et al*., 2002; Thakur and Bajaj, 2006; Capita, 2007).

Fashae*et al*. (2010) recorded a high frequency (87%) of reduced susceptibility to ciprofloxacin among chicken isolates in a study on Antimicrobial susceptibility of*Salmonella*serovarsfrom chickens and humans in Ibadan, Nigeria. In the same way a high frequency of resistance to tetracycline (93%), nalidixic acid (81%), and sulfamethoxazole (87%) was observed. Rare serovars such as S. Apapa, S. Mouschaui, S. Jukestown, S. Oritamerin, and S. Onireke were isolated from both humans and chickens (Fashae*et al*., 2010).

A study by Mathew *et al*. (2012) was undertaken to investigate the antibiotic resistance pattern (antibiogram) of *Salmonella*-like bacterial species isolated from poultry soil of the University of Uyo, Nigeria against some antibiotics. Results revealed that *Salmonella*-like species were highly resistant to Nalidixic acid (66.66%), followed by Tetracycline (63.33%), Cotrimoxazole, Amoxicillin and Augmentin (60%), while the least was

Ofloxacin (26.66%). Multiple resistance to 4 or more antibiotics among the isolates from the soil outside the broilers enclosure was observed, while there was a significant difference ($P < 0.05$) between poultry soil and control soil. This implied that the antibiotics with the highest resistance were most often applied to the birds, the droppings of which contaminated the soil.

Omoigberale*et al*. (2014), in a study on bacteriological quality and prevalence of *Salmonella* in chicken products, fruits and vegetables showed that the percentage susceptibility of *Salmonella* to ten (10) antimicrobial agents revealed 80% susceptibility to ciprofloxacin. None of the isolates was sensitive to septrin, augmetin and chloramphenicol. The isolates showed high multiple antibiotics resistance which is of serious health concern. Hemen*et al*. (2012) reported the isolation of *Salmonella* from poultry litters of selected farms in Benue State. The *Salmonella* isolates were completely resistant to chloramphenicol, augmentin, pefloxacin, amoxicillin.

Antimicrobial resistance profiles of *Salmonella* Gallinarumisolates from free-range chickens inNasarawa state, Nigeria by Salihu*et al*. (2014) revealed that the isolates were resistant to the following antibiotics. Overall, 81.7% resistance was recorded against ciprofloxacin, followed by gentamicin, (76.1%); ampicillin, (65.8%); chloramphenicol and cotrimoxazole, (66.2%); tetracycline, (58.1%), while neomycin, nalidixic acid, colistin, oxytetracycline, norfloxacin, kanamycin and amoxycillin had 43.9%, 42.2%, 35.5%, 33.4%, 30.1%, 24.4% and12.9% resistances respectively.

2.11Classification of Resistance

Antibiotics are important drugs used in the treatment of bacterial infection in both humans and animals. Emerging antibiotic resistance among certain bacteria is now frequently observed, thereby posing a serious threat to public health. Resistance to most traditional, regulatory-approved, or naturally-occurring food antimicrobial agents is difficult to characterize because a precise definition for such resistance is lacking (Cloete, 2003).

From a functional perspective, resistance correlates with failure of a given antimicrobial treatment; whereas from a laboratory perspective, resistance is denoted through a Minimum Inhibitory Concentration (MIC) which is the lowest concentration of an antimicrobial drug, expressed in g/ml or mg/ml, which under defined *in vitro* conditions within a defined period of time inhibits growth of the microbial inoculum value that exceeds a threshold value, which may or may not be associated with a clinical outcome (Courvalin, 2005). A microorganism is resistant if it exhibits significantly reduced susceptibility when compared with that of the original isolate or a group of sensitive strains. Resistance means temporary or permanent ability of a microorganism and its progeny to remain viable and/or multiply under conditions that would destroy or inhibit other members of the strain (Cloete, 2003). Resistance can result from mutations in housekeeping structural or regulatory genes, or alternatively, horizontal acquisition of foreign genetic information. In some cases, resistance may manifest through multiple mechanisms (Courvalin, 2005). The studies of almost 400 different bacteria have demonstrated about 20,000 possible resistance genes (Davies and Davies, 2010).

2.11.1 Innate (Intrinsic) Resistance

Innate (Intrinsic) resistance is inherent to all members of the species. In such cases, the gene that encodes the intrinsic resistance is chromosomal. Innate resistances are related to the general physiology or anatomy of a microorganism and are derived from pre-existing mechanisms or properties. More specifically, Gram negative bacteria are innately resistant to penicillin G by virtue of their double membrane structure, which prevents the antibiotic from accessing the cell wall target. Innate resistance is not considered an important clinical problem because antibiotics were never intended for use against intrinsically resistant bacteria. There are certain circumstances in which antimicrobial agents do not adversely affect bacteria that are generally susceptible to the particular agent. Exposure conditions, such as the environmental conditions (Temperature, pH and food composition) of the antimicrobial application, or interaction of the antimicrobial with components of the suspension medium or food product can influence the efficacy of the antimicrobial agent(Davies and Davies, 2010).

2.11.2 Acquired (Extrinsic) Resistance

Acquired (Extrinsic) resistance results from genetic changes that occur through mutation of the antimicrobial's target site within the bacterium or acquisition of genetic material encoding resistance via plasmids or transposons, genetic element that physically moves from one genetic position within the chromosome or plasmid in which it resides to another.Acquired resistance, the most common type of antibiotic resistance, has been well studied for antibiotics. Acquisition of genes for β-lactamase (An enzyme capable of breaking down and inactivating β-lactam antibiotics such as penicillins and cephalosporins)

and mutation of one of the subunits of DNAgyrase, the target of fluoroquinolones, are examples of this type of resistance (Roe and Pillai, 2003.).

2.11.3 Adaptation

Adaptation may be demonstrated by exposing a microorganism to a stepwise increase in concentration of the antimicrobial agents. This type of resistance, however, is often unstable; the microorganism may revert back to the sensitive phenotype when grown in antimicrobial-free medium. This is also known as back-mutation. In the absence of selective pressure, the mutations associated with resistance may actually reduce fitness of the bacterial strain compared to the wild type, parental strain (Russell, 1991).

2.12 Mechanisms of Antibiotics Resistance in *Salmonella*

Bacteria can evade the actions of antibiotics using diverse mechanisms. Antibiotic resistance reflects the attack and counterattack of complex microbial flora to an antimicrobial agent in order to establish ecological niches and survive (Denyer*et al*., 2011). The major problem in poultry production today is the emergence of multiple-drug resistance, to several types of antimicrobial agent (Amenu, 2014). The mechanisms for antibiotic resistance can be categorized as (i) modification or destruction of the antimicrobial agent, (ii) pumping the antimicrobial agent out from the cell by efflux pumps, (iii) modification or replacement of the antibiotic target, and (iv) decrease in cell membrane permeability. Thus, microorganisms are developing resistance mechanisms through mutations in the gene locations of target proteins or acquiring mobile genetic elements carrying resistance genes such as plasmid, integrons and transposons (Walsh, 2003).

There are many classes of antimicrobial drugs, but the most common antimicrobials that *Salmonella* has developed resistance to at present are namely; aminoglycosides, β-lactams,chloramphenicol, quinolones, tetracyclines, sulfonamides and trimethoprim

2.12.1 Plasmid Mediated Resistance

Plasmids are extra chromosomal pieces of DNA, they are self-replicating circular double stranded DNA that can reside in the host cell. They contain a lot of different genes of benefit to the host cell. Plasmid associated genes have been implicated in resistance to aminoglycosides, chloramphenicol, penicillins, cephalosporins, erythromycin, tetracycline, and sulphonamides (Willey *et al.,* 2013). They are clinically important if they contain virulence or resistance genes. Plasmids are very important in the epidemiology and spread of resistance to antibiotics. Plasmids can be horizontally transferred from one bacterial host to another even between bacterial species (Carattoli, 2009).

2.12.2 Reduced Membrane Permeability

Reduced membrane permeability occurs simply by preventing entrance of the drug into the bacteria. The alteration in membrane permeability occurs when new genetic information changes the nature of proteins in the membrane. Such alterations change a membrane transport system pores, so that an antimicrobial agent can no longer cross the membrane. In *S.* Typhi, resistance to tetracycline, quinolones and some aminoglycosides have occurred by this mechanism. A decrease in permeability can also lead to sulfonamide resistance (Willey *et al*., 2013).

2.12.3 Modification of theTarget Site

Modification of target site resistance arises when the target enzyme or cellular structure of the pathogen is modified so that it is no longer susceptible to the drug. This mechanism is found in *S.* Typhiand other sulfonamide-resistant bacteria. These organisms have developed an enzyme that has a very high affinity for p-aminobenzoic acid (PABA) and a very low affinity for sulfonamide. Consequently, even in the presence of sulfonamides, the enzymes work well enough to allow the bacterium to function (Black, 2005).

2.12.4 Rapid Extrusion or Efflux Pump

Rapid extrusion or efflux of the antibiotic is a resistance mechanism that works by pumping the drug out of the cell after it has entered. Some pathogens have plasma membrane translocases, called efflux pumps, that expels drugs and export the drugs out of the cell. Because they are relatively nonspecific and can pump many different drugs including quinolones, these transport proteins often are called multi drug resistance (MDR) pumps.Resistance to sulfonamides is mediated by a plasmid encoded transport system that actively exports the drug out of the cell (Denyer*et al*., 2011).

2.12.5 Chromosome Mediated-Resistance

Chromosome mediated-resistance has been attributed to a mutation in the gene that codes for either the target of the drug or the transport system in the membrane that controls the uptake of the drug (Denyer*et al*., 2011). The chromosomal-mediated drug resistance phenomenon against fluoroquinolones has been reported recently as a result of selective pressure on the bacterial population due to their uncontrolled use. This has been attributed to single point mutation in the quinolone resistance determining region (QRDR) of the

topoisomerase gene gyrA, which encodes DNA gyrase (Zaki, and Karande 2011). Resistance to trimethoprim is due primarily to mutations in the chromosomal gene that encodes dihydrofolatereductase, the enzyme that reduces dihydrofolate to tetrahydrofolate. Also, resistance to sulfonamides has been found to be mediated by a chromosomal mutation in the gene coding for the target enzyme dihydropteroatesynthetase, which reduces the binding affinity of the drug (Denyer*et al*., 2011).

CHAPTER THREE

3.0 MATERIALSAND METHODS

3.1Study Area and Design

The study was carried out in Katsina State (Figure 3.1).Katsina State, located at the extreme northern margin of Nigeria, covers a total area of about 23,938sqkm (3,370sq) with a total population of 5,801,584 people, going by 2006 census (FGN 2007). It lies between latitudes 11°08'N and 13°22'N and longitudes 6°52'E and 9°20'E. The state is bounded by Niger Republic to the north, by Jigawa and Kano States to the east, by Kaduna State to the South and by Zamfara State to the West. Katsina State has predominantly Hausa-Fulani indigenes. About 75% of the people are farmers and others are traders and livestock owners. There are also some other Nigerian settlers from Southern Nigeria especially the Yoruba's and Ibo's in the state.

The state has varying climate with cool dry season (harmattan) from October to February, hot dry season from March to May and a warm wet season from June to September. Water supply in Katsina State is sourced through damming of rivers and digging of wells and boreholes. The state has thirty four (34) local governments. The local governments are divided into three (3) senatorial zones according to their geographical locations, Namely; Funtua zone (South), Katsina zone (Central), and Daura zone (North) (Dauda*et al.,* 2011).

The study was based on multistage random sampling technique. Therefore, four Local Government Areas from the Katsina South senatorial district were selected for the study. The Local Government Areas wereBakori, Danja, Malumfashi and Sabuwa LGAs. Eight

poultry farms, two (2) eachfrom the four Local Government areas (from the senatorial district) were selected for the study.

3.2Sample Size

The sample size was determined using the equation as described by Niaing*et al*. (2006). The prevalence of 10.9% in a similar study obtained by Agada*et al*. (2014) was used to determine the sample size using theformularbelow:-

$$N = \frac{PQ}{\left(E/Z\right)^2}$$

P = prevalence of previous study 10.9 % (Agada*et al*., 2014)

Q = 100 – p = 100 – 10.9 = 89.1

E = Allowable error = 5%

Z = Standard normal distribution at 95%, CI = Confidence interval, 1.96.

N = Number of sample to be collected

$$N = \frac{109 \times 89.1}{\left(5/1.96\right)^2} = \frac{971.1899}{(2.55)^2} = \frac{971.1899}{6.5} = 149$$

However, for the purpose of obtaining precise results in the research work, 320 droppings and swabs were collected from the poultry farms.

3.3 Sample Design

Simple random sampling technique was employed in the collection of the sample. A total of 320 samples were collected comprising of faecal droppings and cloacal swabs from broiler birds aged six weeks and above and housed in a cage from the four Local Governments Areas selected for the study.

3.4 Inclusion and Exclusion Criteria

A farm that has 200 birds and above, caged, was included in the study. Poultry farms with less than 200 birds and not caged were excluded from the study. Voluntary and informed consent was obtained from the farm owners before samples were collected. Structured questionnaire on *Salmonella*species contamination and the drug resistance in the poultry farms were administered to the farm handlers. The samples were collected from eight commercial poultry farms, 2 each located in Bakori, Danja, Malumfashi, and SabuwaLGA respectively.

3.5 Sample Collection

Faecal droppings (n=160) were collected in a sterile universal bottles. Cloacal swabs (n=160) were collected from the cloacae of the birds using cloacae sticks. All the samples were transported to the laboratory immediately after collection in an insulating foam box with ice maintaining the temperature from 4^{o}C to 6^{o}C (WHO, 2010)



Fig 3.1: Map of Katsina State Showing the Local Government selected for the study (Source: Geography Dept, UMYU, 2013)

3.6Isolation and Identification of *Salmonella* Species

The *Salmonella* species were isolated according to the methods outlined by the WHO (2010).

3.6.1 Culture of the Specimens

A sterilized spatula was used to introduce 1g of the faecal droppings into 9ml buffered peptone water and incubated at 37^{o}C for 24 hours. Cloacal swabs was also inoculated into 9ml of buffered peptone water, thereafter 1 ml of thecultures were transferred into a 9ml of Selenite cysteine Broth and incubated at 37^{o}C for 24 hours (Mitchel and Shane, 2000; WHO, 2010; Al-Abadi and Al-mayah, 2011).A loopful of the overnight Selenite cysteine broth culture was streaked on Xylose lysine deoxycholates Agar (XLD) and the plates incubated overnight at 37^{o}C for 24 hours (WHO, 2010; Menghistu*et al*., 2011).Typical suspected *Salmonella* colonies on XLD appeared red with a black centre. Two suspected*Salmonella* colonies on XLD were purified by sub culturing on nutrient agar and incubated at 37^{o}C for 24 hours for isolation of pure culture and subsequent biochemical confirmation (OIE, 2010; WHO, 2010).

3.6.2 Gram Staining and Microscopy

Dried smear of the isolate obtained was fixed and stained with crystal violet solution (primary dye) for 60seconds, rinsed with tap water and drained to avoid diluting with the mordant. It was further flooded with iodine solution (mordant) for 60seconds and rinsed. Acetone was applied drop wise on the tilted slide for 2-3 seconds until all free colours have been removed and subsequently rinsed with tap water. The slides were further flooded with

safranin for 20-30 seconds, rinsed and air dried. Finally, all slides were examined under the microscope at x100 objective lens (Cheesbrough, 2006).

3.7Biochemical Screening Test of *Salmonella* Species

The following biochemical tests were carried out as recommended for the biochemical screening of *Salmonella* species; TSI, Urease, Indole, Citrate, VogesProskauer, and methyl red (IMVIC) (Kaniz*et al*., 2014; WHO, 2014).

3.7.1 Triple Sugar fermentation test (TSI)

A small amount of growth from the nutrient Agar slant was harvested with a sterile wire loop. The wire loop was used to lightly inoculate the surface of the TSI Agar slant and also used to make a single stab into the butt of the tube. The tubes were incubated at 37°C for 24 hours (WHO, 2014).*Salmonella* forms a red (alkaline) slope and yellow (acid) butt indicating glucose fermentation, crack and bubbles in the medium indicate gas production from glucose. Blackening along the stab line or throughout the slant indicates H_2S production (Cheesbrough, 2006; WHO, 2014).

3.7.2Urease Test

A small amount of the test organism was harvested using a wire loop and lightly inoculated on the surface of the agar slant. The tube was incubated at 37°C for 24 hours. A pink colour indicates positive urease test while no change in colour indicates negative urease test (Cheesbrough, 2006; WHO, 2014).

3.7.3 CitrateUtilizationTest

A sterile wire loop was used to harvest a small amount of growth culture from the nutrient agar slant. The wire loop was used to lightly inoculate the surface of the Simmons citrate agar slant. The tube was incubated at 37°C for 24 h. Positive citrate reaction is indicated by a blue colour while negative test shows a greencolour of the medium (Cheesbrough, 2006; WHO, 2014).

3.7.4 Indoleproduction

The isolates were inoculated into 3mls of indole medium and incubated at 37°C for 24h. After incubation, few drops of Kovac's reagent were added and shaken gently. The presence of yellow colour in the reagent indicated a negative test while the dissolution of indole in the reagent which became red ring and form a layer above the surface of the broth indicated a positive test (Cheesbrough, 2006).

3.7.5Methyl red test

The isolates were inoculated onto 5mls of MRVP broth then were incubated at 37°C for 24h. After incubation, 1ml of the broth was transferred to a small tube and 2-3 drops of methyl red indicator were added. Appearance of a red colour indicated a positive test (Singleton, 1997).

3.7.6 VogesProskauertest

The remaining inoculated and incubated MRVP broth used above, 15 drops of α- napthol reagent was added and followed by 5 drops of 40% KOH. The broth was shaken and the cap of the tube was loosed and placed in a slanting position. The development of a red

colour from the liquid - air interface within 60 minutes indicated a positive test (Singleton, 1997).

3.7.7 Confirmation of the Isolates Using the Commercial Identification Kit (Microgen)

The commercial identification kit (microgen) was prepared according to the manufacturer's instruction. After identifying *Salmonella* Spp. Biochemically, pure cultures was streaked on nutrient Agar slant and stored at 4^0C until needed for antibiotic susceptibility and molecular studies (WHO, 2014).

3.8 Susceptibility Test of *Salmonella* Isolates

The antibiotic susceptibility test of the *Salmonella* isolates was carried out using standard guidelines established by CLSI, 2012 as stated below;

3.8.1 Antibiotic Susceptibility Test

In-vitro susceptibility of *Salmonella* isolates to the antibiotics drugs was performed by the standard modified disc diffusion of Kirby- Bauer technique using guidelines established by CLSI (2012).The antibacterial agents employed were: Amoxicillin- clavulanic acid(AMC) 30µg, Chloramphenicol (C) 30 µg, Sulphamethoxazole- Trimethroprim (SXT) 25 µg, Ciprofloxacin (CIP) 5 µg, Cefotaxime (CTX) 30µg, Gentamicin (CN) 10 µg, Cefuroxime (CXM), 30µg, Tetracycline (TET) 10µg, Ofloxacin (OFX) 5 µg, , Norfloxacin (OFX) 10 µg, (Oxoid, U.K).

3.8.2Standardization of the Inoculum

This was performed as documented by CLSI (2012). Pure colonies of the identified *Salmonella* isolate (s) from a 18- 24 hour plate were selected. Sterile wire loop was used to

pick 2 coloniesof each *Salmonella*and emulsified in 5 ml of sterile normal saline. The tube containing the bacterial saline suspension was adjusted with extra inoculum or diluents, if necessary, until 0.5 McFarland standards which correspond to 1.5x 10^8 CFU/ml was obtained (CLSI, 2012).

3.8.3Inoculation of Test Plates

This was carried out as described by CLSI (2012). Within 5- 10 minutes after adjusting the turbidity of the inoculum suspension, a sterile cotton swab was dipped into the standardized suspension. The cotton swab was rotated several times and pressed firmly on the inside of the wall above the fluid level in order to remove excess fluid from the swab. The dried surface of a 20 ml Mueller- Hinton Agar plate in a 100 mm disposable plate was inoculated by streaking with the cotton swab over the entire sterile Agar surface. The inoculated plates were left open for 5 minutes and air dried at 37ºC to allow for any excess surface moisture to be absorbed before applying the antibiotic discs (CLSI, 2012).

3.8.4Application of Discs to Inoculated Agar Plates

The antibiotic discs (Oxoid, UK) five (5) per plates were evenly dispensed unto the surface of the inoculated Agar plate using a sterile forceps and were gently pressed down to ensure complete contact with the Agar surface. The plates were inverted and incubated at 37ºC for 18 - 24 hours.

3.8.5 Examination of Plates and Interpretation of Results

Each plate was examined after 24 hours of incubation. The diameters of the zones of complete inhibition were measured to the nearest whole millimeter, using a ruler and

compared with the zone interpretation chart (Muragka*et al*., 2004). The interpretation of the results was based on CLSI guidelines (2012).

3.9 Detection of Antibiotics ResistanceGenes in *Salmonella* Isolates by PCR

Inorder to determine the antibiotic resistance genes in the *Salmonella* isolates, the polymerse chain reaction was performed as described below.

3.9.1Bacteria Cell preparation

The preparation of the cell was carried out using the method described by Dubey (2009). LauriaBertani (LB) broth Medium was prepared comprising of 1.5g peptone water, 0.5g yeast extract, 0.5g sodium chloride, 1ml of 1N sodium hydroxide, was dissolved in distilled water and made up to the volume of 100ml.Five milliliter (5ml) of the LB medium was dispensed into each universal sample bottle and autoclaved at 121^{o}C for 15 minutes to sterilize. A single colony was picked from freshly streaked isolate on xylose deoxycholate plate and inoculated into the universal sample bottles and incubated at 37^{o}C for 24 hours.

3.9.2 DNA Extraction using Zymo Research Kits

About 1ml of the cultured broth was dispensed to each 1.5ml Eppendorff tubes, the tubes were centrifuged at 10000g for 1 minute, the supernatant was discarded, and the pellets were re-suspended in 600µl lysis buffer and incubated at 56^{o}C for 30 minutes. The mixture was transferred to spin column in a collection tubes and centrifuged at 10000g for 1minutes, flow through together with the column was discarded. The spin column thereafter was inserted into a new collection tubes. Five hundred (500) µl of wash buffer 1 was added and centrifuged at 10000g for 1minute, afterwhich flow through was discarded,

and 500µl of wash buffer II was also added and centrifuged at 10000g for 1 minute, and the flow through discarded as before.The spin column was then centrifuged empty at 10000g for 2 minutes to remove residual alcohols and salt, the collection tube was discarded and the spin columninserted into a 1.5ml micro-centrifuge Eppendorff tube. One hundred (100) ml of elution buffer was added to the spin column and incubated at room temperature for 2 minutes and centrifuged at 10000g for 2 minutes, and then the spin column was discarded.The resultant DNA in 1.5ml micro-centrifuge tube was stored in -86^{o}C refrigerator for later use in the downstream application.

3.9.3PCR Amplification

Polymerase Chain Reaction was carried out in a total volume of 25µl containing 12. 5µl of master mix containing taq (*thermosaquaticus*) polymerase, dNTPs and 2mM $MgCl_2$ was mixed with 7µl template DNA. One (1) µl forward, 1µl reverse primer and 3.5µl nuclease free water in a PCR micro tube. As for the multiplex PCR (*dhfr 1, sul 1, cat 1 and bla-cmy*2) primer, the PCR conditions were initial denaturation at 94 ^{0}C for 5-10 mins followed by 40 cycle of denaturation at 94 ^{0}C for 30 s, annealing at 58^{0}C for 30sec and extension at 72 ^{0}C for 30 sec. Final extension at 72 ^{0}Cfor 7 mins and finally the reaction was hold at 4^{0}C (Rahn*et al*., 1992).

The cycling parameter for *tet* A involved initial denaturation at 95^{o}C for 5 minutes followed by 30 cycles of denaturation at 95^{o}C 30 seconds, annealing at 58^{o}C for 30 seconds and extention at 72^{o}C for 30 seconds. This was followed by the final extension at 72^{o}C for 7 minutes and finally the reaction was hold at 4^{o}C (Rahn*et al*., 1992). Thesecond well was loaded with negative control then followed byPCRamplicons. PCR was performed in a

DNA thermal cycler (Gene Amp PCR system 9700, applied biosystem) according to the manufacturer instructions. The primers used for PCR are as contained in Table 3.1

3.9.4Agarose Gel Electrophoresis

The casting apparatus was assembled with the combs placed in position, about 1.5g of Agarose powder was weighed and 100ml of 1× TBE buffer was added, the mixture was micro waved and cooled to 65^{o}C. Five (5) µl ethidiumbromide was added and shaken so that it was uniformly distributed. The preparation was carefully poured into the casting tray and allowed to solidify with the comb's in place. The combs were carefully removed and the gel tray was removed and transported to electrophoretic tank containing 1× TBE buffer, the volume of the buffer was adjusted to the maximum.

The wells were loaded with samples, control and DNA ladder. Voltage was set at 80v for 45 minutes. The gel tray was taken into gel documentation units, the gel was carefully slipped down from the tray to the gel documentation system, and the gel was viewed and documented in a computer system

Table 3.1:Oligonucletide Primers and Sequenced Amplicon Sizes for Antibiotic Resistance Genes

Antibiotics	Genes	Primer sequence (5'-3')	Size (bp)	Reference
β-Lactams	*^{bla}CMY2*	5' TGG CCG TTG CCG TTA TCT AC 3' 3' CCC GTT TTA TGC ACC CAT GA 5'	870	Cortez *et al*., 2006
Trimethoprim	*dhfr1*	5' CGG TCG TAA CAC GTT CAA GT 3' 3' CTG GGG ATT TCA GGA AAG TA 5 ,	220	Cortez *et al*., 2006
Sulfonamides	*sul1*	5' TCA CCG AGG ACT CCT TCT TC 3' 3' CAG TCC GCC TCA GCA ATA TC 5'	331	Cortez *et al*., 2006
Chloramphenicol	*cat1*	5' CTT GTC GCC TTG CGT ATA AT 3' 3' ATC CCA ATG GCA TCG TAA AG 5'	508	Cortez *et al*., 2006
Tetracycline	*tet A*	5'GCT ACA TCC TGC TTG CCT TC3' 3'CAT AGA TCG CCG TGA AGA GG 5'	210	Ng *et al*., 2001.

CHAPTER FOUR

4.0 RESULTS

4.1 Cultural, Morphological and Biochemical Characterization of the Isolates

On xylose lysine Deoxycholate (XLD) plates, the presumptive*Salmonella c*oloniesappeared red with black centre. In Gram staining, the morphology of the isolated bacteriumwassmall, Gram negative rod, appearing singly.Appendix VIII shows the presumptive and the biochemical characteristics of the *Salmonella* isolates..

4.2Microgen GNA Identification of *Salmonella* Species and other Enteric Bacteria.

The twenty isolates showing typical *Salmonella* like reactions were confirmed using the microgen GNA Identification system. A total of twenty species were identified. The species identified were *Salmonella*Arizonae, *Proteus mirabilis, Escherichia coli, Proteus vulgaris, Cronobactersakazakii, Citrobacterfreundii* and*Pantaoeagglomerans*.Out of the twenty suspected colonies; three were confirmed to be *Salmonella* speciesas shown in Appendix IX.

4.3 Distribution of *Salmonella*Species and Other *Enterobacteria*

A total of three*Salmonella* species and seventeen other enteric bacteria wereisolated from cloacal swab and faecal droppings. *Salmonella*Arizonae accounted for 3 (15%), while for the other enteric organism were,*Proteus mirabilis*7(35%),*Escherichia coli 2*(10%), Proteus*vulgaris*3(15%), *Cronobactersakazakii*1(5%),*Citrobacterfreundii*1(5%) *and Pantoaeagglomerans*3(15%) *were* isolated. A prevalence of 6.3% was obtained for all the *Enterobacteriaceae* (Table 4.1).

4.4Distribution of *Salmonella*Arizonae based on Type of Sample

Out of the 320 samples collected, three*Salmonella*Arizonae were isolated, giving rise to an overall prevalence of 0.94%.There was no positive sample recorded in faecal droppings. The three positive samples obtained from cloacal swabs gave a prevalence of 1.88%. There was a statistically significant difference in the occurrence of *Salmonella* in the different types of samples analyzed (χ^2 = 3.028, df = 1, p=0.0409) as shown in Table 4.2.

4.5Distributionof *Salmonella*Arizonaewith Respect to the Study Poultry Farms

The occurrence of *Salmonella* obtained in Sabuwa poultry farms was 3.75%. There was no occurrence of *Salmonella*Arizonae in Bakori, Danja and Malumfashi poultry farms. There was a statistically significant difference in the occurrence of *Salmonella* in the poultry farms ($p<0.05$) as shown in Table 4.3.

Table 4.1: Prevalence ofSalmonellaArizonaeand other Enteric Organisms in Relation to Sample Source.

Sample source	Enteric organism	Number Positive (%)
Faecal	*E.coli*	2(10)
Droppings	*P.agglomerans*	2(10)
	P. mirabilis	2(10)
	P. vulgaris	1(5)
	S. Arizonae	3(15)
Cloacal swab	*C. freundii*	1(5)
	C. sakazakii	1(5)
	P.agglomerans	1(5)
	P. mirabilis	5(25)
	P. vulgaris	2(10)

Table 4.2:Prevalence of SalmonellaArizonae Isolated from Cloacal Swab and Faecal Droppings

Sample type	Number examined	Number positive (%)
Cloacal swab	160	3(1.88)
Faecal droppings	160	0(0.00)
Total	320	3(0.94)

χ^2=3.028, df = 1, p=0.0409.

Table 4.3: Prevalence of *Salmonella*Arizonaewith Respect to the Poultry Farms

Farms	Number of Samples examined	Number Positive (%)
Sabuwa	80	3 (3.75)
Danja	80	0 (0.00)
Bakori	80	0 (0.00)
Malumfashi	80	0 (0.00)
Total	320	3 (0.94)

χ^2= 9.085, df = 3, p = 0.0282

4.6Percentage Susceptibility of *Salmonella*Serovars

The *Salmonella* isolates was categorized as resistant, intermediate and susceptible according to the CLSI, (2012) guideline. The overall results of antimicrobial susceptibility tests revealed that the*Salmonella*Arizonaewere 33.3% susceptible to ofloxacin, norfloxacin, gentamicinand cefotaxime each.

The result also showed that the serovar was 66% resistant to ofloxacin, norfloxacin, gentamicin and cefotaxime.While, 100% resistant was recorded for cefuroxime, Amoxicillin – clavulanic acid, ciprofloxacin, tetracycline, chloramphenicoland sulfomethozole – Trimethroprim (Table 4.4).

4.7Multidrug Resistance Pattern of *Salmonella*Arizonae

All the *S.* Arizonaeisolates (100%) exhibited multidrug resistance, which means showing resistance to at least one agent in threeor more classes of antibiotics. S2C18 had the highest resistance pattern followed byS2C19 andS2C12which wereresistant to 10, 9 and7 antibiotics respectively (Table 4.5).

4.8Antimicrobial Resistance Genes

All the*Salmonella*Arizonae isolates were screened for the presence of resistance genes. The results of the analysis indicated that the isolates possess all the five genes namely:*Tet*A, *sul*1, ^{bla}*CMY*-2, *cat*1, *dhfr*1 genes. Out of the tetracycline resistant isolates, 2(66.7%) were positive for *tet*A. Among the sulphonamides resistance isolates, 2 (66.7%) were positive for *sul* 1, While, 2(66.7%) strains of *Salmonella* showed the presence of cat1 genes. All the *Salmonella* isolates exhibited (100%) presence of*blaCMY*-2 and *dhfr*1 genes (Table 4.6).

Plate 1, shows PCR amplicon of tet A resistance genes. M is a DNA ladder of 100 base pair. Lane 1-6 shows amplicons sizes for tet A genes. Plate 11, shows the photo of a multiplex PCR amplicons for bla-cmy2, sul 1, cat 1, and dhfr 1 resistancegenes in *Salmonellaarizonae*isolates.Lane M is a 100bp ladder; lane 1 is a negative control, while lanes 2-4 areamplicon for the resistance genes.

Table 4.4: Percentage Susceptibility of SalmonellaArizonae from the Poultry Farms in Southern Part of Katsina State.

Antibiotics	Resistant(%)	*Salmonella*Arizonae Intermediate (%)	Susceptible(%)
OFX	66.7	0	33.3
CXM	100	0	0
AMC	100	0	0
CIP	100	0	0
TE	100	0	0
NOR	66.7	0	33.3
CN	66.7	0	33.3
CTX	66.7	0	33.3
C	100	0	0
SXT	100	0	0

Key: OFX = Ofloxacin; CXM = Cefuroxime, AMC = Amoxicillin-Clavulanic acid; CIP = Ciprofloxacin; TE = Tetracycline, NOR = Norfloxacin; CN = Gentamicin; CTX = Cefotaxime; C = Chloramphenicol; STX = Trimethroprim-sulfamethoxazole.

Table 4.5: Multidrug Resistance Pattern of SalmonellaArizonae from the Poultry Farms in Southern Part of Katsina State

No of Isolates Code	Resistance Pattern	MARI
S2C18	OFX, CXM, AMC, CIP, TE, NOR, CN, CTX, C, STX	1.0
S2C19	OFX, CXM, AMC, CIP, TE, NOR, CN, C, STX	0.9
S2C12	CXM, AMC, CIP, TE, CTX, C, STX	0.7

Key: OFX = Ofloxacin; CXM = Cefuroxime, AMC = Amoxicillin-Clavulanic acid; CIP = Ciprofloxacin; TE = Tetracycline, NOR = Norfloxacin; CN = Gentamicin; CTX = Cefotaxime; C = Chloramphenicol; STX = Trimethroprim-sulfamethoxazole. MARI = Multiple Antibiotic Resistance Index. S2= Sabuwa 2 poultry farm. C= Cloacal swab.

Table 4.6: Genotypic AntibioticResistance Pattern of the SalmonellaArizonae using PCR

Strain	Serovar	Genotypic resistance patterns	Percentage Resistance(%)
S2C20	*S. Arizonae*	*BlaCMY*-2, *Sul* 1, *dhfr*1, *cat*1.	*Tet* A (66.7), *Cat* 1 (66.7)
S2C12	*S. Arizonae*	*Tet* A, *BlaCMY*-2, *dhfr*1, *cat*1	*Sul* 1 (66.7), *dhfr*1 (100)
S2C19	*S. Arizonae*	*Tet* A, *Bla*CMY-2, *dhfr1*, *Sul* 1.	*BlaCMY*-2 (100)

Key: Tet A – resistance gene for tetracycline, *Sul*1 – resistance gene for sulphonamides, *Bla-CMY*-2 – resistance genes for Beta – lactams, *dhfr*1 – resistance genes for trimethroprim, *cat*1 –resistance genes for chloramphenicol, S2 – Sabuwa poultry 2, C – Cloacal swab.

4.9Risk Factor for the Colonization of the Farms with *Salmonella*

Various risks factors for the colonization of flocks with *Salmonella* were examined using a structured questionnaire. Knowledge of *Salmonella* among the farm handlers is statistically significantly associated with *Salmonella* (OR 13.00; CI 95% 334.5-505.25). Other factors such as administration of antibiotics (OR 0.73; CI 95% 0.02-25.09), faeces in water (OR 0.20; CI 95% 0.04-56.14), hand washing (OR 0.2; CI 95% 0.003-4.65) were protective against *Salmonella* contamination. Statistically, there was no significant association ($p > 0.05$). Feed on the ground in the farm (OR 3.86; CI 95% 0.12-126.74) and contamination of food during transportation or storage (OR 5.00; CI 95% 0.07-366.35) were not statistically significantly associated with*Salmonella* contamination ($p > 0.05$) as shown in Table 4.7.

Results on drug resistance shows that Addition of antibiotics to feed (OR 2.60; CI 95% 0.04-170.4); misuse of drugs (OR 13.60; CI 95% 0.02-26.91), sensitivity testing before drug prescription (OR 2.60; CI 95% 0.04-170.4), taking of drugs according to prescription (OR 13.60; CI 95% 0.02-26.91) and Non-therapeutic use of antibiotics (OR 2.33; CI 95% 0.67-76.7) were not statistically associated with antibiotics resistance ($p > 0.05$), although high odds were recorded (Table 4.8).

Table 4.7: RiskFactorsAssociated with Flocks Colonization by*Salmonella*.

Risk factor	*Salmonella*		OR	(95% CI)	P Value
	Positive(%)	Negative(%)			
Knowledge on Salmonellosis					
Yes	1(50.0)	1(50.00)	13.00	334.50-505.25	0.17
No	0(0.0	6(100)			
Administering antibiotics to their feed					
Yes	0(0.0)	2(100)	0.73	0.02-25.09	0.86
No	1(16.7)	5(83.3)			
Faeces in water and salmonellosis					
Yes	1(12.5)	7(87.5)	0.20	0.003-14.65	0.46
No	0(0.0)	0(0.0)			
Leaving dead chickens in the farm					
Yes	0(0.0)	1(100)	1.44	0.04 - 56.14	0.84
No	1(14.3)	6(85.7)			
Sweeping of feed on the ground in the farm					
Yes	1(25.0)	3(75.0)	3.86	0.12 -126.74	0.45
No	0(0.0)	4(100)			
Washing of hands after handling the birds					
Yes	1(12.5)	7(87.5)	0.20	0.003 - 4.65	0.46
No	0(0.0)	0(0.0)			
Contamination of feed during transportation or storage					
Yes	0(0.0)	0(0.0)	5.00	0.07 - 366.35	0.46
No	1(12.5)	7(87.5)			

OR – Odds ratio CI- Confidence Interval P- P value.

Table 4.8: Some Risks *Factors Associated with Drug Resistance in Salmonella*.

Risk factor	*Salmonella*		OR (95% CI)	P Value
	Positive(%)	Negative(%)		
Addition of antibiotics to feed daily				
Yes	0(0.0)	2(100)	2.60 0.04 -	0.65
No	0(0.0	6(100)	170.39	
Overuse or Misuse of drugs	1(14.3)	6(85.7)	13.60 0.02 - 26.91	0.84
Yes	0(0.0)	1(100)		
No				
Sensitivity testing before drug prescription	0(0.0)	2(100)	2.60 0.04-170.39	0.65
Yes	0(0.0)	6(100)		
No				
Taking of drugs according to prescription	1(14.3)	6(85.7)	13.60 0.02-26.91	0.84
Yes	0(0.0)	1(100)		
No				
Practicing of self-medication				
Yes	1(20)	4(80)	2.33 0.67 - 76.67	0.63
No	0(0.0)	3(100)		
Addition of antibiotics to feed for productivity				
Yes	1(14.3)	6(86.7)	13.60 0.02 - 26.91	0.84
No	0(0.0)	1(100)		
Buying of drugs in shop or pharmacy				
Shop	1(50.0)	1(50.0)	13.00 334.5-	0.17
Pharmacy	0(0.0)	6(100)	505.25	

OR – Odds ratio CI- Confidence Interval P- P value.

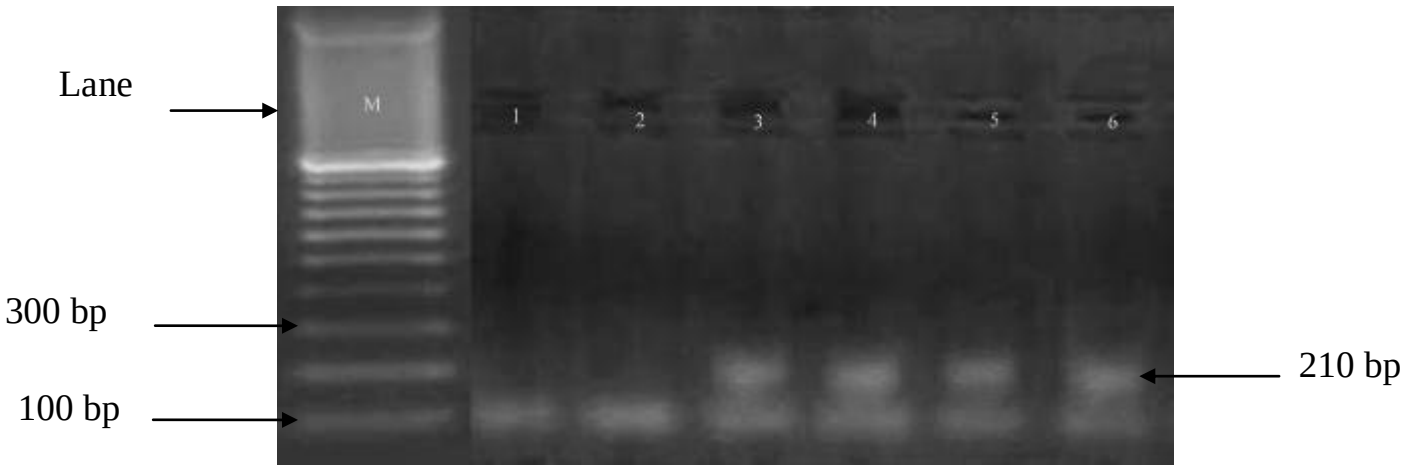


***Plate I**:* Gel electrophoresis of PCRamplicon ofTetA gene inSalmonella*Arizonae*isolates.

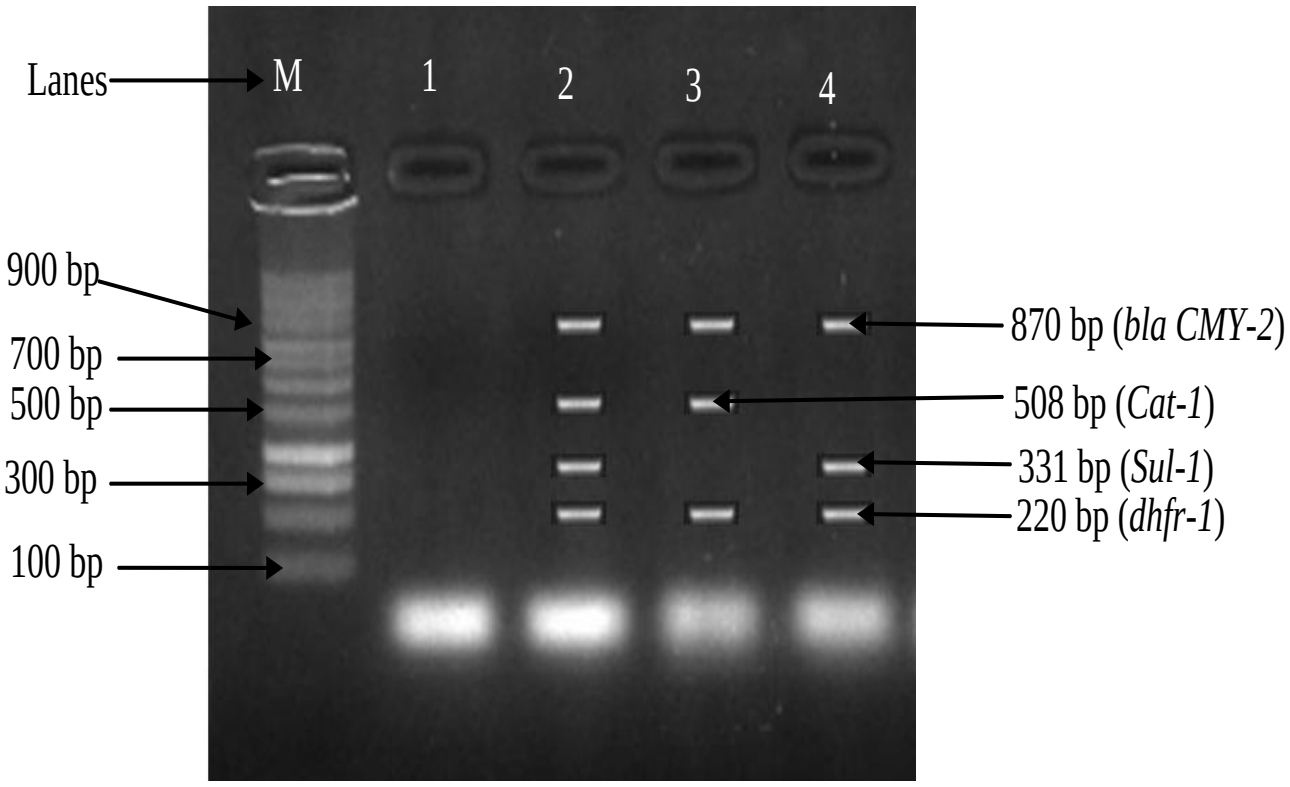


Plate II:Gel electrophoresis of multiplex PCR amplicon of Bla- CMY-*2,* Cat *1,* Sul *1, and* dhfr *1in* Salmonella*Arizonae*isolates.

CHAPTER FIVE

5.0 DISCUSSION

A total of three (3)*Salmonella* isolates belonging to serovar*Salmonella*Arizonae were isolated from160 cloacal swab sample and 160 faecal droppings across the four (4) Local Government Area in Southern part of Katsina State. The isolation of invasive *Salmonella* serotype such as *S.* Arizonae in our study indicate the public health significance of these serovar(Manoj *et al*., 2015) as poultry product contaminated with *Salmonella* may pose serious health hazards (Butaye*et al*., 2006;Grazziani *et al*., 2008;CDC, 2013).*S.*Arizonaehas been implicated in bacteremia, septicemia and gastroenteritis.The isolation of *S.*Arizonae from poultry products as observed in this present study could be due to contamination of the feed by *Salmonella* during transportation or storage (Jones and Richardson 2004; EFSA, 2008). This findings is in line with the report ofJones (2011) and Rickie *et al*. (2013) who however indicated that horizontal transmission of *Salmonella* via faecal matter can occur among poultry flock that are transmitted by insects and rodents.

The overall prevalence of*Salmonella* Arizonae isolated from cloacal swab and faecal droppings in the poultry farms in Southern part of Katsina State was 0.94%. The low prevalence of *Salmonella*Arizonae recorded in this study may be attributed to the improved hygienic condition in the poultry farms and theadministration of antibiotics into the feeds and water in the farm (Carrique and Davis, 2008). The low prevalence of 0.94%obtained in this study could be attributed to the age of the birds that were six weeks and above. In broiler flocks, samples taken at the age of three weeks and below was reported to be more successful in detecting infection than sampling later in the production stage (Gradel *et*

al.,2002). Our study focused on birds that were six weeks and above. This may accountfor the lower prevalence obtained in our study.

Salihu *etal*. (2014) in Nassarawa State, Nigeria, reported a prevalence of 2.5% which was higher than 0.94% reported in this study. In addition, Obi and Ike (2015), reported a prevalence of 1.3% in IntensivelyReared and Backyard Chickens in Nsukka Area, Nigeria.However, relatively lower but similar prevalence of 0.85 % was reported by Aiyelu (2015) who isolated *Salmonella* Arizonae from chicken in Zaria, Kaduna State. The difference in prevalence observed may be due to the fact that the presence and distribution of *Salmonella* serotypes vary from region to region as reported by Uyttendaele*et al*.(1998), Dominquez *et al*.(2002), Agada *et al*. (2014) and Manoj *et al*. (2015). Similarly, the isolation rates might depend on the sampling plan as well as the detection limit of the methods of isolation adopted (Roberts, 1982; Uyttendaele*et al*., 1998; Agada *et al*., 2014; Manoj*et al*., 2015).

Isolation of *Salmonella* from cloacal swab (0.94%) was higher compared to isolation from faecal droppings (0/160). There was a statistically significant difference ($p<0.05$) in the isolation rate between cloacal swab and faecal droppings. The observed differences may be due to the fact that cloacal swab is most ideal in the recovery of *Salmonella* thandroppings as reportedbyLankagbel *et al*. (2014). The overall prevalence of 1.88% recorded from the cloacal swab in the study was higher than 1.33% obtained in intensively reared chicken in

Nsukka, by Obi and Ike (2015). The observed differences may be due to differences in geographical location that may affect serovar distribution.

However, good hygienic practice maintained in the poultry farm may have accounted for the 0% prevalence recorded in the faecal dropping. Meanwhile, an overall prevalence of 38.3% against 0% in our findings was obtained by Orji *et al*. (2005) in Akwa, Anambra state from faecal droppings. The difference observed may be due to good hygienic practice maintained in the poultry farm.The difference in the isolation could be due to the nature of the samples; cloacal swab were direct samples from the birds while faecal droppings could be contaminated with other floral present on the litter. Similarly, since cloacal swabs only obtain a small amount of faeces and *Salmonella* may be present in low numbers or may be non-uniformly distributed in faeces. This method may be likely or relatively insensitive in the recovery of *Salmonella* (Frunk *et al*., 2000; Kotton *et al*., 2006). On the other hand, the lower prevalence obtained may be due to intermittent shedding and low number of organisms excreted by infected birds (Carrique and Davis, 2008).

The result of the study showed that the isolation in the four local government areas were statistically significantly different ($P<0.05$). The isolates in Sabuwa were significantly higher compared to Bakori, Danja and Malumfashi. The isolation may be due to lack of knowledge on the part of the farm handlers on the possible transmission of *Salmonella* amongst the poultry birds (Conan *et al*., 2012). Similarly, leaving dead carcasses in the farm, presence of faeces in water and Lack of proper hygienic conditions on the farms (Conan *et al*., 2012) may have accounted for the isolation of Salmonella from Sabuwa

poultry farms. The isolation of *Salmonella* Arizonae as observed in this study might also be due to contamination of the feed by *Salmonella* via dust during transportation or storage (Jones and Richardson, 2004; EFSA, 2008).

Salmonella were only isolated in one out of the eight farms visited across the four local Government areas in the present study. This could be attributed to the incorporation ofantimicrobials in poultry feeds, intermittent shedding of the Salmonellae, or maintenance ofgood hygiene in the poultry environment. A similar pattern of isolation has been reported byObi and Ike (2015) in Nsukka, Enugu state, Nigeria fromtwo out of the threefarms sampled. Aragaw *et al*. (2010) in Ethiopia also failed to isolate *Salmonella*spp. in two of the three farmssampled and also Curtello *et al*. (2013) in Jamaica alsofailed to isolate *Salmonella* spp. from 1500 cloacal swabs.

Antibiotic administration for the prevention and treatment of infected animals are widely practiced in the chicken industry. But, unfortunately, antibiotics resistances were resulting from the misuse of antibiotics in the poultry industries (Berends *et al*., 2001; WHO, 2014).

The result of the antibiotic resistance test in our present study showed that the three (3) *Salmonella* isolates were resistant to the various antibiotics used in the study. These organisms harbour multiple resistance genes. These findings agree with that of Kristiansen *et al*. (2003) who reported multiple resistance characteristics to chloramphenicol, amoxicillin, sulfonamides and tetracycline. These therefore indicate that fewer drugs would be available to treat infectious diseases caused by *Salmonella* Arizonae. Resistance to

sulphamethoxazole – Trimethoprim among *Salmonella* isolates from poultry have been previously reported in Jos, Nigeria (Agada *et al*., 2014), in Ibadan, (Fashae *et al*., 2010), Senegal (Bada-Alambedji *et al*., 2006), Mexico (Zaidi *et al*., 2006) and in USA (Zhao *et al*; 2003). A lower resistance to SXT (65.2% and 37.5%) than that observed in our study has been reported by Salihu *et al*. (2012) in Nassarawa state, Nigeria and Mengistu *et al*. (2014), in Addis Ababa. The observed resistance to sulphamethoxazole – Trimethoprim is likely due to its high usage in animal production (Threlfall *et al*., 2000).

Among the Fluoroquinolones, resistance to ciprofloxacin (100%) was higher in this study compared to 81.6% in Nassarawa State, Nigeria (Salihu *et al*., 2014), 35% resistance in the USA and 10.2% to 16.8% in Germany (Malorny *et al*., 2003). Also resistance to norfloxacin(66.6%) was higher compared to 30.1% resistance in Nassarawa State, Nigeria (Salihu *et al*., 2014) .The high resistant rate to these antibiotics could be due to the fact that most of the antibiotics used to treat animals or prevent diseases are also used to promote growth. Also, resistance is also connected to the availability of these drugs to people because it is less expensive in the local drug stores and often sold without prescription (Kwaga and Adesiyan, 1984; Salihu *et al*., 2014).The highfluoroquinoloneresistance may also be linked to the fact thatfluoroquinolone antibiotics have found their way intofeeds as a result of increasing smuggling of animal feedsinto Nigeria (Fasure *et al*., 2012). This has threatened theefficacy of fluoroquinolones, the preferred antibiotics fortreatment of *Salmonella*-associated diseases.

Prescription pattern, availability and cost-effectiveness of quinolones as drugs that are usually prescribed in the management of most resistant – bacterial infections were suggested as factors that could be responsible for continued rapid evolution of fluoroquinolones resistant bacteria in Nigeria (Lamikanra *et al*., 2011). The *Salmonella* showed resistance (66.6% to 100%) to the cephalosporins. This finding agrees with the 59.5% found in *Salmonella* species from poultry in the United Arab Emirates, (Vincent *et al*., 2008). Agada *et al.* (2014) also reported a high resistance to cephalosporins in Jos, Nigeria. Also Arlet *et al*. (2006) documented that *Salmonella* species is the most common serovars associated with extended spectrum cephalosporins resistance in poultry. The resistance to cefotaxime (100%) to *Salmonella* Arizonae observed in this finding is higher than 94.73% observed by Sivakumar *et al*., 2012. *Salmonella* serotypes with reduced fluoroquinolones (F) and cephalosporins (C) resistance from poultry have been documented (Akinyemi *et al*., 2007). The emergence of F and C resistant *S.*Arizonae among poultry is a serious threat because this resistant bacterium from food producing animal increase the chances of these bacteria transferring to humans (Tollefson and Flynn, 2002). Resistance to multiple classes of antimicrobials, particularly those involving the cephalosporins and fluoroquinolones (Angulo *et al*., 2000) have severely limited treatment options.

The resistance to tetracycline (100%) observed in the *Salmonella* Arizonae isolates is higher than those reported in various studies; 63.3% in Jos, Nigeria (Agada *et al*., 2014), 46% in Senegal (Bada-Alambedji *et al*., 2006) and 36% in Portugal (Antunes *et al*., 2003). The results also contradicts 33.3%, 6% 52.5% and 58.1% reported by Addis *et al.* (2011), Wandili *et al*. (2013) , Mengistu *et al*.(2014) and Salihu *et al*. (2014). Tetracycline is the

most commonly used antibiotics for prevention and treatment of common diseases in poultry. Resistance to these agents is likely because these drugs are approved for use in broilers for the purpose of growth promotion (Jones, 2003).

The susceptibility to Gentamicin (33.3%) observed in the *Salmonella* Arizonae isolate is lower than 97.5% susceptibility reported by Mengistu *et al*., (2014). However, the resistance to gentamicin (66.6%) reported in this study is lower than the results of Salihu *et al*. (2014), Asrat (2008), and Beyene *et al.* (2011) who reported 76.1%, 75.6% and 74.3% resistances respectively. However, 66.6% resistance observed in our findings was higher than 10.58% resistance by the agent reported in Cote d Ivoire by Bonny *et al*. (2015). The differences in the pattern could be due to the geographical location where the isolate was obtained.

Asrat (2008), Beyene *et al*. (2011) and Salihu *et al*. (2014) reported 83.7%, 81.4% and 66.2% resistance against chloramphenicol. This result disagrees with the resistance to chloramphenicol (100%). However, a lower level of resistance (6%) against chloramphenicol was observed in Kenya by Wandili *et al*., (2013). This may be due to the fact that the antibiotic resistance genes have altered the microbial community by continuous antibiotic usage and the effects still persist for years even after discontinued use (Sommer and Dantas, 2011).

The result of these findings showed that the isolatesrecovered from the broiler birds were resistant to the antibiotics used in this study. This statement is consistent with that ofDiarra *et al*.(2007) whofound that isolatesrecovered from broiler chickens showed some level of

multipleantibiotic resistances.Additionally, our results of multi drug resistant *Salmonella*recorded in this study was similar to the findings of other researchers (Davis *et al*., 2005; Akinyemi *et al*., 2007; Mohammed *et al.,* 2010). In many of these studies, non typhoidal *Salmonella* species were found to be resistant to Amoxicillin, co-trimoxazole, chloramphenicol, ciprofloxacin, sulphamethoxazole–Trimethroprim, Tetracycline, Cefotaxime. Similarly Okeke *et al*. (2005) also reported that multiple antimicrobial resistancesare common in *Salmonella* species in developing countries.

Multidrug resistant *Salmonella*Arizonae reported in this study agrees with the findings of Hemen *et al*. (2012) in Benue State Nigeria who reported the isolation of multi drug resistant *Salmonella* in poultry environment,thatwas resistant to chloramphenicol, Augmentin and Amoxicillin.

Fasure *et al*. (2012) also revealed the emergence of multi - drug resistant *Salmonella* serotypes in Ogun state, Nigeria. In his study, resistant to ampicillin (100%), Tetracycline (90.6%), and Fluoroquinolones (100); resistant strains were observed. Similarly, Mohammed *et al*. (2010), also reported that *Salmonella* isolate from a poultry farm in Jos was resistant to Gentamicin and Ciprofloxacin. It was observed that 100% of the *Salmonella* species were resistant to the antibiotics in the study. This is similar to the findings of Emam and Mohammed (2008), who reported the same (100%). However, the findings disagrees with the reports of Addis *et al*. (2011), Wandili *et al*. (2013) and Mengistu *et al*. (2014) who reported 83.3%, 25% and 27.5% resistances among *Salmonella* species respectively. The routine use of antibiotics for agricultural purposes, and disease

prevention could be responsible for the emergence of multi-drug antibiotic resistance (Akinyemi *et al*., 2007; Oluyege *et al*., 2010; Fasure *et al*., 2012; Shah and Korejo, 2012).

The *Salmonella* isolates were resistant to 7-10 antibiotics respectively. This is at variance with the findings of Emam and Mohammed who found 68% of *Salmonella* isolates to be resistance to 4-5 antibiotics. Similarly, the antibiotic resistance to 7-10 antibiotics agrees with that reported by Agada *et al*. (2014) who reported that five of the *Salmonella* serovars incriminated in animal salmonellosis was resistant to more than five different antibiotics. Also the antibiotic resistance profile to 7-10 antibiotics in this study is similar to the one obtained by Kohinur *et al*., 2010. In his study, he reported that strains isolated from cloacal swab exhibit wider antibiotic spectrum resistance than strains isolated from other sources. The report is consistent with the isolation of *S.* Arizonae from cloacal swabs.

The antimicrobial susceptibility pattern of the *Salmonella* strain isolated indicated that a large proportion of the isolates were resistant to the drugs tested. It is estimated that nearly 90% of all antibiotics used in food animals are given at sub therapeutic concentration prophylactically or to promote growth (Shroeter *et al*., 2004; Su *et al*., 2004; Shah and Korejo, 2012). This therefore supports a high level of resistance in the study. The result of the antibiotic resistance in our study implies that this organism harbours multiple resistance genes *(*among other possible resistance mechanism).These findings agree with that of Kristianseen *et al*. (2003) who reported multiple resistance characteristics to chloramphenicol, Amoxicillin, Sulfonamides and tetracycline.

Polymerase Chain Reaction detection of antibiotic resistance genes analysis revealed that 100% of the isolates had *bla-CMY* genes of the AmpC family. *Salmonella* resistance to cefuroxime, cefotaxime and amoxicillin-clavulanic acid could be due to the secretion of beta-lactamases into the periplasmic fluid. This enzyme hydrolyses the beta-lactam ring in beta-lactams into beta – amino acids which have no anti-microbial activity. The genes encoding for beta lactamases are typically carried on plasmids (Mascaretti, 2003). The presence of beta-lactamases in this study is in line with the findings of Abdel-Maksoud *et al.,* (2015); Miko *et al*., (2005); and Chen *et al*., (2004) who reported the presence of these genes in *Salmonella* isolates from poultry samples. The detection of these genes in this study may be due to the widespread use of antibiotics in animals for disease prevention and animal production. Thus, the spread of resistantce genes in poultry flock (Angulo *et al*., 2000) that can infect human directly or transfer antibiotic resistance genes to other human pathogen (Sturenberg and Mark, 2003).

Chloramphenicol has been in use in human and veterinary medicine for the treatment of salmonellosis for a long time but has now given rise to resistance strains. Up to 66.7% of the *Salmonella* Arizonae possesses *Cat* 1 genes. *Salmonella* resistance to chloramphenicol is mediated by the presence of plasmid coded genes coding for the enzymes called chloramphenicol acetyl trasferases (Guerra *et al.,* 2000; Abatcha *et al*., 2014). The presence of this gene in the *Salmonella*Arizonae in this finding is consistent with those reported by other researchers; Alcaine *et al*. (2005), Doublet *et al*. (2004) also reported the presence of these genes in *Salmonella* that are multi-drug resistant. These genes are found in

Salmonella genomic islands as well as on different plasmids due to its high mobility (Mounier *et al*., 2003). The observed similarity in this finding with the earlier ones may be likely due to the presence of the genes on plasmid carrying multiple resistance genes (Abatcha *et al*., 2014).

Tetracycline is clinically known as a broad spectrum antibiotics that is effective against many gram positive and gram negative bacteria (Chopra and Roberts, 2001; Mascaretti, 2003). However, the rise of antibiotic resistant bacteria has seriously inhibited their usage (Abatcha *et al.,* 2014).Up to 66.7% of the *Salmonella* isolates possess *Tet*A gene, indicating that this gene is diffused in *Salmonella* strains. This suggests that *tet*(A) tetracycline resistance circulating *Salmonella* strains are found in poultry. Similarly, *Tet* A genes are located on transferable plasmids (Guerra *et al*., 2002), allowing them to be easily transferred and widespread among *Salmonella*. Also, Carattoli *et al*. (2002), Chen*et al*. (2004), and Pezzella *et al*. (2004) reported that *Tet*A genes are found mostly in isolates that displays multi-drug resistance. This is consistent with our findings of 7,9,10 multi-drug resistant phenotypes recorded. Therefore, the presence of these genes is an important identification marker in identifying serious *Salmonella* infection (Abatcha *et al*, 2014).

Sulfonamides are classes of antibiotics that are bacteriostatic. They act by inhibiting enzymes involved in the synthesis of tetrahydrofolic acid (Abatcha *et al*, 2014). Sulfonamides inhibit dihydropterate, synthetase (DHPS). 66.7% of the *Salmonella* isolates detected in the study have *Sul* 1 gene. The resistance of *Salmonella* to sulfonamide is

attributed to the presence of an extra *Sul* gene which expresses an insensitive form of DHPS (Mascaretti, 2003; Antunes *et al*., 2005). Also class I integrons that carry antibiotic resistance genes have been associated with *Sul* 1 gene. Thus, the present result of 66.7% *Salmonella* possession of *Sul* 1 gene is lower than 100% *Sul* 1 gene present in *Salmonella* isolates reported by Adesiji *et al*. (2014) in their study on antimicrobial resistance genes associated with *Salmonella* spp. isolated from human, poultry and sea foods. The difference may be due to difference in location where study was conducted.

Abatcha *et al*. (2014) reported that the combinations of trimethoprim and sulfonamides have been used in veterinary practice for long because of their wide broad spectrum of activity against gram positive and gram negative bacteria.All(100%) of the *Salmonella* isolates possess *dhfr* 1 gene. Trimethoprim resistance is attributed to the expression of dihydrofolate reductance (*dhfr*) which does not bind trimethoprim (Mascaretti, 2003).

Knowledge of salmonellosis among farm handlers have been reported to decrease the risk of *Salmonella* infection in poultry farms (Conan *et al*., 2012). Our findings showed that knowledge of salmonellosis is associated or at high odds of*Salmonella* infection among poultry flocks (OR 13.00, CI 95% 334.50-505.25). Although, the association is not statistically significant ($p>0.05$).This association could be attributed to the low level of education and lack of awareness on the prevention and control of *Salmonella* infection among the farm handlers (Charles and Takayuki, 2010; Mai *et al*., 2013). Similarly, Agada *et al*. (2014) also reported an association between knowledge of salmonellosis and *Salmonella* infection in Jos, Nigeria.

Administration of antibiotics against bacterial infection had been reported to reduce the number of colonized birds and shedding of bacteria in birds (Velge *et al*., 2005). Administration of antibiotics (OR 0.73; CI 95% 0.02-25.09) is significantly protective against *Salmonella* infection. Therefore, the lower prevalence recorded in the study might be due to administration of antibiotics to the birds prior to the collection of the sample. However, abuse in theadministration of antibiotics promotes drug resistance leading to longer and prolonged treatment of infected birds.

The presence of feaces in water is a high risk factor in the contamination of flock with *Salmonella* (Manoj *et al*., 2015). However, contaminated water through faecal material showed a protective effect against *Salmonella* infection (OR 0.20, CI 95% 0.003-14.65). Statistically, the association is not significant (p>0.05).Contaminated water through faecal matter has been reported as an horizontal means of *Salmonella* infection in poultry farms(Nakamura*et al*., 1997; Jones, 2011; Rickie *et al*., 2013). The prevalence of 0.94% of *Salmonella*Arizonae reported could be due to the fact that the *Salmonella* infection originated from water contaminated by faeces. Despite the lower prevalence and a protective effect obtained in the study, poultry owners are encouraged to use clean water in the farms to prevent contamination.

Leaving dead chickens (carcasses) in the farm is a source of contamination of other flocks with *Salmonella* in the poultry farm (Baggesen *et al*., 1992; Angen *et al*., 1996; Orji*et al*.,

2005; Payne *et al*., 2006;Fashae*et al*., 2010; Selvaraj *et al*., 2010; Hassanein *et al*., 2011). Although our findings showed that presence of carcasses in the farm are at high odds of contaminating the poultry farms with *Salmonella* (OR 1.44, CI 95% 0.04-56.14). Although, the association is not statistically significant (p>0.05). Despite this, a prevalence rate of 0.94% *Salmonella* Arizonae was recorded. This may be due to the continual presence of *Salmonella* in the carcasses left in the farm that could be transmitted to other flock as a result of poor hygienic practice in the farms (Davis and Breslin, 2003b). Our findings agrees with the report of Agada *et al*. (2014) who reported that poultry farms previously contaminated have high risks of infection than that not infected.

Presence of feed on the ground in the poultry farm is associated with *Salmonella* contamination (Manoj *et al*., 2015). The result of our finding showed that the factor is associated withthe *Salmonella* infection (OR 3.85; CI 95% 0.12-126.74).The association is not statistically significant (P>0.05).However, a lower prevalence of *Salmonella* was reported. This may be due to the fact that some birds shed the *Salmonella* that could contaminate the feeds and further spread by insect vectors within the farm (Manoj *et al*., 2015). A similar result by Okonkwo *et al*. (2010) in Calabar, Cross River State Nigeria, also showed that *Salmonella* contamination within poultry farm could be associated with the presence of feed on the ground that attracted insect vectors that are carriers of *Salmonella*.

Horizontal transmission of *Salmonella* has been reported to occur via farm handlers while handling the birds (Nakamura *et al*; 1997). Hand washing after handling the birds is statistically protective against *Salmonella* contamination in the farms (OR 0.20; CI 95% 0.002-4.65). Although a lower prevalence rate of 0.94% was recorded. This perhaps may be due to the hand washing technique employed by the farm handlers which helps to reduce the rate of transmission of the bacteria. Health authorities are encouraged to enforce hand washing procedures after handling the birds in the poultry farms in order to reduce the rate of *Salmonella* contamination in the farms.

Our findings showed an association between feed contamination and *Salmonella* infection in the poultry farms (OR 5.00; CI 95% 0.07-366.35). Statistically, the association is not significant (P>0.05). Although recent findings reported that contamination of food during transportation or storage is an important risk factor for *Salmonella* infection in poultry (Manoj *et al*., 2015). However, a prevalence of 0.94% *Salmonella* Arizonae was reported in this work. This could be due to inefficient transportation of feeds and storage that might favour dust and rodents gaining access to the feeds in the poultry house (Hoelzer *et al*., 2011; Mather *et al*., 2013; Manoj *et al*., 2015).

The findings showed that addition of antibiotics to feed is a risk factor for drug resistance (OR 2.60; CI 95% 0.04-170.39). Addition of antibiotics has been reported to reduce the number of colonized flocks and shedding of *Salmonella* (Agada *et al*., 2014). Meanwhile, 7-10 multi-drug resistance profile was recorded in the findings. This could be attributed to

indiscriminate use of antibiotics in the poultry farms. Antibiotics as growth promoters are of utmost concern because its usage in sub-therapeutic levels, stimulate survival of resistant bacteria in the ecosystem (Al-Ferdous *et al*., 2013).

The WHO observed an alarming rateof resistant *Salmonella* strains due to abusive use of antibiotics (WHO, 2014). Overuse and misuse of drugs showed high odds of causing resistance to antibiotics (OR 13.60; CI 95% 0.02-26.91). Previous finding had shown that misuse or over use of drugs are linked to antibiotic resistance and even high mortality rate in poultry farms (Muhammed *et al*., 2010). The present study showed a high multi-drug resistance to the antibiotics used in the study which could be attributed to the abusive use of the drugs, although the association was not statistically significant ($p>0.05$).

Susceptibility testing before drug prescription showed a high risk of causing drug resistance (OR 2.60; CI 95% 0.04-170.40). The importance of susceptibility testing cannot be over emphasized. It provides guidance to the selective use of antibiotics for therapy and choice in managing diseases with fewer side effects (Edward and Ewing, 2003). However, 7-10 multi-drug resistance profile was recorded in the study. This may be due to negligence in the routine culture of major zoonotic pathogens such as *Salmonella*, by the health authorities which have reduced awareness on the type of antibiotics to be employed in managing *Salmonella* infection (Muktar *et al.,* 2015). Health authorities should be advised to employ stringent measures in regulating susceptibility testing before administering drugs.

Taking of drugs according to prescription is not statistically significant with drug resistance strains ($p>0.05$).Although, it is a risks factor for drug resistance strain (OR 13.60; CI 95% 0.02-26.91). However, the increase in antimicrobial resistance in developing countries is possibly caused by availability of drugs without prescription (Muktar *et al*., 2015).The isolates obtained showed resistance to the antibiotics used in the study. This may be due to the availability of drugs to people in Nigeria without prescription in the local store. In view of these health authorities are enjoined to apply stringent measures in the control of antibiotics used in humans and poultry houses.

There is association between Non-therapeutic use of antibiotics and drug resistance (OR 2.33 CI 95% 0.67-76.7). However, in a study by GAO (2011), it was documented that the non-therapeutic use of antibiotics in animals in the absence of bacterial disease is among the practice of greatest concern for the development of antibiotic resistance. The high antibiotic resistance to the bacterial agent may be due to addition of antibiotics to the water or feed in the farms in the absence of disease.

Buying of drugs in shops or pharmacy is associated with drugs resistance (OR 13.00; CI 95% 334.5-505.25). Buying of drugs from local shops has been linked with drug resistance. This probably explains the multi-drug resistance to the antibiotics employed in the study. This association is likely due to lack of knowledge on the effect of buying drugs in shop rather than pharmacy by the poultry owners. Poultry owners are thereby encouraged to buy drugs in pharmacy.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATION

6.1 Conclusions

The study concludes that *Salmonella*Arizonae was present in the poultry farms with a prevalenceof 0.94%. Cloacal swab was discovered to provide a better source of *Salmonella* recovery than dropping. A prevalence of 1.88% was recorded for the *Salmonella* Arizonae from the swabs. The *Salmonella* Arizonae (100%) were resistant to all the antibiotics used in the study namely; amoxicillin clavulanic acid, ciprofloxacin, chloramphenicol, cefuroxine, cefotaxine, gentamicin, norfloxacin, ofloxacin, sulphamethoxazole – trimethroprim, and tetracycline. The *Salmonella* Arizonae possess antibiotic resistance genes to tetracycline, chloramphenicol, Beta-lactams, sulphomethoxazole and trimethroprim. The presence of these genes confirms that the isolates were genotypically multi-drug resistant to all the antibiotics employed in the study. Knowledge of *Salmonella*, contaminated feed during transport or storage, presence of feed on the ground in the farm, presence of carcasses are strongly associated with transmission of *Salmonella*. Administering of antibiotics to the birds, faeces in water, washing of hands after handling the birds are not statistically associated with *Salmonella* infection in the poultry farms.

Misuse of drugs, addition of antibiotics to feed, sensitivity testing, non-therapeutic use of drugs and buying of counterfeit drugs are associated with drug resistance. Although the association are not statistically significant ($P>0.05$).

6.2 Recommendations

Based on the findings from the study the following recommendations were made;

1. Health authorities should restrict antibiotic use in the farm. They should educate the poultry owners, farm handlers and the consumers (especially in the preparative processes) on the dangers posed by birds that possess antibiotic resistance genes in the food chain
2. Poultry litter, chicken carcasses and other related samples should be equally screened for*Salmonella* spp.
3. More researches should be conducted on the susceptibility patterns of *Salmonella* spp to determine the most suitable antibiotics for treating *Salmonella* infection in poultry farms.
4. Detection of other antibiotic- resistance genes should be tried on the *Salmonella* isolates to avoid the spread of resistant strains.
5. Further studies should be conducted on risks factors associated with contamination and drug resistance in *Salmonella* infection in poultry farms.

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APPENDICES

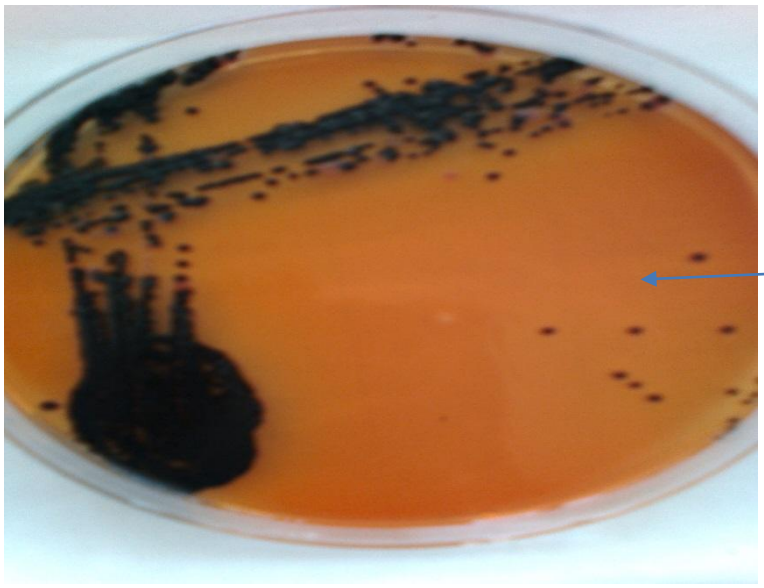
Appendix I: Percentage susceptibility of the Enterobacteriaceae from the poultry farms in southern Part of Katsina state.

Isolates		Antibiotics									
		OFX (%)	CXM (%)	AMC (%)	CIP (%)	TE (%)	NOR (%)	CN (%)	CTX (%)	C (%)	SXT (%)
	Resistant	28.6	100	100	57.1	85.7	100	57.1	28.6	42.9	100
Proteus	Intermediate	71.4	0.0	0.0	42.9	0.0	0.0	42.9	57.1	0.0	0.0
mirabilis (7)	Susceptible	0.0	0.0	0.0	0.0	14.3	0.0	0.0	14.3	57.1	0.0
	Resistant	0.0	100	100	50	100	100	50	50	0.0	100
Escherichia	Intermediate	100	0.0	0.0	50	0.0	0.0	50	5	50	0.0
coli (2)	Susceptible	0.0	0.0	0.0	0.0	0.0	0.0	0.0	5	50	0.0
Proteus	Resistant	66.7	100	100	66.7	100	100	66.7	33.3	66.7	100
vulgaris (3)	Intermediate	33.3	0.0	0.0	0.0	0.0	0.0	33.3	33.3	0.0	0.0
	Susceptible	0.0	0.0	0.0	33.3	0.0	0.0	0.0	33.3	33.3	0.0
Cronobacter	Resistant	0.0	100	100	0.0	0.0	0.0	0.0	100	100	0.0
sakazakii (1)	Intermediate	0.0	0.0	0.0	100	0.0	0.0	0.0	0.0	0.0	0.0
	Susceptible	100	0.0	0.0	0.0	100	100	100	0.0	0.0	100
Citrobacter	Resistant	0.0	100	100	0.0	100	100	0.0	100	0.0	100
freundii (1)	Intermediate	100	0.0	0.0	100	0.0	0.0	0.0	0.0	0.0	0.0
	Susceptible	0.0	0.0	0.0	0.0	0.0	0.0	100	0.0	100	0.0
Pantoae	Resistant	33.3	100	100	0.0	66.7	66.7	33.3	66.7	33.3	66.7
agglomerans	Intermediate	66.7	0.0	0.0	100	0.0	0.0	33.3	0.0	0.0	0.0
(3)	Susceptible	0.0	0.0	0.0	0.0	33.3	33.3	33.3	33.3	66.7	33.3

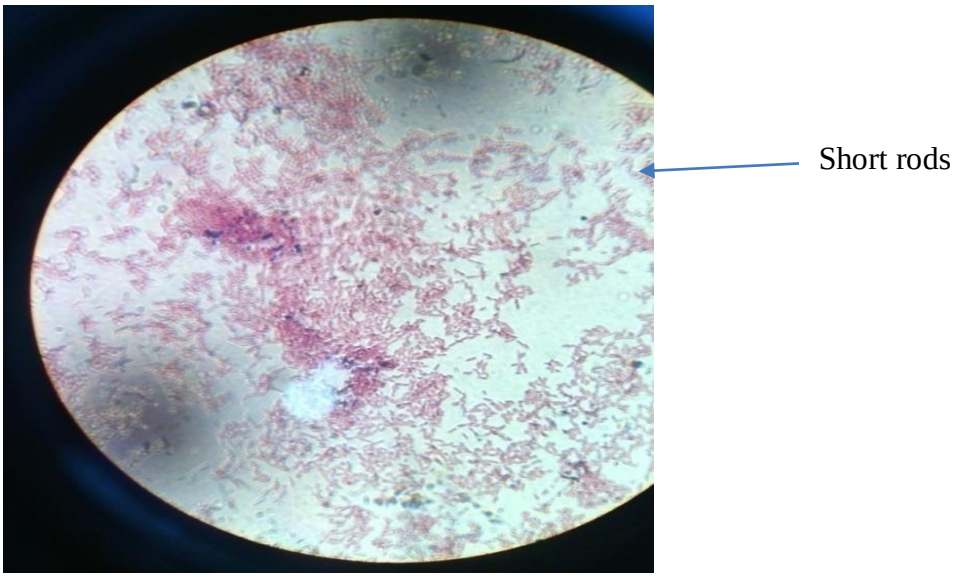
Key: OFX = Ofloxacin; CXM = Cefuroxime, AMC = Amoxicillin-Clavulanic acid; CIP = Ciprofloxacin; TE = Tetracycline, NOR = Norfloxacin; CN = Gentamicin; CTX = Cefotaxime; C = Chloramphenicol; STX = Trimethroprim-sulfamethoxazole

Appendix II: Multidrug Resistance patterns of the Enterobacteriaceae from the poultry farms in southern part of Katsina state

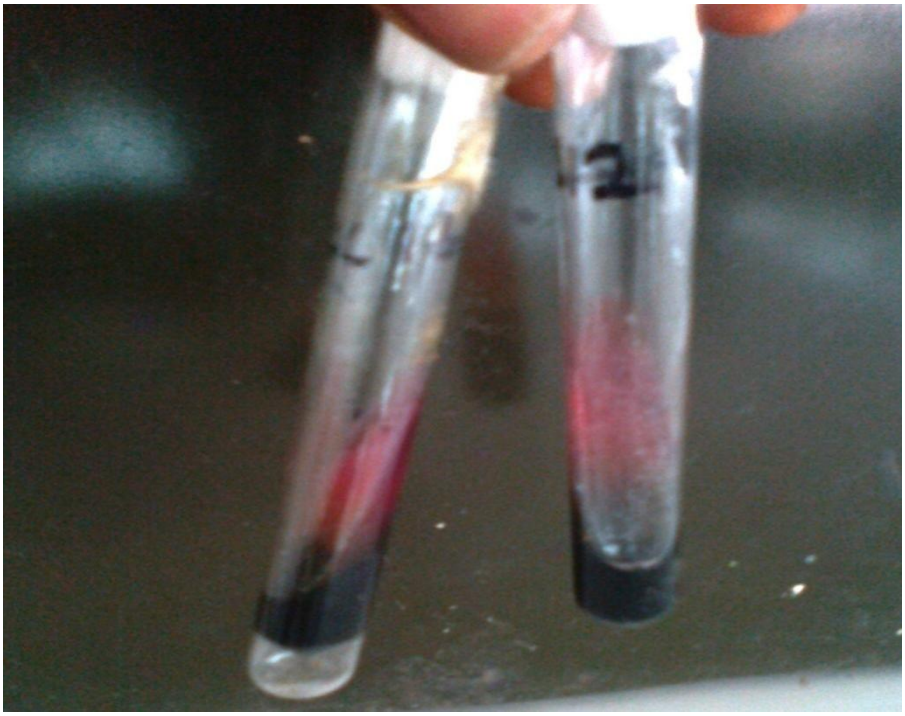
Species	No of isolates	No of antibiotics	Resistance pattern	Percentage (%)
P. mirabilis (7)	1	7	OFX, CXM, AMC, CIP, TE, NOR, SXT	14.3
	1	4	CXM, AMC, NOR, SXT	14.3
	1	9	CXM, AMC, CIP, TE,NOR, CN, CTX, C, SXT	14.3
	1	8	CXM, AMC, TE, NOR, CN, CTX, C, SXT	14.3
	1	7	CXM, AMC, TE, NOR, CN, C, SXT	14.3
	1	6	CXM, AMC, CIP, TE, NOR, SXT	14.3
	1	8	OFX, CXM, AMC, CIP, TE, NOR, CN, SXT	14.3
				100%
E. coli (2)	1	5	CXM, AMC, TE, NOR, SXT	50
	1	8	CXM, AMC, CIP, TE, NOR, CN, CTX, SXT	50
				100%
P. vulgaris (3)	1	9	OFX, CXM, AMC, CIP, TE, NOR, CTX, C, SXT,	33.3
	1	9	OFX, CXM, AMC, CIP, TE, NOR, CTX, C, SXT,	33.3
	1	6	CXM, AMC, TE, NOR, CN, SXT	33.3
				100%
C. Sakazakii(1)	1	4	CXM, AMC, CTX, C	1**00%**
C. freundii (1)	1	6	CXM, AMC, TE, NOR, CTX, SXT	**100%**
P. agglomerans	1	3	CXM, AMC, CTX	33.3
(3)	1	8	OFX, CXM, AMC, TE, NOR, CN, C, SXT	33.3
	1	7	CXM, AMC, TE, NOR, CN, CTX, SXT	33.3
				100
TOTAL	20			**100%**



Appendix III: Characteristic red colonies with black center on XLD plate of SalmonellaArizonae.



Appendix IV: Gram staining of Salmonella using microscopy × 40 objectives lens



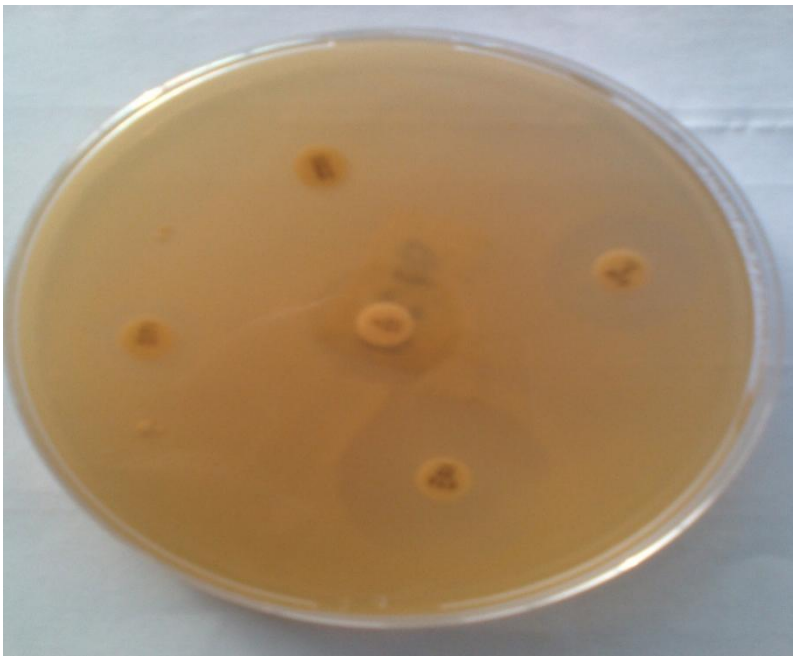
Appendix V: Triple sugar fermentation for the preliminary identification of *Salmonella* spp.



Appendix VI: Microgen GNA identification of *Salmonella* spp. The colour signifies the positive and negative reactions of the isolates to the biochemical reagents in the Microgen kit.



Appendix VII: Biochemical test; Citrate and TSI test for the preliminary identification of *Salmonella* spp.



Appendix VIII: Antibiotic susceptibility test for *Salmonella* Arizonae.

AppendixIX: Cultural, Morphological and Biochemical Characteristics of the Isolates

Isolate code	Growth on XLD	GRM	U	TSI	MR	VP	C	I	Inference
S_2C_{12}	Red colonies with black centre	Gram negative rods, appearing singly	-	$\frac{K}{AG} + H_2S$	+	-	+	-	*Salmonella* spp
S_2C_{18}	Red colonies with black centre	Gram negative rods, appearing singly	-	$\frac{K}{AG} + H_2S$	+	-	+	-	*Salmonella* spp
S_2C_{20}	Red colonies with black centre	Gram negative rods, appearing singly	-	$\frac{K}{AG} + H_2S$	+	-	+	-	*Salmonella* spp

Key: K –Alkaline TSI- Triple sugar iron agar

A-Acid MR- Methyl red

G-Gas VP- Voges proskauer

H_2S-Hydrogen sulphide XLD - Xylose lysine Desoxycholate

U - Urease GRM – Gram reaction and morphology

C – citrate I - indole

Appendix X: Microgen GNAIdentificationof *Salmonella* SpeciesandOtherEntericBacteria. N =20

Code	Lys	Orn	H_2S	Glu	Mann	Xy1	ONPG	Ind	Urea	V.P	Cit	TDA	Octal code	ID
DJC_9	-	-	+	+	+	+	+	-	+	-	+	+	1753	*C. freundii*
S_2C_{14}	-	+	+	+	+	+	+	-	+	+	+	+	3757	*C. sakazakii*
B_IFD_{20}	+	+	-	+	+	+	+	+	-	-	-	-	6760	*E. coli*
B_IFD_6	+	-	-	+	+	+	+	+	-	-	-	-	4760	*E. coli*
B_2C_{16}	-	-	+	+	-	+	+	+	+	+	-	+	1575	*P. vulgaris*
S_1D_{18}	-	-	-	-	-	+	-	-	+	-	-	+	0111	*P. vulgaris*
S_1C_{20}	-	-	+	+	-	+	+	-	-	-	+	+	1 543	*P .vulgaris*
DJ19	-	-	+	+	+	+	+	-	+	+	+	+	1757	*P.agglomerans*
S_1C_{11}	-	-	+	+	+	+	+	-	+	+	+	+	1757	*P.agglomerans*
B_1D_1	-	-	+	+	+	+	+	-	+	+	+	+	1757	*P.agglomerans*
S_2D_{18}	-	+	+	+	-	-	-	-	+	-	+	+	3413	*P. mirabilis*
S_1D_{11}	-	+	+	+	-	-	-	+	-	+	+	+	3413	*P. mirabilis*
B_1C_{18}	-	+	+	+	-	-	-	-	+	-	+	+	3413	*P. mirabilis*
S_2C_2	-	+	+	+	-	+	-	-	+	-	+	+	3513	*P .mirabilis*
S_1C_{13}	-	+	+	+	-	+	-	-	+	-	+	+	3513	*P .mirabilis*
DJC_{10}	-	+	+	+	-	+	+	+	-	+	+	+	3567	*P. mirabilis*
B_1C_{20}	-	+	+	+	-	+	-	+	+	+	-	+	3535	*P .mirabilis*
S_2C_{18}	+	+	+	+	+	-	+	+	-	-	+	+	7663	*S. Arizonae*
S_2C_{12}	+	+	+	+	+	+	+	+	-	-	+	+	7773	*S. Arizonae*
S_2C_{19}	+	+	+	+	+	+	+	+	-	-	+	+	7763	*S. Arizonae*

Key: DJ – Danja, C – Cloacal swab, S_1 – Sabuwa poultry 1, B_1-Bakori Poultry 1 B_2 – Bakori Poultry 2, S_2 – Sabuwa poultry 2, FD – Faecal dropping, Lys- Lysine, Orn- Ornithine, H_2S- Hydrogen sulphide, Glu- Glutamine, Mann- Mannose, Xyl- Xylose, ONPG-, Ind- Indole, Urea- urease, V.P- Voges proskaeur , Cit- Citrate, TDA- Octal code= isolate code, n= number of isolates, Id= Identification of organism.

Appendix XI

QUESTIONNAIRE

Ahmadu Bello University Zaria,

Department of Microbiology,

Kaduna state.

09 January, 2015.

Dear respondent,

I am a student of the above mentioned institution, conducting a research study on the isolation and molecular detection of antibiotic resistant Salmonella species from some poultry farms in southern part of Katsina state, Nigeria.

Instruction: Do not write your name, respond to the question and statement on this paper by ticking (√) or writing on the space provided, all information collected would be use for research purpose only and would be treated as confidential. The questionnaire is divided into two (2) sections A and B.

SECTION A (Risk factors on drug resistance)

1. Do you add antibiotics to the feed daily?
   a. Yes [ ] b. No [ ]
2. Do your doctors prescribed drugs before you use them?
   a. Yes [ ] b. No [ ]
3. Do your doctors take cultures for sensitivity testing before prescribing antibiotics?
   a. Yes [ ] b. No [ ]

4. Do you take your drugs according to doctor's prescription?

a. Yes [ ] b. No [ ]

5. Do you take drugs in the absence of disease(self medication)?

a. Yes [ ] b. No [ ]

6. Do you add antibiotics to animal feed to increase productivity?

a. Yes [ ] b. No [ ]

7. Where do you buy your drugs?

a. Shop [ ] b. Pharmacy [ ]

SECTION B (The risk factors associated with contamination of farm with *Salmonella)*

8. Do you have any knowledge whether salmonella can be transmitted from animals to man?

a. Yes [ ] b. No [ ]

9. Do you add antibiotics to there feed?

a. Yes [ ] b. No [ ]

10. Whenever you observe faeces in water do you change the water?

a. Yes [ ] b. No [ ]

11. Do you leave dead chickens in the farm for sometimes before disposing it?

a. Yes [ ] b. No [ ]

12. When the feed fell on the ground do you sweep it immediately?

a. Yes [ ] b. No [ ]

13. Do you wash your hands after handling the birds?

a. Yes [ ] b. No [ ]

14.Is the feed contaminated during transport or storage?

a. Yes [ ] b. No [ ]