

**PREVALENCE AND ANTIMICROBIAL SUSCEPTIBILITIES OF
SALMONELLA SPP IN POULTRY IN**

ZAMFARA STATE NIGERIA

BY

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**DEPARTMENT OF COMMUNITY MEDICINE,
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DECLARATION

I declare that this Thesis entitled “Prevalence and antimicrobial susceptibilities of *Salmonella* spp. in poultry in Zamfara, Nigeria”, has been performed by me in the Department of Community Medicine, Ahmadu Bello University, Zaria under the supervision of Prof. Jacob Kwaga and Prof. E.C. Okolocha. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented to another degree or diploma at any University.

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CERTIFICATION

This Thesis entitled “PREVALENCE AND ANTIMICROBIAL SUSCEPTIBILITIES OF *SALMONELLA* SPP IN POULTRY IN ZAMFARA STATE, NIGERIA” by Samuel Sha’aibu meets the regulations governing the award of the degree of Masters of Public health (Field Epidemiology) of the Ahmadu Bello University, and is approved for its contribution to knowledge and literacy presentation.

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DEDICATION

To my parents, my best friend, wife Ade, and my son Shamir for their tireless support and understanding during my study.

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LIST OF ACRONYMS

| | |
|--------|---|
| BPW | Buffered Peptone Water |
| CDC | Centers for Disease Control and Prevention |
| CLSI | Clinical and Laboratory Standards Institute |
| DNA | Deoxyribonucleic Acid |
| ELISA | Enzyme-linked Immunosorbent Assay |
| FAO | Food and Agricultural Organization |
| FDA | Food and Drug Administration |
| FSIS | Food Safety Inspection Service |
| HACCP | Hazard Analysis and Critical Control Point |
| LBM | Live Bird Market |
| MOH | Ministry of Health |
| MHA | Mueller Hinton Agar |
| MRSI | Methicillin-Resistant <i>Staphylococcus</i> Infections |
| MR-VP | Methyl Red- Voges Proskauer |
| NAFDAC | National Agency for Food and Drugs Administration and Control |
| ONPG | o-nitrophenyl β - d-galactopyranoside |
| OIE | World Organization for Animal Health |
| PCR | Polymerase Chain Reaction |
| PAHO | Pan American Health Organization |
| RNA | Ribonucleic Acid |

| | |
|------|---|
| RVB | Rappaport Vassiliadis Broth |
| SIM | Sulphur Indole Motility |
| TDA | Tryptophan Deaminase |
| TSI | Triple Sugar Iron |
| USDA | United States Department of Agriculture |
| WHO | World Health Organization |
| WTO | World Trade Organization |

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SUMMARY

Salmonella is an important zoonotic pathogen and its prevalence in animals poses a continuous threat to man. A characteristic feature of this organism is its wide host range, which comprises most animal species including mammals, birds and cold-blooded animals in addition to humans. The organism's route of infection is the faecal-oral route via food or water contaminated with faeces or urine of previously infected persons or animals.

A cross-sectional study was carried out to determine the prevalence and antimicrobial susceptibilities of *Salmonella* species in poultry in Zamfara State. From February to April 2015, a total of 300 samples were taken from poultry slaughtered and dressed in live bird markets in Zamfara State using multistage sampling method. Carcass swab and cloacal samples were collected from each bird sampled. The laboratory procedure was carried out using ISO 6579:2002. Conventional biochemical test as well as Microbact™ 12E was used for bacterial identification. Data was analyzed using Epi info 7 and Microsoft Excel 2007.

A prevalence rate of 3.3% (95% CI:0.02-0.05) was observed from the 300 samples. Subspecies of *Salmonella* detected were *S. arizonae* and *Salmonella* spp. Statistically significant difference ($p < 0.05$) was observed between isolates and occurrence at different sample sites. The isolates were 100% resistant to vancomycin, amoxicillin/clavulanic acid, 87.5% to erythromycin, 81.3% to doxycycline, 75% to chlororamphenicol and 25% to kanamycin. Five of the isolates were resistant to more than five different antibiotics. There was statistically significant difference ($p < 0.01$) in antimicrobial resistance patterns exhibited by the subspecies. However, the isolates showed 100% sensitivity to ceftriaxone, ciprofloxacin, imipenem and ofloxacin.

In conclusion, the study revealed the existence of multiple drug resistant *Salmonella* spp from poultry. We therefore suggest further epidemiological studies and enforcement of the food hygiene regulations.

Key words: Multiple drugs, resistant, *Salmonella*, poultry

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND INFORMATION

Salmonella causes infections/diseases in humans and animals¹. The widespread occurrence of Salmonella in the natural environment and the intensive husbandry practice has been a significant problem in public health¹. Human *Salmonella* infection can lead to several clinical conditions including enteric fever, enterocolitis and systemic infections². *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. Poultry and poultry meat are considered one of the main carriers of the organism and represent a significant share of the attributed sources of salmonellosis in humans³.

Salmonella is generally identified as being a non-lactose fermenting, Gram negative rod shaped organism, ranging 0.7 to 1.5 x 2 to 5 µm in size. *Salmonella* is oxidase negative, catalase positive, indole and Voges Proskauer(VP) negative, methyl red and Simmons citrate positive, H₂S producing and urease negative. Some of these characteristics are used for the biochemical confirmation of *Salmonella*¹.

Salmonella infection in poultry generally causes no clinical symptoms, but nevertheless it can cause severe disease¹. In most cases, the birds are not affected and production is not affected¹. Salmonellosis is also considered as one of the most widespread food borne zoonoses in industrialized as well as developing countries, even though the incidence seems to vary between countries³. The emergence of antimicrobial-resistant *Salmonella* spp is

associated with supplementation of antibiotics to animal feed and for their treatments⁴. Nigeria is a developing country with possible abuse of antibiotics in animal husbandry and this may cause antimicrobial resistance of bacteria from animals.

Salmonellae are widely distributed in nature. The main reservoir of these bacteria is the intestinal tract of man and warm-and cold-blooded animals, except for fish, molluscs and crustaceans, which may get contaminated after being harvested. Among warm-blooded animals, chickens, geese, turkeys and ducks are the most important reservoirs⁵.

The natural habitat of *Salmonella* may be divided into three categories based on the specificity of the host and clinical pattern of the disease: highly adapted to man: *Salmonella* Typhi and *Salmonella* Paratyphi A, B and C, agents of typhoid fever; highly adapted to animals: *Salmonella* Dublin (bovines), *Salmonella* Choleraesuis and *Salmonella* Typhisuis (swine), *Salmonella* Pullorum and *Salmonella* Gallinarum (birds), responsible for poultry paratyphoid. The third category includes most of the serovars that affect man and animals, called zoonotic *Salmonella*, responsible for worldwide-distributed food borne diseases, and detected in most species of animals used for human consumption, wild and domestic animals⁶.

Prevention and control programs for infections caused by paratyphoid salmonellae aim at protecting the health of the birds, ensure the safety of the consumers, and strengthen the reliability of the poultry production chain⁷. In the case of *Salmonella*, measures recommended for prevention and controls are not specific due to the large number of species and their complex epidemiological behaviour. Similarly, variability in the implementation of

these measures depends on the requisites determined by the international market, or the adaptation of the industry to the chronogram of production⁸.

In the past 10 years, there have been important outbreaks of emerging food borne diseases all over the world^{4, 5}. These outbreaks showed sanitary authorities of the countries affected that there is an increasing need for measures to prevent the risk of transmission. This led the Food and Agriculture Organization (FAO) to create the WTO, which motivated countries to review their innocuousness policies, rules and strategies to ensure that the food consumed by the population had appropriate sanitary conditions for international trade⁵.

1.2 PROBLEM STATEMENT

Zoonoses and animal diseases in poor countries are being 'massively' under-reported and 99.9% of all livestock losses in sub-Saharan Africa are never registered in official disease reports⁹. Salmonellosis epidemiology and control are highly complex, and hygienic and sanitary standards vary with the region, based on feeding and cooking habits, and animal raising practices. Control of the disease is a challenge to public health because of the emergence/re-emergence of serovars in different areas, both in developing and developed countries¹⁰.

Contamination of poultry products (meat and eggs) destined for human consumption may occur at the slaughterhouse, during food preparation, or by cross-contamination with material from poultry with intestinal and systemic infections. As for poultry meat, even a small number of infected birds may contaminate the whole slaughter line, multiplying the chances of occurrence of food borne disease^{9, 11}. Because of that, slaughterhouses where carcasses are not correctly processed are a threat to public health and current practices of broiler slaughtering and processing may spread microorganisms from one carcass to another¹².

Contaminated foods of animal origin are one source of human bacterial infections; therefore, the presence of antibiotic-resistant strains in food animals such as poultry has raised concerns that the treatment of human infections will be compromised^{9, 13}.

The poultry meat is largely consumed throughout the country in order to meet the nutritional requirements in the form of animal protein. However, the industry is facing major problems such as lack of disease control programs mainly associated with poor handling of raw material from production to marketing facilities^{9, 102}. Increase in demand for meat without the

infrastructure for proper sanitary handling may lead to transfer of pathogenic microorganisms from animals to the consumer. These bacterial hazards are of major concern in the hygienic production of food of animal origin⁹.

Among the food-borne pathogens the genus *Salmonella* is one of the most common causes of foodborne infections worldwide. It has been reported that *Salmonella* is one of the most important pathogens responsible for human food poisoning in the developed world, and chicken products are widely acknowledged to be a significant reservoir for *Salmonella*^{9,98}.

1.3 JUSTIFICATION

Growth in international trade and current facilities for travelling increased not only the dissemination of pathogenic agents and contaminants in foodstuffs, but also our vulnerability. Nowadays, the world is interrelated and interdependent. Thus, local food borne disease outbreaks have become a potential threat for the whole world¹⁴.

Globalization, commercialization and distribution make 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 discard of literally tons of food, considerable economic losses to the production sector, restrictions to trade, and effects on the tourism industry¹⁵.

Microbiological analysis of meat for food-borne organisms like salmonellae is of principal importance in ensuring the supply of safe food for the consumers¹⁶. Approximately 95% of cases of human salmonellosis are associated with the consumption of animal products. Increase in demand for meat without the infrastructure for proper sanitary handling may lead to transfer of pathogenic organisms from the animals to the consumer^{13, 16}.

Poultry and poultry meat products are one of the main carriers of the organism and sources of salmonellosis in humans¹⁷. Poultry can become infected with many different serotypes of *Salmonella*¹⁸. About 50 percent of all *Salmonella* spp. have been detected in poultry¹⁷. There may be irrational widespread use of antimicrobials in the booming poultry industry in Zamfara State.

Studies have shown high incidence of human salmonellosis in neighbouring states, and no work has been carried out on the level of salmonellosis in animals in Zamfara State. And also because of the increasing poor hygienic practices among the poultry workers in the processing and handling of poultry meat and in the live birds markets (LBMs). Moreover, there is paucity of information on the prevalence of animal salmonellosis in the state and the study was designed to provide information on which will form a basis for future quality control of poultry meat in the state.

1.4 RESEARCH QUESTIONS

1. What is the prevalence of *Salmonella* spp in poultry in Zamfara State?
2. What are the antibiotic profiles of *Salmonella* spp in poultry in Zamfara State?
3. What is the level of knowledge, attitude and practice of salmonellosis and antibiotic use among poultry workers in live bird market in Zamfara State?

1.5 GENERAL AND SPECIFIC OBJECTIVES

1.5.1 General Objective

- To evaluate the prevalence and antimicrobial resistance patterns of *Salmonella* spp isolated from poultry in Zamfara State

1.5.2 Specific Objectives

1. To determine the prevalence of *Salmonella* in poultry in Zamfara State
2. To determine the antimicrobial resistance profiles of *Salmonella* isolated from poultry in Zamfara State.
3. To assess the knowledge, attitude and practice of disease control and use of antibiotics among the poultry workers of live bird markets in Zamfara State.

1.6 SCOPE OF STUDY

This study focused on the prevalence of *Salmonella* infection in poultry that are slaughtered and prepared in live bird markets. It also determined the level of awareness, hygiene and sanitary practices towards salmonellosis among poultry workers.

CHAPTER TWO

2. LITERATURE REVIEW

2.1 HISTORICAL BACKGROUND

A pioneer worker on the disease salmonellosis was William Budd, the British physician, who before the bacteriologic era supported the idea that typhoid fever was a contagious disease. His findings published between 1856 and 1878 and supporting arguments were based on carefully observed outbreak occurring in his practice area¹⁹. The typhoid bacillus was described in tissue in 1880 and isolated by Gaffky in 1884²⁰. Salmon and Smith in 1885 isolated *Salmonella Cholerasuis* (*B. cholera suum*, *Bact. Suipestifer*, the Hog cholera bacillus) from hogs suffering cholera, which was thought to be the cause of the disease²¹. Gartner in 1888 isolated *Salmonella* Enteritidis from a man who died from eating a raw meat of a diseased cow^{20, 22}. De Nobele in 1889 isolated *Salmonella* Typhimurium from a food poisoning outbreak¹⁹.

In the early 19th century, the association of human intestinal ulceration with a contagious agent was reported by clinical pathologists in France²³. The agent later was identified as typhoid fever. During the first 2 decades of the 20th century, a great step forward occurred with the serological detection of somatic and flagellar antigens within the *Salmonella* groups²⁴. An antigenic scheme for the classification of salmonellae was first proposed by White and Kauffmann; nowadays more than 2,500 serovars are included in the Kauffmann-White scheme²⁵.

2.2 MORPHOLOGY

Salmonellae are Gram-negative, straight rods not exceeding 1.5 micrometers in width. They are facultative anaerobes usually motile by peritrichous flagella. Most salmonellae form common fimbriae and most of them possess type-1 fimbriae associated with mannose-sensitive adhesive properties²⁶. These fimbriae are composed of fimbrillin subunits containing a high proportion of hydrophobic amino-acids. *Salmonella* are routinely classified by stereotype on the basis of expression of three surface antigens, the somatic O antigen, the flagella H1 and H2 antigens and the capsular Vi antigen, according to Kauffman-White scheme^{25, 27}. The absence of flagella may consequently affect complete identification of the serotype; *Salmonella enterica* serovar Typhimurium exhibits morphological differences dependent on the peptone constituent of the culture medium. However, in media containing soy-based peptone as the primary nutrient, *Salmonella* displays a normal flagellated morphology²⁶.

Table 2.1: Salmonella species and subspecies (WHO 2001)

| Salmonella species and subspecies | No of serotypes |
|---|------------------------|
| <i>Salmonella enteric</i> | 2,480 |
| <i>S. enterica</i> subspecies <i>enterica</i> | 1,478 |
| <i>S. enterica</i> subspecies <i>salamae</i> | 498 |
| <i>S. enterica</i> subspecies <i>arizonae</i> | 94 |
| <i>S. enterica</i> subspecies <i>diarizonae</i> | 327 |
| <i>S. enterica</i> subspecies <i>houtenae</i> | 71 |
| <i>S. enterica</i> subspecies <i>indica</i> . | 12 |
| <i>Salmonella bongori</i> | 21 |
| Total | 2,501 |

Salmonellae are chemoorganotrophic, with the ability to metabolize nutrients by both respiratory and fermentative pathways²⁵. *Salmonella* grows at temperature of between 2 – 47 °C, with rapid growth occurring between 25 to 43 °C. The minimum temperature for growth prevails at neutral pH and increases sharply with increasing acidity or alkalinity of the suspending medium²⁸. The optimum pH for growing is between 6.5 and 7.5. At concentrations of $\geq 3\%$ (w/v), NaCl generally inhibits the growth of salmonellae^{25, 28}. *Salmonella* catabolizes D-glucose and other carbohydrates with the production of acid and gas. *Salmonella* are oxidase negative and catalase positive, grow on citrate as a sole carbon

source, generally produce hydrogen sulphide, decarboxylate lysine and ornithine and do not hydrolyze urea. Many of these traits have formed the basis for the presumptive biochemical identification on *Salmonella* isolates²⁵.

2.3 SALMONELLA NOMENCLATURE

According to the latest nomenclature, which reflects recent advances in taxonomy^{29, 37}, the genus *Salmonella* consists of only two major species: *S. enterica* and *S. bongori*. A third putative species, *S. subterranea* has also been proposed following the isolation of a single unusual environmental strain, but more recent unpublished data suggest that this organism does not actually belong in the genus *Salmonella*^{29, 30, 37}. *Salmonella enterica* is divided into six subspecies, which are distinguishable by certain biochemical characteristics and susceptibility to lysis by bacteriophage Felix O1. These subspecies are: Original subgenera

Current nomenclature

- Subspecies I = subspecies *enterica*
- Subspecies II = subspecies *salamae*
- Subspecies IIIa = subspecies *arizonae*
- Subspecies IIIb = subspecies *diarizonae*
- Subspecies IV = subspecies *houtenae*
- Subspecies VI = subspecies *indica* for the serovars of *S. bongori*, the symbol V was retained to avoid confusion with the serovar names of *S. enterica* subsp. *enterica*. Strains of *Salmonella* are classified into serovars on the basis of extensive diversity of lipopolysaccharide (LPS) antigens (O) and flagellar protein antigens (H) in accordance with the Kauffmann–White scheme; currently over 2500 serovars are recognised³¹. This number is

constantly being increased. The most common serovars that cause infections in humans and food animals belong to subspecies *enterica*^{32, 35}. The serovars of the other subspecies are more likely to be found in poikilothermic (cold-blooded) animals and in the environment, but are occasionally associated with human disease. Some serovars of subspecies *arizonae* and subspecies *diarizonae* have been associated with disease in turkeys and sheep and others may be carried by free-living or captive reptiles and amphibians^{32, 35, 58}. Names are retained only for subspecies *enterica* serovars. These names must no longer be italicised. The first letter is a capital letter. In clinical practice the subspecies name does not need to be indicated as only serovars of subspecies *enterica* bear a name, e.g. Typhimurium, London or Montevideo are serovars of subspecies *enterica*. The genus *Salmonella* followed by the serotype name may be used for routine practice (e.g. *Salmonella* Typhimurium). Serovars of the other subspecies are designated by an antigenic formula, including subspecies designated by Roman numerical (e.g. *Salmonella* IV 48:g.z51)^{37, 58}.

Subspecies and serovars important in human disease

Most of the isolates that cause disease in humans and other mammals belong to *S. enterica* subsp. *enterica*. A few serovars - *Salmonella* ser. Typhi, *Salmonella* ser. Paratyphi and *Salmonella* ser. Hirschfeldii - are human pathogens. They are transmitted mainly from person to person and have no significant animal reservoirs³³. The remaining *Salmonella* serovars, sometimes referred to as non-typhoidal *Salmonella*, are zoonotic or potentially zoonotic. *S. bongori*, *S. enterica* subsp. *salamae*, *S. enterica* subsp. *arizonae*, *S. enterica* subsp. *diarizonae*, *S. enterica* subsp. *houtenae* and *S. enterica* subsp. *indica* are usually found in

poikilotherms (including reptiles, amphibians and fish) and in the environment. Some of these organisms are occasionally associated with human disease³⁴.

2.4 GEOGRAPHIC DISTRIBUTION

Salmonellosis can be found worldwide but seems to be most common where intensive animal husbandry is practiced. *Salmonella* eradication programs have nearly eliminated the disease in domestic animals and humans in some countries (e.g. Sweden), but reservoirs remain in wild animals. Serovars vary in their distribution. Some, such as *Salmonella* ser. Enteritidis and *Salmonella* ser. Typhimurium, are found worldwide. Others are limited to specific geographic regions^{14, 20}.

2.5 DISEASE AND PATHOGENESIS

Salmonella are well known pathogens, highly adaptive and potentially pathogenic for humans and/or animals. *Salmonella* infections are capable of producing serious infections that are food borne and present as gastroenteritis. However, a small percentage of these infections may become invasive and result in bacteremia and serious extra intestinal disease⁶⁷. The main reservoirs for non-typhoidal *Salmonella* are animals such as poultry, livestock, pets and reptiles. *Salmonella* Typhi and *Salmonella* Paratyphi colonize only in humans so they can be acquired only in close contact with a person who has typhoid fever, from a chronic carrier or from water or food contaminated by human faeces⁶¹.

While certain serovars of *Salmonella enterica* cause disease in humans and a variety of animals, other serovars are highly restricted to a specific host. *Salmonella* infections range from gastrointestinal infections that are accompanied by inflammation of intestinal epithelia,

diarrhoea and vomiting, to typhoid fever, a life threatening infection⁶⁸. The outcome of *Salmonella* infection is determined by the host and the status of the bacterium. Whereas, age, genetic and environmental factors mainly determine the status of the host, the status of the bacterium is determined by so-called virulence factors⁸⁷.

Serotypes adapted to man, such as *Salmonella* Typhi and *Salmonella* Paratyphi, usually cause severe diseases in humans as a septicaemic typhoidic syndrome (enteric fever). These serotypes are not usually pathogenic to animals. Serotypes that are highly adapted to animal host, such as *Salmonella* Gallinarum (poultry) or *Salmonella* Abortus-Ovis (sheep), usually produce very mild symptoms in man⁶⁸. However, *Salmonella* Choleraesuis which has pig as a primary host also cause severe systemic illness. In the same way, *Salmonella* Dublin, which has a preference for bovines, is primarily responsible for the systemic form of Salmonellosis. In young calves, this disease causes high mortality and in adult cattle, it results in fever, reduced milk yield, diarrhoea, abortion and occasionally death. Ubiquitous serotypes such as *Salmonella* Enteritidis or *Salmonella* Typhimurium which affect both man and animal generally cause gastrointestinal infection usually less severe than enteric fever. However, they also have the capacity to produce typhoid-like infections in mice and in humans, or asymptomatic intestinal colonization in chickens^{77, 84}. *Salmonella* avoids host defence in the stomach and reaches the intestine^{61, 58}, and the bacteria interact with the non-phagocytic cells such as epithelial cells of the intestinal mucosa⁶⁸. They adhere to the intestinal epithelial cells by adhesive structures (fimbriae) that promote binding and invade epithelial cells to provoke gastroenteritis. The organisms have virulence factors such as virulence-plasmids, toxins, fimbriae and flagella that help in establishing an infection⁷⁸.

The mechanism of pathogenesis has been described in the following steps

a) *Bacterial mediated endocytosis*: A highly coordinated series of interaction between proteins released by salmonellae and proteins of the host cells causes host cellular surface membrane ruffling and engulfment of bacteria in cellular vacuoles.

b) *Neutrophil recruitment and migration*: *Salmonella* associated with gastroenteritis induce a secretory response in intestinal epithelium and initiate recruitment and transmigration of neutrophils into the intestinal lumen.

c) *Epithelial cell cytokine secretion*: In tissue culture models of *Salmonella* Enteritidis translocation of SPI-1 proteins into intestinal epithelial cells leads synthesis and polarizes secretion of inflammatory mediators and neutrophil chemo attractants.

d) *Fluid and electrolyte secretion*: Several translocated SPI-1 proteins contribute to intestinal inflammation and fluid secretion. Intestinal inflammation probably contributes to fluid secretion and diarrhoea by disrupting the epithelial barrier and increasing water flux by an exudative mechanism. Innate immune system activation also contributes to intestinal inflammation.

e) *Systemic infection*: *Salmonella* Typhi invades macrophages and the migration of infected macrophages to reticuloendothelial organs via the lymphatic system and blood produces systemic illnesses with less diarrhoea.

2.6 SALMONELLOSIS IN HUMANS

With respect to human disease, *Salmonella* serotypes can be divided into three groups that cause distinctive clinical syndromes, typhoid fever, bacteraemia and enteritis. The non-typhoid *Salmonella* serotypes can cause protean manifestations in humans, including acute

gastroenteritis, bacteraemia, and extra intestinal localized infections involving many organs. Within *Salmonella enterica* subspecies I (*Salmonella enterica* subspecies *enterica*), the most common O-antigen serogroups are A, B, C1, C2, D and E. Strains within these serogroups cause approximately 99% of *Salmonella* infections in humans and warm-blooded animals. Serotypes in other subspecies are usually isolated from cold-blooded animals and the environment but rarely from humans.

Following ingestion of contaminated food or water, the pathogenesis of both typhoid and *Salmonella* Enteritidis begins with the intestinal phase, while only typhoid progresses to a systemic phase. Transmission of this disease within the human population is generally a result of poor sanitation of food and water supplies in developing nations. The broad host-range *Salmonella* serovars are prevalent within warm-blooded animal populations that make up the human food supply, and bacterial transmission generally results from consumption of raw or undercooked food products.

The vast majority of *Salmonella* infections are transmitted from animals to humans through food and occasionally from person to person through the faecal-oral route. In general, *Salmonella* causes one or more of four broad clinical syndromes such as gastro-enteritis, enteric fever, septicaemia with associated focal lesions, and asymptomatic long-term carriage.

2.7 SALMONELLOSIS IN ANIMALS

Salmonella serotypes have a broad host range, prevalent in the warm-blooded animal population, including rodents, snakes, and free living terrestrial and aquatic turtles, and the pathogenicity of *Salmonella* serovars is known to be specific for animal species. Some

serotypes are highly adapted to animal hosts, such as *Salmonella* Gallinarum in poultry and *Salmonella* Abortus-Ovis in sheep. Many non-typhoidal salmonella strains such as *Salmonella* Typhimurium and *Salmonella* Enteritidis infect a wide range of animal host including poultry, cattle and pigs. These serotypes generally cause self limiting gastrointestinal infections usually less severe than enteric fever in humans. However, they also have the capacity to produce typhoid-like infections in mice and in humans or asymptomatic intestinal colonization in chickens. At the abattoir, the initial source of contamination is the carrier animal. Transmission at the abattoir occurs by direct contact by carrier and non-carrier animals and also by exposure to contaminated environment. It has been suggested that stress associated with transportation, overcrowding and feed withdrawal experienced by animals before slaughter increase the shedding of *Salmonella*.

During slaughtering operations these carrier animals are able to contaminate the area, the equipments and personnel, and eventually the final products.

2.8 SALMONELLOSIS AND PUBLIC HEALTH

Salmonellosis is also considered as one of the most widespread food borne zoonoses in industrialized as well as developing countries, even though the incidence seems to vary between countries³⁵. The majority of human infections are caused by a limited number of the more than 2,600 serovars described to date, and the prevalence of specific serovars differ by geographical region, but only a limited number of serovars are of public health importance^{36,17,38,39}.

Growth in international trade and current facilities for travelling increased not only the dissemination of pathogenic agents and contaminants in foodstuffs, but also our

vulnerability. Nowadays, the world is interrelated and interdependent. Thus, local food borne disease outbreaks have become a potential threat for the whole world³⁴.

Globalization, commercialization and distribution make 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 discard of literally tons of food; to considerable economic losses to the production sector; restrictions to trade; and effects on the tourism industry²⁸.

Salmonella spp. is an intestinal bacterium responsible for severe food borne infections. It is one of the most important agents involved in outbreaks reported in several countries⁴¹. Salmonellosis is an important socioeconomic problem in several countries, mainly in developing countries, where this etiological agent is reported as the main responsible for food borne disease outbreaks³⁶. There are reports of food borne salmonellosis in humans since the 19th century, caused by the ingestion of contaminated bovine meat³⁷. It is one of the most problematic zoonosis in terms of public health all over the world because of the high endemicity, but mainly because of the difficulty in controlling it and the significant morbidity and mortality rate^{38, 39, 40}.

According to the World Health Organization, *Salmonella* is the bacterial agent most frequently involved in cases of food borne disease all over the world. The agent is normally transmitted to humans by means of foods of animal origin, such as meat, eggs and milk³⁴. In the past, the main motivations for controlling *Salmonella* spp. infections in poultry were the losses caused by clinical (pullorum disease and fowl typhoid) and subclinical diseases

(paratyphoid infections)³⁶. Nowadays, due to the public health implications, prevention of food borne transmission of *Salmonella* spp. is a priority for the poultry sector^{27,30}.

Historically, *Salmonella* Typhimurium was the most common agent of the food borne disease in humans, although in the past decades *Salmonella* Enteritidis has been most frequently involved in salmonellosis outbreaks^{41,42}.

There is a growing concern about human infections caused by other serovars, such as Infantis, Agona, Hadar, Heidelberg and Virchow⁴³.

Concerns about the presence of *Salmonella* spp. in foodstuffs of poultry origin increased in the 1980s, when *Salmonella* Enteritidis phage type 4 was responsible for several outbreaks of food borne disease in England, caused by the ingestion of foods containing poultry ingredients^{31,43,44}. The vertical transmission of *Salmonella* Enteritidis in commercial poultry was responsible for the increased number of cases of human infection in Europe, North America and other parts of the world^{45,46}. These serovars replaced *Salmonella* Typhimurium, which was the most common agent of human food borne infection until the 1980s^{47,49}.

2.9 TRANSMISSION

Salmonella spp. are mainly transmitted by the faecal-oral route. They are carried asymptotically in the intestines or gall bladder of many animals, and are continuously or intermittently shed in the faeces. They can also be carried latently in the mesenteric lymph nodes or tonsils; these bacteria are not shed, but can become reactivated after stress or immunosuppression. Fomites and mechanical vectors (insects) can spread *Salmonella*⁵⁰.

Vertical transmission occurs in birds, with contamination of the vitelline membrane, albumen and possibly the yolk of eggs. *Salmonella* spp. can also be transmitted *in utero* in mammals.

Animals can become infected from contaminated feed (including pastures), drinking water or close contact with an infected animal (including humans). Birds and rodents can spread *Salmonella* to livestock. Carnivores are also infected through meat, eggs and other animal products that are not thoroughly cooked. Cats sometimes acquire *Salmonella* Typhimurium after feeding on infected birds or spending time near bird feeders⁵⁰.

People are often infected when they eat contaminated foods of animal origin such as meat or eggs. They can also be infected by ingesting organisms in animal faeces, either directly or in contaminated food or water. Most directly transmitted human infections are mostly often acquired from the faeces of reptiles, chicks and ducklings. Livestock, dogs, cats, adult poultry and cage birds can also be involved⁵⁰.

Salmonella spp. can survive for long periods in the environment, particularly where it is wet and warm. They can be isolated from many sources including farm effluents, human sewage and water. *Salmonella choleraesuis* has been isolated for up to 450 days from pig meat and for several months from faeces or faecal slurries. *Salmonella* Typhimurium and *Salmonella* Dublin have been found for over a year in the environment⁵⁰.

2.10 DETECTION METHODS

Diagnosis is based on the isolation of the organism either from tissues collected aseptically at necropsy or from faeces, rectal swabs or environmental samples, food products and feedstuffs; prior or current infection of animals by some serovars may also be diagnosed serologically⁵⁸. When infection of the reproductive organs, abortion or conceptus occurs, it is necessary to culture foetal stomach contents, placenta and vaginal swabs and, in the case of poultry, embryonated eggs. Salmonellae may be isolated using a variety of techniques that

may include pre-enrichment to resuscitate sub lethally damaged salmonellae, enrichment media that contain inhibitory substances to suppress competing organisms, and selective plating agars to differentiate salmonellae from other enterobacteria⁵⁸. Various biochemical, serological and molecular tests can be applied to the pure culture to provide a definitive confirmation of an isolated strain. Salmonellae possess antigens designated somatic (O), flagellar (H) and virulence (Vi), which may be identified by specific typing sera, and the serovar may be determined by reference to the antigenic formulae in the Kauffman–White scheme. Many laboratories may need to send isolates to a reference laboratory to confirm the full serological identity and to determine the phage type and genotype of the strain, where applicable⁵⁸.

Salmonellae are short Gram-negative bacilli, about 0.7-1.5 x 2-5 µm, readily stained, and nonsporulating. Most of them move using peritrichial flagella, although serotypes such as *Salmonella Pullorum* and *Salmonella Gallinarum* are nonmotile. They are either aerobic or facultative anaerobic, and grow between 5 and 45°C. Optimum growth occurs at 37°C. Ideal pH for multiplication is 7, but *Salmonella* survives in pH values between 4 and 9. They grow in culture medium for enterobacteria and in blood agar. Colonies are 2-4 mm in diameter, with smooth and round edges. They are slightly raised in medium containing carbon and nitrogen. Colonies may remain viable for a long time when stored in peptone^{37, 38}.

Biochemically, *Salmonella* strains have the ability to metabolize nutrients, and catabolise D glucose and other carbohydrates, except lactose and sucrose, with production of acid and gas. They are catalase positive and oxidase negative, as are all genera in the *Enterobacteriaceae* family. They do not ferment malonate, do not hydrolyze urea, do not produce indole, use

citrate as a sole source of carbon, reduce nitrate to nitrite, and may produce hydrogen sulphide⁴⁰.

Conventional culture methods for isolating *Salmonella* spp. in poultry or animal feed or in feed ingredients have been reported in a number of studies, which were summarized by Williams⁵⁹. Although all methods follow the basic strategy of pre-enrichment followed by selective enrichment, differential plating and biochemical or serological confirmation, there is no single internationally accepted procedure for *Salmonella* spp. detection⁵⁸.

The Food and Drug Administration (FDA), for example, recommends lactose broth for Pre-enrichment⁵⁹, while Wyatt used buffered peptone water⁶⁰. Cox reported that pre-enrichment decreased the recovery of *Salmonella* spp. from artificially contaminated poultry feed when compared with direct enrichment⁶¹. Suggested protocols also vary with the substrate: Kafel suggested the use of anaerobic lactose broth, followed by selection in tetrathionate brilliant green broth and plating on brilliant green agar, in the analysis of fish meal⁶². Allen reported that the sensitivity of Rappaport Vassiliadis medium depended on the substrate in the detection of *Salmonella* spp. in high moisture foods, compared with tetrathionate or selenite cystine broth⁶³. Eckner added novobiocin to tetrathionate selective enrichment and increased the incubation temperature to 42°C⁶⁴.

The conventional technique for the detection of the microorganism includes the following steps: pre-enrichment, selective enrichment, isolation and selection, biochemical characterization, serological characterization and final identification. This technique requires at least four days for a negative result and six to seven days for the identification and

confirmation of positive samples⁶⁵. The presence of *Salmonella* has to be determined in at least 25g or ml of sample⁶⁶.

New methodologies, such as immunological tests, have been proposed as alternatives for direct detection of this pathogen. For example, ELISA (Enzyme-linked Immunosorbent Assay) was used by Loguercio⁶⁷. Immunoenzymatic technology may be combined with other rapid methods in order to decrease total assay time. Luk combined a digoxigenin-based ELISA with the polymerase chain reaction (PCR) to detect amplified *rfbS*, a lipopolysaccharide gene of *Salmonella* spp.; in this case, pre-enrichment was no longer than 16 hours⁶⁸.

Other types of assays have also been used: techniques based on molecular biology, such as nucleic acid hybridization or PCR, which was used by Flôres⁶⁹; and tests based on metabolism measurements (impedance and radiometry)⁷⁰.

Ribotyping is the most recent addition to the automated identification of bacteria. The RiboPrinter™ Microbial Characterization System is based on the highly conserved nature of the rRNA operon. Ribotyping provides a reproducible method by which rRNA and polymorphic fragments can be compared with a database for identification of genus, species and strain⁷¹. The system is almost completely automated, requiring only picking up the colonies, suspending them in buffer and submitting them to heat treatment in a special carrier. Once heated, the sample is placed in the device, which automatically lyses the bacteria, releasing DNA; digests it with restriction enzymes; transfers the sample to agarose gel; and separates restricted fragments by electrophoresis. DNA fragments separated by size

are then transferred to a nylon membrane, which is hybridized with a chemically-labelled and treated DNA antibody/alkaline phosphatase conjugate⁵⁰.

Resulting stained bands are then photographed, and the image is stored in the computer database and compared with other images in it. The database for this system is less comprehensive than that of other automated systems, but is still adequate for *Salmonella* spp. The system would, however, be invaluable in epidemiological studies related to incidents⁵⁰.

Serotyping is an important epidemiological tool that complements the identification of *Salmonella*, making it possible to determine the prevalence/emergence or to show trends of a given serovar in different geographical regions, as well as to identify outbreaks, and discover sources of infection and routes of transmission. Serotyping is based on the Kauffmann & White classification and involves the identification of somatic and flagellar antigens⁵⁰.

The somatic structure is identified based on the recognition of the serovars, which are represented by uppercase letters. For example, group A (O:2), group B (O:4); group C1 (O:6,7), group C2 (O:6,8,20), group D (O:9), group E1 (O:3,10), group E2 (O:3,15), group E4 (O:1,3,19), etc. Some factors identify the antigenic group, for example, O:4, O:9. Other factors have little or no discriminatory value, and are normally associated because they represent a complex, such as O:12 (121, 122, 123), with O:2, O:4 and O:9. For example, *Salmonella* Paratyphi A (O:1,2,12), *Salmonella* Typhimurium (O:1,4,5,12) and *Salmonella* Enteritidis (O:1,9,12)^{50,72}.

Some antigens appear as a consequence of a change in the structure, such as O:1, which is a result of the insertion of galactose in the polysaccharide; O:5 a result of the acetylation of

abequose, found in the repetitive units of the polysaccharide responsible for specificity, such as in serovar *Salmonella* Typhimurium O:4,12 and O:1,4,5,12⁵⁰.

As for the characterization of flagellar antigens, it should be taken into account the fact that some *Salmonella* serovars have only one flagellar phase. They are called monophasic: *Salmonella* Enteritidis (9,12: g,m:-), *Salmonella* Typhi (9,12 [Vi]:d:-); however, most serogroups show two flagellar phases, that is, they are diphasic strains, such as *Salmonella* Typhimurium (1,4,5,12: i: 1,2) and *Salmonella* Hadar (6,8: z10: e,n,x), which express phase 1 (antigens i or z10) and phase 2 antigens (respectively, antigens 1,2 or e,n,x). Non motile strains, which have no flagella, have also been recognized⁷³.

2.11 DRUG RESISTANCE

Microbial resistance is related to strains of microorganisms that are able to multiply in the presence of concentrations of antimicrobial compounds even higher than those given as therapeutic doses to humans. Development of resistance is a natural phenomenon that followed the introduction of antimicrobial agents in clinical practice. The irrational and widespread use of these agents has added to the problem, and resistance rates vary from place to place, depending on the local use of antibiotics^{74, 50}.

One of the major concerns of the poultry industry is maintaining the sanitary status of the flocks. In the incubators where birds are born, there is an attempt to reduce contamination to minimum levels in all phases of the process. Lack of contact with natural biota soon after birth interferes with the normal development of bird intestines⁷⁵. Generally, antimicrobial substances (antibiotic or chemotherapeutic agents), called growth promoters, are used in the feed from the first day of life to the moment of slaughter of the birds, respecting the

recommended withdrawal period⁷⁶. These growth promoters improve performance because they “modulate” intestinal microbiota and improve feed efficiency⁵⁰.

Suppliers of growth promoters guarantee that these substances are not absorbed through the intestinal walls and are shed in faeces, where they are quickly biodegraded. Thus, they do not leave residues in the animal, and do not pose risks to human health or the environment⁷⁵. However, consumers are constantly concerned on the possible risks that antimicrobial resistance poses to human health.

Since antimicrobials started to be widely used by humans at the end of the 1940s, the emergence of resistant strains was observed in most bacterial species, and against all drugs available⁷⁷. The use of antimicrobials, combined with improvements in sanitation, nutrition and immunization, has led to a dramatic decrease in deaths and a major gain in human life expectancy⁷⁸. However, with the increased use of antimicrobials, antimicrobial resistance has emerged as one of the greatest threats to the safety of human health⁷⁸, and as a most pressing problem for public health, animal health and food safety authorities^{79,80}.

The increase in antimicrobial resistance has narrowed the potential uses of antibiotics for the treatment of infections in humans and animals⁸¹. As a striking example, the CDC estimated that the total of methicillin-resistant *Staphylococcus* Infections (MRSI) in US hospitals and communities have increased from 2 % in 1974 to almost 63% in 2004⁸².

In the US, more than 40% of the antibiotics produced are used in animal feed. This nontherapeutic use of antibiotics is a way to promote the selection of a growing number of resistant bacteria⁸³. As more strains responsible for poultry infections become resistant to therapeutic drugs, these compounds become less available for human treatments. Similarly,

with *Salmonella* being an important cause of food borne diarrhoeal disease in humans, the reduction in the number of antibiotics available for effective treatment of *Salmonella*-related infections in humans and animals has become a serious concern⁸⁴.

In Europe, besides this concern with resistance, several recent public health episodes were branded on the mind of the consumers. Amongst them, there is connection between eggs and *Salmonella* Enteritidis, BSE/“mad cow disease” and cattle meat, and more recently avian flu in Asia. Therefore, zoonoses and restricted use of additives and antimicrobials as growth promoters in feeds, together with the occurrence of resistant microorganisms, have become an important challenge in the control of detrimental microorganisms found in the digestive system of birds^{85, 50}.

There is a consensus in several countries that the indiscriminate use of antimicrobials in animal production is one of the causes of the increased resistance to antimicrobials. Human infections are more severe when a strain of a given microorganism is resistant to the drug of choice for its treatment. The use of antimicrobials may stimulate the selection of resistant bacteria in this ecosystem. Human pathogens and resistant genes may cross species and ecosystems by contact with, or consumption of contaminated food and water⁸⁶. Due to the little knowledge on single, multiple or cross-resistance mechanisms in microorganisms that are highly pathogenic to humans, the WHO has recommended careful use and restrictions to antimicrobials in animal production⁷⁸.

Before *Salmonella* Enteritidis outbreaks related to traditional drugs in Europe, different antibiotics – such as nitrofurazone, furazolidone, novobiocine and tetracyclines - were used in drinking water and in feed offered to poultry. In Brazil, tetracyclines, penicillins,

chloramphenicol, sulphonamides, furazolidone, nitrofurazone and avoparcin were banned as additives in animal feed in 1998. However, the use of several other drugs is still allowed: 3-nitro acid, arsanilic acid, avilamycin, colistine sulfate, enramycin, flavomycin, lincomycin, spiramycin, tylosin sulfate and zinc bacitracin^{50, 78}.

Extensive use of quinolones in birds was made possible by very flexible prescription regulations, use of generic, lower cost drugs in feed and water, and, without a doubt, because of the efficiency of these agents against *Salmonella*. The use of fluoroquinolones, which have a similar mechanism of action, followed quinolones⁸⁷.

Strains of *Salmonella* Enteritidis may become resistant because of the indiscriminate use of drugs in their country of origin, imports of foodstuffs contaminated with bacteria carrying resistance genes, or infected people returning from international trips. Finnish researchers observed increased antimicrobial resistance in strains of *Salmonella* Enteritidis isolated from travellers after they came back from Asian countries where quinolones were used indiscriminately⁸⁸. There was an increase from 3.9% to 23.5% in the resistance to fluoroquinolones in samples analyzed between 1995 and 1999 in Finland⁷⁸.

These facts, suggest that drug resistance genes may be associated with virulence, or that human strains have an improved resistance profile compared with *Salmonella* of animal origin, making the whole situation even more concerning from a public health viewpoint⁵⁰.

The frequency and extent of *Salmonella* resistance to antimicrobials vary based on the use of antibiotics in humans and animals, and on ecological differences in the epidemiology of *Salmonella* infections⁸⁸. Globally, *Salmonella* exhibits extensive resistance profiles which have been associated both with higher rates of morbidity and mortality and the use of

antimicrobials in food-producing animals⁸⁹. Antibiotics suppress normal intestinal microbiota, breaking its protective effect, increasing the competitive advantage of antibiotic-resistant *Salmonella*, and favouring the occurrence of salmonellosis⁹⁰.

Salmonellosis surveillance has been described all over the world, especially after the emergence of strains resistant to multiple antibiotics, making control and treatment even more difficult. The WHO observed an alarming increase in the number of strains of *Salmonella* resistant to antibiotics due to the abusive use in intensive animal raising⁹¹. This finding is a concern for surveillance and environmental control organisms, once the use of antibiotics in animal feed as growth promoters contributes for the emergence of resistant and pathogenic strains⁹².

Antibiotics may be either bactericidal or bacteriostatic agents. Bactericidal agents cause changes incompatible with bacterial survival, whereas bacteriostatic agents inhibit bacterial growth and reproduction, without immediately killing microorganisms⁹³.

The mechanism of action of antibiotics is essentially related to interference with cell wall synthesis. Cell wall constitution varies in Gram-positive or Gram-negative bacteria, leading to differences in permeability to drugs. Antibiotics that affect the permeability of the cytoplasmic membrane are similar to cationic detergents, due to the presence of basic groups (NH₃⁺) in a lateral chain of the fatty acid⁵⁰.

Insertion of antibiotic molecules disorganizes the membrane, producing leakage of cell components and death. Antibiotics that interfere with DNA replication generate toxic products that get inserted in the DNA molecule, breaking it up and preventing its synthesis. Other compounds loosen the DNA spiral structure, making it larger and breaking the

bacterial cell. Agents that affect protein synthesis act on the ribosome, inhibiting protein synthesis by different mechanisms^{94,95}.

Some bacterial species are considered naturally resistant to antibacterial compounds (primary resistance), because only concentrations that would be unviable *in vivo* would affect them. Under continuous exposure to antimicrobials, microorganisms show acquired resistance (secondary) caused by the development of new mechanisms of defence⁹⁶.

Resistance mechanisms may emerge because of changes in bacterial DNA, or biochemical mechanisms of molecule production, reactions and behaviours, which may be transmissible or not to the daughter cells. Resistance is observed when an antibiotic is administered to patients who are carriers of sensitive, mutant strains. Antimicrobials eliminate microorganisms that are sensitive, “selecting” the ones that are resistant. The rate of emergence of mutant strains is highly variable, and the mutation process may occur quickly in some cases, and slowly and gradually in other cases, taking years to appear. Some cells may present random genetic changes that may lead to resistance to a given antibiotic⁹⁷. The process is called single resistance when the bacterium is resistant to only one drug; multiple resistances, when it is simultaneously resistant to two or more drugs⁹⁸.

Acquired resistance to antibiotics is a necessary gain, or temporary or permanent change of bacterial genetic information. Most resistance genes are found in plasmids, which may be swapped with chromosomal elements. Acquired resistance is caused by mutations in the bacterial chromosome (which leads to the emergence of resistance genes in a sensitive bacterium), or by the transfer of resistance genes from one cell to another, with DNA fragments with these genes being inserted in the receptor cell. Both types of resistance,

mutation (chromosomal) and transferable (plasmidial) may be found in the same bacterium¹⁰¹.

Antimicrobial resistance is one of the most important problems for human and veterinary medicine, and it is recognized by the WHO as an important public health problem¹⁰³. There was a significant increase in the occurrence of *Salmonella* Enteritidis in poultry carcasses from 2000 to 2005 in the US. Studies in Brazil between 2000 and 2009 show the predominance of this serovar in poultry¹⁰⁰. More than half of the strains were resistant to multiple antibiotics, and *Salmonella* Enteritidis was the only serovar that showed different degrees of resistance to all antimicrobial compounds. Studies carried out with *Salmonella* Heidelberg demonstrated that all strains showed multiple resistance, including marked resistance to third generation cephalosporins. In the past years in the US, increased resistance to ceftiofur was observed in poultry strains. In 1997, resistance to this antibiotic was 1.6%, and in 2003, 7.4%¹⁰⁴. During decades, ampicillin, chloramphenicol and trimetoprim-sulfamethoxazole were the most frequent antimicrobials used in salmonellosis treatment. However, the increase in the number of strains resistant to these drugs reduced their use in medical practice⁷⁸.

Consequently, fluoroquinolones became the main antimicrobials used in the treatment of human infections¹⁰⁵. Resistance to *Salmonella* spp transmitted by contaminated foods of animal origin is undesirable, but it is an inevitable consequence of the use of antimicrobials in animals used in food production¹⁰⁶. Bacterial resistance is a natural process, but it should and can be prevented with the rational use of antimicrobials in animal production. Therefore,

it is very important to follow the evolution of resistance in order to use efficient methods for *Salmonella* control^{50, 78}.

2.12 PREVENTION AND CONTROL

Prevention and control programs for infections caused by paratyphoid salmonellae aim at protecting the health of the birds, ensure the safety of the consumers, and strengthen the reliability of the poultry production chain. In the case of *Salmonella*, measures recommended for prevention and controls are not specific due to the large number of species and their complex epidemiological behaviour. Similarly, variability in the implementation of these measures depends on the requisites determined by the international market, or the adaptation of the industry to the chronogram of production^{50, 78}.

In the past 10 years, there have been important outbreaks of emerging food borne diseases all over the world. These outbreaks showed sanitary authorities of the countries affected that there is an increasing need for measures to prevent the risk of transmission. This led the Food and Agriculture Organization (FAO) to create the WTO, which motivated countries to review their innocuousness policies, rules and strategies to ensure that the food consumed by the population had appropriate sanitary conditions for international trade¹⁰⁷.

General regulations issued all over the world for *Salmonella* control and prevention are: Proposed Guidelines for the Control *Campylobacter* and *Salmonella* in chicken meat, from the Codex Alimentarius; Prevention, Detection and Control of *Salmonella* in poultry, Chapter 6.5 of the Terrestrial Animal Health Code of 2010, from the World Organization for Animal Health (OIE); Compliance Guideline for Controlling *Salmonella* and *Campylobacter* in

Poultry, of May 2010, from the Food Safety Inspection Service and United States Department of Agriculture (FSIS/USDA)⁷⁸.

Together with many other biosafety measures, monitoring of these bacteria, which may be associated with food borne disease in humans, is one of the great objectives of the poultry industry. Health education actions that emphasize personal hygiene habits, mainly correct hand washing, care in food preparation, handling, storage and distribution, are recommended for food handlers. Main prevention strategies should be: selection of raw materials; careful cleaning of equipment and utensils; adequate supply of potable water; adequate garbage disposal and sewage treatment; adoption of good manufacturing practices and implementation of the HACCP; removal of asymptomatic carriers from the production area, and adequate methods for transportation and preservation. All these actions are in compliance with the recommendations of public health authorities from all over the world^{108, 109, 110}.

Literature information show that one year after the implementation of *Salmonella* control in Finland, prevalence was below 1% in egg and bovine, swine and poultry meat production, decreasing the occurrence of salmonellosis outbreaks¹¹¹. Food hygiene, therefore, is based on the adoption of preventive and control measures. The HACCP system is an efficient tool to remove disease-causing agents. The system provides specific protection against food borne disease, and leads to reduction in costs and warranties of microbiologically safe foods⁷⁸.

The risk of vertical transmission may be minimized by bacteriological and serological monitoring of breeding chicken lots, resulting in *Salmonella*-free birds; by purchasing birds more resistant to *Salmonella* infection¹¹²; by culling birds that are carriers of the

microorganism; by treatment of eggs that are still in the sheds, and careful incubation of dirty and cracked eggs¹¹³.

Biosafety and sanitary management are important to reduce the environmental presence of *Salmonella*. According to Gast, one of the methods employed to achieve this aim is cleaning and disinfection of the sheds with chemical disinfectants¹¹⁴. However, not all disinfectants are efficient and depend, for example, on their behaviour in the presence of large amounts of organic material¹¹⁵. Together with this, it is important to control rodents found in bird sheds. These animals have an important role in *Salmonella* infection by contaminating the environment and transmitting the microorganism to birds and eggs¹¹⁶.

Specific procedures that aim at controlling *Salmonella* in bird feed include pelleting and use of organic acids¹¹⁷. According to Gama, as pelleting is carried out at temperatures over 60°C, the process may eliminate *Salmonella* from poultry feed, provided that the feed is not recontaminated by handling, rats or insects¹¹⁸. Iba and Berchieri Jr, observed that a mixture of formic and propionic acids was efficient in controlling *Salmonella* Typhimurium in artificially contaminated feed¹¹⁹.

Another important tool in *Salmonella* prevention and control is the use of quantitative thresholds. These values vary from country to country and correspond to the measures and control systems that are adequate for local production. These limits should be established based on scientific research and special attention should be paid to the use of antibiotics, detergents, disinfectants and process temperature⁷⁸.

Indiscriminate use of antibiotics and addition of growth promoters in animal feed contributed to the emergence of resistance among strains of *Salmonella* and other bacteria¹²⁰. Besides,

according to Barrow, after the therapeutic agent is removed, there may be a period in which birds may become susceptible to *Salmonella* infection, because their normal microbiota – which would inhibit *Salmonella* naturally – is also affected by the use of the antibiotic¹²¹.

Competitive exclusion is based on oral inoculation of the caecum contents of adult birds in newborn chicks, speeding the establishment of desirable intestinal microbiota¹²². The process attempts to prevent the establishment of pathogenic microorganisms in the intestinal mucous membrane. This is an important method in the control of *Salmonella* infection in birds with immature or debilitated intestinal microbiota⁷⁸.

Another measure for *Salmonella* control and prevention is vaccination of susceptible birds¹²³. Nowadays, several studies have been carried out in order to evaluate to the efficacy of live attenuated vaccines. These studies support the use of vaccination, in a safe and efficient manner, as part of the prevention of infection in birds and contamination of eggs by *Salmonella* Enteritidis¹²⁴.

Notification and epidemiological records are important sources of information for inspection and control agencies, which may estimate which pathogens and foods that may possibly be involved in food borne disease outbreaks. For example, the presence of several *Salmonella* serotypes that did not show high prevalence some years ago, are found now in poultry flocks and represent an important public health problem worldwide⁷⁸.

Control of salmonellosis will be achieved by the adoption of some measures, such as frequent and systematic surveillance of food production and distribution. An efficient program both provides warranties in the production of safe foods and reduces costs.

2.13 SALMONELLOSIS IN FOOD ANIMALS IN NIGERIA

Epidemiological reports suggest that meat product is one of the major causes of diarrhoeal illness which account for 36% of mortality cases in Nigeria¹²⁵. Ojeniyi in 1984 conducted an epidemiological study of salmonellosis in free-range village poultry. *Salmonella* Hirschfeldi (invalid) was isolated from four birds in a village on the outskirts of the city of Ibadan, Nigeria¹²⁶. This is the first report of an isolation of *S. Hirschfeldi* in poultry in Nigeria. The same organism was found in an adult male in the village in that study. Kwaga *et al* in Zaria, Nigeria reported thirteen different serotypes of *Salmonella* from the lymph nodes of slaughter cattle and ten from raw beef. Out of these, *Salmonella* Ealing, *Salmonella* Eppendorf, *S. Tilene*, *S. Widemarsh*, *Salmonella* group E1 3, 10: b and *Salmonella* 17: K: e, n, Z15 were reported for the first time in Nigeria¹²⁷.

Poultry is an essential component of the Nigerian economy, providing income for small scale farmers and a good source of high quality protein for the ever-growing population of Nigeria. In livestock production, poultry occupies a prominent position in the provision of animal protein and this accounts for about 25% of local meat production in Nigeria¹²⁸. With the great expansion of poultry rearing and farming, salmonellosis have become an important public health problem in Nigeria and other parts of the world, causing heavy economic losses through substantial morbidity and mortality¹²⁹.

CHAPTER THREE

3. METHODOLOGY

3.1 STUDY AREA

Zamfara State consists of 14 LGAs with Gusau as the State capital with a population of 3,278,873 (2006 National Census) located between latitude 10°50 N and 13°58 N and longitudes 4°16 E and 7°13 E with an area of 38, 418 square kilometres. It is bordered in the north by Sokoto State and Niger republic, to the west by Kebbi State, in the east by Kastina State and to the south by Kaduna and Niger Sates. It has a warm tropical climate between March and May. The mean annual rainfall in the State varies slightly, from the northern to the southern parts of the State. The onset of the rains, on the average, is between mid-March and May, lasting for about six months till the end of October, while the cold season (Harmattan) last from November to February. The vegetation of Zamfara State consists of Sudan and Northern Guinea Savannah. The Sudan Savannah occurs in the western, northern and eastern parts of the State. On the southern part of the state, is found the Northern Guinea Savannah.

It is mainly populated by Hausa and Fulani people, with other tribes. The State capital is an important commercial center with a heterogeneous population of people from all over Nigeria. Agriculture is the most important occupation with majority of the population engaged in crop and livestock production. The State has a livestock population of nearly six million comprising of over one million cattle; 857,000 sheep, over two million goats; about 18,000, 21,000 and 46,000 horses, camels and donkeys respectively and an estimated poultry population of 5,845,508⁵⁸. The poultry industry is characterized mainly by backyard, small

scale to medium scale commercial production system and a growing area of large commercial system.



Figure 3-1: Map of Nigeria showing Zamfara State in green

3.2 STUDY DESIGN

A descriptive cross-sectional study was employed

3.3 STUDY POPULATION

Poultry in Live bird markets and slaughter slabs in Zamfara State

3.3.1 Inclusion criteria

- All LBMs within the selected LGAs that have poultry currently stocked within their cages and the willingness of the birds owners to participate

3.3.2 Exclusion criteria

- Sick birds or birds on antimicrobial therapy

3.4 Study period

The study was conducted from January to March 2015

3.5 SAMPLE SIZE DETERMINATION

Using the formula described by Kish and Leslie (1965)

$$N = Z^2 p (1 - p)/d^2$$

N = Desired sample size

Z = Reliability coefficient put at 1.96 at 95% CI

p = Prevalence of *Salmonella* in poultry 11% by Fashae *et al.*¹³²

d = Desired absolute precision of 5%

$$N = (1.96)^2 \times 0.11 \times (1-0.11) / (0.05)^2$$
$$= 150 \text{ Samples}$$

plus 10% of non response = 165 samples

3.6 Preparation for sample collection

Advocacy visits was first carried out by the researcher to the Director, Veterinary services, Zamfara State, the State Director, Federal Ministry of Agriculture, Zamfara State Office, Poultry Farmers Association, poultry sellers in Zamfara State.

3.7 Data Collection Methods

The following instruments were used to obtain necessary information from the samples:

- Data sheet: were used to obtained basic data such as sex, breed and species, of sampled poultry
- Interviewer administered semi-structured questionnaires with five sections A-E as outlined below were used to obtain information from poultry sellers

A: Socio-demographic data

B: Occupational History

C: Knowledge of salmonellosis and zoonoses

D: Attitudes related to salmonella transmission and protective measures

E: Management practices related to in *Salmonella* prevention and control

3.8 Data Quality

The questionnaires will be checked for errors and also the data will be double entered

3.9 Data Management

Data was entered and managed using EpiInfo™ (Epi-Info version 7 database, US Centers for Disease Control and Prevention, Atlanta GA.) and Excel 2010 software (Microsoft inc. USA). Data was checked for consistency and cleaned before analysis.

3.10 Statistical Analysis

Univariate analysis was conducted to obtain frequencies, means \pm SD and proportions as appropriate. Bivariate analysis was conducted to determine factors associated with farm antimicrobial sensitivity for *Salmonella* in Poultry. Chi-square (X^2) test was used to compare categorical data at 95% Confidence interval with a level of significance of $P \leq 0.05$. Data was presented in tables, cross tabulations and graphs as appropriate.

3.11 Sample collection

- Sample included cloacal samples and poultry carcass swab
- Cloacal samples were collected in clear, transparent sterile, wide mouthed bottles.

- Sample of poultry meat were collected by swabbing the skin of slaughtered and defeathered chicken
- The cotton swab was placed in a clean, sterile container
- Data sheet was used to collect (species, origin) information for bird sampled

3.12 Sample processing

3.12.1 Pre-enrichment

Cloacal swaps were collected and placed into 3ml buffered peptone water (BPW) in screw capped bottles, incubated at 37°C for 24 hour.

3.12.2 Enrichment

1 ml of the pre-enrichment broth was transferred into tubes containing 9ml Rappaport Vassiliadis Broth (RVB) (Oxoid UK), incubated at 37°C for 24 hour.

3.13 ISOLATION AND IDENTIFICATION OF *SALMONELLA*

3.13.1 Presumptive isolation of *Salmonella*

A loopful of culture from RVB was sub cultured by streaking onto Salmonella-Shigella agar (SSA) (Oxoid UK), and were incubated at 37°C for 24-48 hour. Colony morphology (size, shape, margin, elevation, colour, etc.) were carefully examined after incubation, and presumptively identified *Salmonella* colonies (that is transparent colonies with black centres and or dome-shaped colonies which may have central black spot due to hydrogen sulphide production) were selected.

3.13.2 Purification of isolates

The isolates were re-plated onto SSA and nutrient agar for isolation of pure culture and for further biochemical characterization and profiling.

3.13.3 Biochemical identification of isolates

The following biochemical tests were conducted, urease test, citrate test, TSI, SIM, methyl red test, Voges-Proskauer test, sugars (arabinose, manitol, sucrose, lactose and maltose).

3.13.4 Microbact (Oxoid)

One to three isolates of the 24 hours culture was picked and emulsified in 5ml sterile peptone water and incubated at 37°C for 4 hours. The wells of the individual substrate set were exposed by cutting the end tag of the sealing strip and slowly peeling it back. The plate was placed on a holding tray and using Pasteur pipette 4 drops (100µl) of the bacterial suspension were added to the wells. Using a sterile pipette a drop of sterile mineral oil was added to well 1, 2 and 3. The inoculated row was resealed with the adhesive seal and specimen identification number was written on the end of the tag with marker pen. This was then incubated at 37°C for 18-24 hours.

After 18-24 hours incubation, to well 8 (Indole production) 2 drops of Indole (Kovacs) reagent were added and evaluated within 2 minutes of addition of reagent. To well 10, (Voges-Proskauer reaction), one drop of VPI reagent and VP II reagent were added and evaluated within 15-30 minutes of the addition of the reagents. To well 12 (Tryptophan Deaminase, TDA), 1 drop of TDA reagent was added and evaluated immediately.

Results were interpreted as + or – by comparing them with the colour chart and were recorded under the appropriate heading on the reporting form. Results were recorded in

forms containing the substrate that were tested. Twelve (12) substrates were tested; Ornithine, Hydrogen sulphide, Glucose, Mannitol, Xylose, o-Nitrophenyl β - D-galacotopyranoside (ONPG), Indole, Urease, VP, Citrate and TDA.

3.14 DETERMINATION OF ANTIBIOTIC SUSCEPTIBILITY OF *SALMONELLA* ISOLATES

Susceptibility of *Salmonella* isolates to different antimicrobial agent was measured in vitro by the Kirby-Bauer method. It allowed rapid determination of the efficacy of drug by measuring the zone of inhibition that result from diffusion of antimicrobial agent into the medium surrounding the disc.

3.14.1 Antimicrobial Susceptibility Testing

All isolates were tested for 13 antimicrobial drugs (oxoids); kanamycin (30 μ g), ciprofloxacin (5 μ g), chloramphenicol (30 μ g), vancomycin (30 μ g), Amoxicillin/clavulanic acid (30 μ g), doxycycline (30 μ g), erythromycin (15 μ g), ofloxacin (5 μ g), ceftriaxone (30 μ g), enrofloxacin (5 μ g), ceftazidime (30 μ g), imipenem (10 μ g) and gentamicin (10 μ g). Susceptibility testing was performed according to recommendations of Clinical and Laboratory Standards Institute (CLSI) ⁵⁹. To standardize bacterial suspension (in 0.8% NaCl), the density of suspension was adjusted to 0.5 McFarland and spread over the entire surface of Mueller Hinton agar (MHA) plates using a sterile cotton swab. Antimicrobial discs were placed on the agar surface followed by incubation of the plates at 37 °C for 24 hours. Inhibition zones were measured by Venier Calliper and interpreted accordingly by CLSI recommendations.

3.15 ETHICAL CONSIDERATION

Ethical clearance was sought and obtained from the Ethical Committee of Zamfara State Ministry of Health, Gusau. Similarly, permission was obtained from bird owners before sampling of their animals.

3.16 Limitations

Identification of subtypes will be limited to those subtypes for which antigens are available

CHAPTER FOUR

4. RESULTS

4.1 Demographic information

A total of 150 poultry workers were interviewed, with a median number of respondents of 4 and a range of 1 to 29 workers interviewed in each live bird market (LBM). The average age of respondents was 26 years; 65.3% of the respondents were male. About half the respondents were single and had never married, while the majority (54) had attended at least a secondary school. The median duration of work in the LBM was 6 years, with a range from 1 to 22 years.(Table 4.1)

Table 4.1: Socio-demographic characteristics of poultry workers in LBMs in Zamfara State, Nigeria. N=150

| Factor | Frequency(N) | Percentage (%) |
|---------------------------------|--------------|----------------|
| Sex | | |
| Male | 98 | 65.3 |
| Female | 52 | 34.7 |
| Marital status | | |
| Married | 68 | 45.3 |
| Single | 80 | 53.3 |
| Others | 2 | 1.4 |
| Educational status | | |
| Primary | 30 | 20.0 |
| Secondary | 81 | 54.0 |
| Tertiary | 29 | 19.3 |
| No formal education | 10 | 6.7 |
| Role in live bird market | | |
| Poultry seller/market | 65 | 43.4 |
| Buyer | 66 | 44.0 |
| Processor/butcher | 14 | 9.3 |
| Market official | 5 | 3.3 |
| Total | 150 | 100.0% |

Tables 4.2: Awareness of salmonellosis among poultry workers in LBMs in Zamfara State, Nigeria. N=150

| Characteristic | Percent (%) |
|---|--------------------|
| Ever heard of salmonellosis? | |
| Yes | 35.1 |
| No | 64.9 |
| Does it affect only animals? | |
| Yes | 15.6 |
| No | 11.0 |
| I don't know | 73.4 |
| Knowledge of mode of transmission? | |
| Animal bite | 8.3 |
| Direct contact with infected birds | 49.5 |
| Eating poorly cooked meat | 6.2 |
| Inhaling infected air | 6.2 |
| Utensils contaminated with faeces of infected birds | 30.0 |

4.2 Knowledge

A small proportion of the respondents (35.14%) had heard about salmonellosis. However, only 7.7% of respondents correctly defined salmonellosis as a bacterial infection that occurs in all species of birds. Knowledge of transmission of the disease varied: 49.48% knew that the disease could be transmitted from an infected bird, and 66.67% knew it could be transmitted from bird to human and through handling of uncooked poultry. Only 8.25% suggested the possibility of animal bite transmission. (Table 4.2)

Table 4.3: Attitude towards salmonellosis among poultry workers in live bird markets in Zamfara State, Nigeria. N=150

| Characteristics | Percent (%) |
|---|--------------------|
| Do you think such organism cause disease in man? | 66.7 |
| Agree | 19.4 |
| Disagree | 13.9 |
| Uncertain | |
| Do you think salmonellosis can be prevented? | 72.2 |
| Agree | 13.0 |
| Disagree | 14.8 |
| Uncertain | |
| Does washing utensils reduce transmission? | 42.7 |
| Agree | 19.4 |
| Disagree | 37.9 |
| Uncertain | |
| Does disinfecting surfaces reduce transmission? | 69.4 |
| Agree | 13.3 |
| Disagree | 17.3 |
| Uncertain | |

4.3 Attitude

One hundred (66.7%) of respondents perceived that the disease can be transmitted from poultry to man, 108(72.2%) of the respondent perceived that such disease can be prevented, while 63 (42.0%) said quarantining new birds is important in reducing the spread of the disease. Many of the respondents, 104 (69.4%) perceived that disinfecting surfaces after work is important as a means of prevention.(Table 4.3)

Table 4.4: Hygiene and sanitary practices towards salmonellosis among poultry workers in LBMs in Zamfara State, Nigeria. N=150

| Characteristics | Percentage (%) |
|---|-----------------------|
| How often do you wash hands with soap and water before and after handling poultry? | |
| Always | 29.6 |
| Often | 5.6 |
| Sometimes | 12.0 |
| Rarely | 45.4 |
| Never | 7.4 |
| How often do you wash and disinfect surfaces and utensils? | |
| Always | 30.6 |
| Often | 6.5 |
| Sometimes | 13.0 |
| Rarely | 45.4 |
| Never | 4.5 |
| How often do you practise all-in all out system? | |
| Always | |
| Often | 15.5 |
| Sometimes | 4.9 |
| Rarely | 23.3 |
| Never | 5.8 |
| How often do you segregate/isolate sick birds? | 50.5 |
| Always | |
| Often | 37.8 |
| Sometimes | 25.2 |
| Rarely | 13.6 |
| Never | 9.7 |
| When your birds are sick, do you consult a veterinarian? | 13.6 |
| Always | |
| Always | 16.7 |
| Often | 8.8 |
| Sometimes | 15.7 |
| Rarely | 2.9 |
| Never | 55.9 |

4.4 Hygiene Practices

Only 44(29.6%) regularly wash their hands with soap and water before and after handling birds, 46(30.5%) always wash and disinfect surfaces and utensils after work or use. 76(50.5%) never practice all-in-all out system of selling birds, 57(37.8%) regularly isolate sick birds from the rest of the flock. 84(55.9%) never consult a veterinarian when their birds are sick. (Table4.4)

Table 4.5: Use of antibiotics among poultry workers in LBMs in Zamfara State, Nigeria. N=150

| Characteristics | Percentage (%) |
|---|-----------------------|
| Do you use antibiotics for your birds? | |
| No | 65.7 |
| Yes, but regularly | 20.4 |
| Yes, but rarely | 13.9 |
| When do you use it? | |
| To treat animals when they are sick | 65.8 |
| I give it to prevent animals from getting sick | 21.1 |
| To make them grow fast | 13.1 |
| How do you choose which antibiotic to use? | |
| I use different antibiotics depending on what I can find or afford | 42.1 |
| I use what the salesperson recommend | 26.3 |
| I use antibiotics after consulting a veterinarian | 31.6 |
| Are you aware of resistance of some bacteria to Antibiotics? | |
| No | 31.1 |
| I have heard but don't know how it affects my birds | 58.3 |
| Yes, I am aware and try to restrict my use of certain antibiotics | 10.7 |

4.5 Use of Antibiotics

99(65.7%) of respondents said they do not use antibiotics for their bird, 32(21.1%) use it to prevent their birds from getting sick while 20(13.1%) use it as growth promoter. 63(42.1%) of the respondents said their choice of antibiotics is based on affordability, only 47(31.6%) use antibiotics after consulting a veterinarian. 16(10.7%) are aware of resistance of some bacteria to antibiotics and try to restrict their use.

Table 4.6: Number of samples collected and prevalence of *Salmonella* spp in each LBM.

| Live bird market | No of Samples | <i>Salmonella</i> Positive | % Positive n=10 |
|-------------------------|----------------------|-----------------------------------|------------------------|
| Tudun wada | 90 | 4 | 4.4 |
| Sabon Kasuwa | 60 | 2 | 3.3 |
| Tsafe | 40 | 1 | 2.5 |
| Kaura | 35 | 1 | 2.9 |
| Anka | 30 | 0 | 0 |
| Zurmi | 20 | 1 | 5 |
| Mafara | 25 | 1 | 4 |
| Total | 300 | 10 | 3.3 |

Table 4.7: Resistance of 16 *Salmonella* isolates to 13 antimicrobial agents

| Antibiotic | Concentration (µg) | Number(%) Resistant |
|------------|--------------------|------------------------|
| AMC | 30 | 16(100.0) |
| VA | 30 | 16(100.0) |
| E | 15 | 14(87.5) |
| DO | 30 | 13(81.3) |
| C | 30 | 12(75.0) |
| K | 30 | 4(25.0) |
| CN | 10 | 2(12.5) |
| CAZ | 30 | 2(12.5) |
| ENR | 5 | 2(12.5) |
| IPM | 10 | 0(0.0) |
| CIP | 5 | 0(0.0) |
| OFX | 5 | 0(0.0) |
| CRO | 30 | 0(0.0) |

F-value -216.53

P-value- 0.001

LOS: Level of significance

**: Significant at 5 % level of probability*

CRO: Ceftriaxone, AMC: Amoxicillin-clavulanic acid, C: Chloramphenicol, DO: Doxycycline, VA: Vancomycin, K: Kanamycin, CN:Gentamicin, IPM: Imipenem, CAZ: Ceftazidime, CIP: Ciprofloxacin, E: Erythromycin, OFX: Ofloxacin, ENR: Enrofloxacin.

Table 4.8: Microbact Results

| Sample No | Microbact Identification | Percentage (%) Probability |
|------------------|---------------------------------|-----------------------------------|
| 225 | <i>Salmonella</i> spp | 59.4 |
| 160 | <i>Salmonella arizonae</i> | 76.4 |
| 161 | <i>Salmonella arizonae</i> | 85.0 |
| 267 | <i>Salmonella</i> spp | 33.5 |
| 127 | <i>Salmonella</i> spp | 96.7 |
| 36 | <i>Salmonella arizonae</i> | 74.4 |
| 67 | <i>Salmonella arizonae</i> | 85.0 |
| 60 | <i>Salmonella arizonae</i> | 76.4 |
| 57 | <i>Salmonella arizonae</i> | 76.44 |
| 289 | <i>Salmonella</i> spp | 42.30 |
| B | <i>Salmonella arizonae</i> | 98.3 |

Microbact TM 12E test kit confirmed 11 of the 16 *Salmonella* suspects identified by conventional biochemical tests carried out.

CHAPTER FIVE

5. DISCUSSION

From the demographic analysis, the poultry workers in the LBMs have average secondary education and an average 6 years work experience that does not necessary translate to high knowledge and awareness level of salmonellosis and zoonoses. Certain occupations might have a higher risk of contracting zoonoses, for example the livestock farmers, veterinarians, abattoir workers, butchers, poultry workers, etc. This agrees with study in Edo that showed the level of awareness and knowledge of zoonoses to be low⁵⁴, even though a few of the poultry workers are aware of diseases like Avian Influenza, rabies as zoonoses.

Management and prevention practices were also poor to nonexistent, this might be attributed to lack of knowledge on the transmission of *Salmonella* infection, improper orientation on biosecurity measures among poultry farmers and the lack of good hygienic practices among poultry farm-handlers. Most importantly, there was no consistent follow-up program put in place by regulatory agencies to educate poultry farmers on how to prevent and control salmonellosis in the farms and LBMs (Table 4.4).

In this study 3.3 % of the apparently healthy poultry sold and slaughtered at live bird markets in Zamfara State were positive for *Salmonella*. This shows that they harbour *Salmonella* organisms, and also implies that apparently healthy birds could be carriers of *Salmonella* .

The prevalence of *Salmonella* spp. was relatively low, similar to other studies in Edo State with a prevalence of 3.8%, 6% in Maiduguri, 4% in Ibadan, 4.5% in UK and 2.1% in Morocco^{63, 64,74,78,88}. Other studies have shown high prevalence in some regions within and

outside the country. The prevalence of *Salmonella* spp. is said to vary in different species and LBMs. The variation may be explained by the fact that each LBM is supplied by different farms and sources with different levels of contamination.

Poultry meat is an important source of protein and a valuable commodity for the local consumers in Zamfara State. This study on poultry meat sales points revealed that majority of the live birds markets do not operate in a safe and clean environment. Moreover, the practice of using the same cutting knives for the uninfected and infected carcasses, results in a further chance of cross-contamination¹³¹.

The processing of carcasses as per consumer demand further spreads contamination by exposing carcass surfaces and susceptible fleshy parts to the contaminants through the use of the same cutting tables. In addition, the water used for washing of carcasses is often from the same container and it could be contaminated with *Salmonella* from faeces or from the butcher's hands during washing¹³¹.

The difference between studies on the occurrence, prevalence and serotype resistance profiles of *Salmonella* in poultry can be explained by the fluctuation of dominant serotypes that occur between geographical regions. Additionally, the amplitude of serotypes within the same region may be related to propagation of *Salmonella* by feed, derived from supplies from different locations, collaborating to increase the diversity of serotypes^{130, 120}. Also the differences between the data of prevalence of *Salmonella* serotypes can be associated with the age of the chickens, differences in origin, variation in sampling procedures, poultry contamination, and differences in sample size among others¹²⁰.

The main subspecies in the carcasses surveyed were *S. enterica* subsp. *arizonae* and *Salmonella* spp⁶. *Salmonella* spp caused a worldwide increase in the number of cases of human infection, mainly related to consumption of chicken⁷; it is known to be resistant to several drugs.

Basically, in the livestock industry, antibiotics have been used successfully in human and veterinary medicine in the past sixty years to turn many of life threatening bacterial infections into treatable conditions. However, in recent times, antibiotic resistance has become an important health and food safety issue with emergence of many drug-resistance species of microbial pathogens in humans⁵⁵. The use of several antibiotics for therapeutic or prophylactic administration or for growth enhancement, 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 reduce efficacy of such drugs sometimes administered by non-qualified personal⁵⁶.

The results of antimicrobial susceptibility testing in this study revealed that all the *Salmonella* isolates were resistant to vancomycin, amoxicillin/clavulanic acid, 87.5% to erythromycin, 81.3% to doxycycline, 75% to chlororamphenicol, and 25% to Kanamycin (Table 4.7). There was a statistically significant difference at $p < 0.01$ in antimicrobial resistance patterns exhibited among the *Salmonella* subspecies. High resistance to those antimicrobial drugs were consistent to previous observations from various countries^{16, 18}, which implies that the wide consumption of such antimicrobials as feed additives in livestock contributes to emergence and dissemination of resistance in *Salmonella*. In addition it had also been reported the sub-therapeutic doses of antimicrobial drugs in animal

husbandry as a responsible factor in emergence and maintenance of multiple antimicrobial resistant pathogenic bacteria¹⁹⁻²¹. The bacterium develops resistance to most of these commonly used antibiotics because of their inadvertent use for long duration or in suboptimal doses. Therefore, it is recommended to use antibiotics based on their antibiogram pattern only. Use of antibiotics based on earlier report of their effectiveness may not be effective at all times because of the presence of resistant bacterium¹³.

According to several reports, our results demonstrated that the fluoroquinolone groups such as ciprofloxacin and norfloxacin are still the most effective drugs to treat *Salmonella* infection²²⁻²⁴. In recent years, evidence for decreasing susceptibility to fluoroquinolones in *Salmonella* has been reported. Increasing resistance to fluoroquinolones is growing as an issue receiving special attention, since fluoroquinolones are effective drugs against *Salmonella* in clinical performance and are usually considered as treatment of choice in life threatening cases²⁵.

A similar trend in resistance was recorded for non-typhoidal *Salmonella* ($p < 0.05$) by Akinyemi *et al.*¹²⁴, with susceptibility to both ciprofloxacin and ofloxacin. Ciprofloxacin and gendox, showed low resistance patterns in human and animal isolates, as also reported by Okoli *et al.*¹²⁶. Quite worrisome is the fact that five of the *Salmonella* subspecies were resistant to more than five different antibiotics (appendix 4). Similar report in USA shows that 18.0% of isolates from all sources were also found resistant to two or more antimicrobials. The detection of multidrug resistant *Salmonella* spp suggests that antibiotic resistance can pose a risk to both humans and animals. Thus, strict guidelines for the use of

antibiotics will be necessary to prevent the dissemination and acquisition of antibiotic resistance²⁶.

This study also found that the *Salmonella* isolates were susceptible to four antibiotics ceftriaxone, imipenem, ciprofloxacin and ofloxacin. Ciprofloxacin is a fluoroquinolone antimicrobial that is increasingly and successfully used for the treatment of septicaemic salmonellosis in human, worldwide¹³². The low resistance level to ciprofloxacin was an important finding since ciprofloxacin is clinically essential for the treatment of serious gastrointestinal infections in adults²⁴.

CHAPTER SIX

6. CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusion

The prevalence of *Salmonella* spp. found in this study was relatively low (3.4%); however there was a high proportion of multidrug-resistant strains including resistance to cephalosporins used in the treatment of salmonellosis.

Resistance of *Salmonella* strains to antimicrobials normally used in poultry production may serve as a warning against the indiscriminate use of antibiotics in the treatment of infections. Data obtained shows the urgent need for a more judicious use of the antibiotics in poultry production.

6.2 Recommendations

Based on findings from this study, the following general and specific recommendations are made:

6.2.1 General recommendations

1. The national and local health authorities should enforce the food hygiene regulations to reduce the spread of zoonotic diseases.
2. The Ministry of Animal Health and Livestock Development in collaboration with NAFDAC should re-examine the use of antibiotics.

3. Collaborative efforts spearheaded by the MOH are needed and should focus on public education and training through media resources.
4. Public enlightenment programmes on the transmission and prevention of salmonellosis should be conducted by the Human and Veterinary Public Health Services

6.2.2 Specific recommendations

1. The Local Government should construct more befitting slaughter slabs for the LBMs.
2. Public health awareness should be created to educate the poultry workers in LBMs on salmonellosis prevention.
3. The butchers/processors should clean their knives and also other utensils including work benches during dressing and processing in-between different batches of birds to reduce contamination.
4. Processing tables and utensils should be disinfected every day at the close of work.
5. The poultry farmers should not administer drugs to birds without recommendations from a veterinarian or paravet.
6. The poultry farmers/workers should avoid handling and consumption of sick birds.
7. The poultry farmers/workers should also practise good environmental hygiene and ensure poor disposal of litter.

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Appendix 1

QUESTIONNAIRE ON KNOWLEDGE, ATTITUDE AND PRACTICES RELATED TO SALMONELLOSIS AMONG POULTRY WORKERS IN ZAMFARA STATE, NIGERIA

Date.....

Name of live bird Market.....

Good day. I am Samuel Sha'aibu, a student of Ahmadu Bello University, Zaria. I am conducting a study to determine the prevalence of *Salmonella* spp. in poultry in Zamfara State and to assess knowledge, attitude and practices related to Salmonella in poultry among poultry workers in the State. The aim of my study is to provide baseline data that could be useful in policy formulation; create awareness/advocate for effective prevention/control of this disease and protect public health including your health. You are not compelled to participate, however we would be grateful if you could kindly oblige us a few minutes of your time. All information supplied would be treated as strictly confidential.

SECTION A: SOCIO-DEMOGRAPHIC DATA

1. Age in years at last birthday: _____
2. Sex (do not ask, tick as appropriate): Male Female
3. Marital status (Tick whichever applies)
 - a) Married
 - b) Single (never married)
 - c) Others (specify)_____

4. What is your highest educational attainment

a) No formal education

b) Primary

c) Secondary

d) Tertiary

SECTION B. OCCUPATION HISTORY

5. What is your type of work/role in the live bird market?

a) Poultry seller/marketer

b) Buyer

c) processor/butcher

d) Market official

6. How long (in years) have you been working in a live bird market/farm?

7. Where do you source the birds you sell? (List all sources).....

8. How many poultry (birds) do you have?

9. What species of the poultry do you trade in? (list all spp)

a) Chicken

b) Geese

c) Ducks

d) Cattle

e) Guinea fowl

f) Pigeon

SECTION C: KNOWLEDGE RELATED TO SALMONELLA

10. Have you heard of Salmonellosis? a. Yes [] b. No []
11. If yes, what causes the disease?
12. Does *Salmonella* affect all type of animals? a. Yes [] b. No [] c. I don't know []
13. Does *Salmonella* affect only animals? a. Yes [] b. No [] c. I don't know []
14. How is *Salmonella* transmitted?
- a. Direct contact with infected bird
 - b. Utensils contaminated with faeces of infected birds
 - c. Inhaling infected air
 - d. Animal bites
 - e. Eating poorly cooked meat
15. What are the symptoms of Salmonellosis in poultry?
- a. Loss of appetite
 - b. Diarrhoea
 - c. Increased thirst
 - d. In coordination including loss of ability to walk and stand
 - e. Drop in egg production
 - f. Depression

SECTION D: ATTITUDES RELATED TO SALMONELLA CONTROL AND PREVENTION

I would like to know your attitudes towards *Salmonella* in birds. Try to answer the following questions as truthfully as possible

16. Salmonellosis may be prevented? Agree Uncertain Disagree
17. Salmonella in poultry can cause disease in man? Agree Uncertain Disagree
18. Selling sick birds in the market can spread *Salmonella* Agree Uncertain Disagree
19. Washing utensils can reduce transmission of *Salmonella* Agree Uncertain Disagree
20. Consuming sick birds can cause human sickness Agree Uncertain Disagree
21. Quarantine new birds before adding to pen Agree Uncertain Disagree
22. Washing/disinfecting surfaces reduces transmission Agree Uncertain Disagree

SECTION E: PRACTICES RELATED TO SALMONELLA CONTROL AND PREVENTION IN POULTRY

23. Wash hands with soap and water before and after handling poultry/entering a pen

Always Often Sometimes Rarely Never

24. How often do you wash and disinfect surfaces and utensils

Always Often Sometimes Rarely Never

25. How often do you practice all-in-all-out system

Always Often Sometimes Rarely Never

26. How often do you segregate/isolate sick birds

Always Often Sometimes Rarely Never

27. When your birds are sick, do you consult a veterinarian

Always Often Sometimes Rarely Never

USE OF ANTIBIOTICS

28. Are antibiotics used in your live bird market? (Please choose one)

- a) No
- b) Yes, but rarely
- c) Yes, regularly

29. If yes in above question, when do you use it? (Choose one or more)

- a) To treat animals when they are sick
- b) I give it to prevent animals from getting sick
- c) I give it to animals as it makes them grow faster

30. If yes in question 28 , how do you choose which antibiotics to use? (Please choose one)

- a) I use different antibiotics depending on what I can find or afford
- b) I use what the salesperson recommends
- c) I use antibiotics depending on what illness is present and/or after consultation with a veterinarian

31. Are you aware of resistance of some bacteria to antibiotics? (Please choose one)

- a) No
- b) I have heard of it but don't know how it affects my birds
- c) Yes, I am aware of the problem and try to restrict my use of certain antibiotics

Thank you very much for your participation.

Appendix 2

Plate showing the isolation of *Salmonella* species through sensitivity

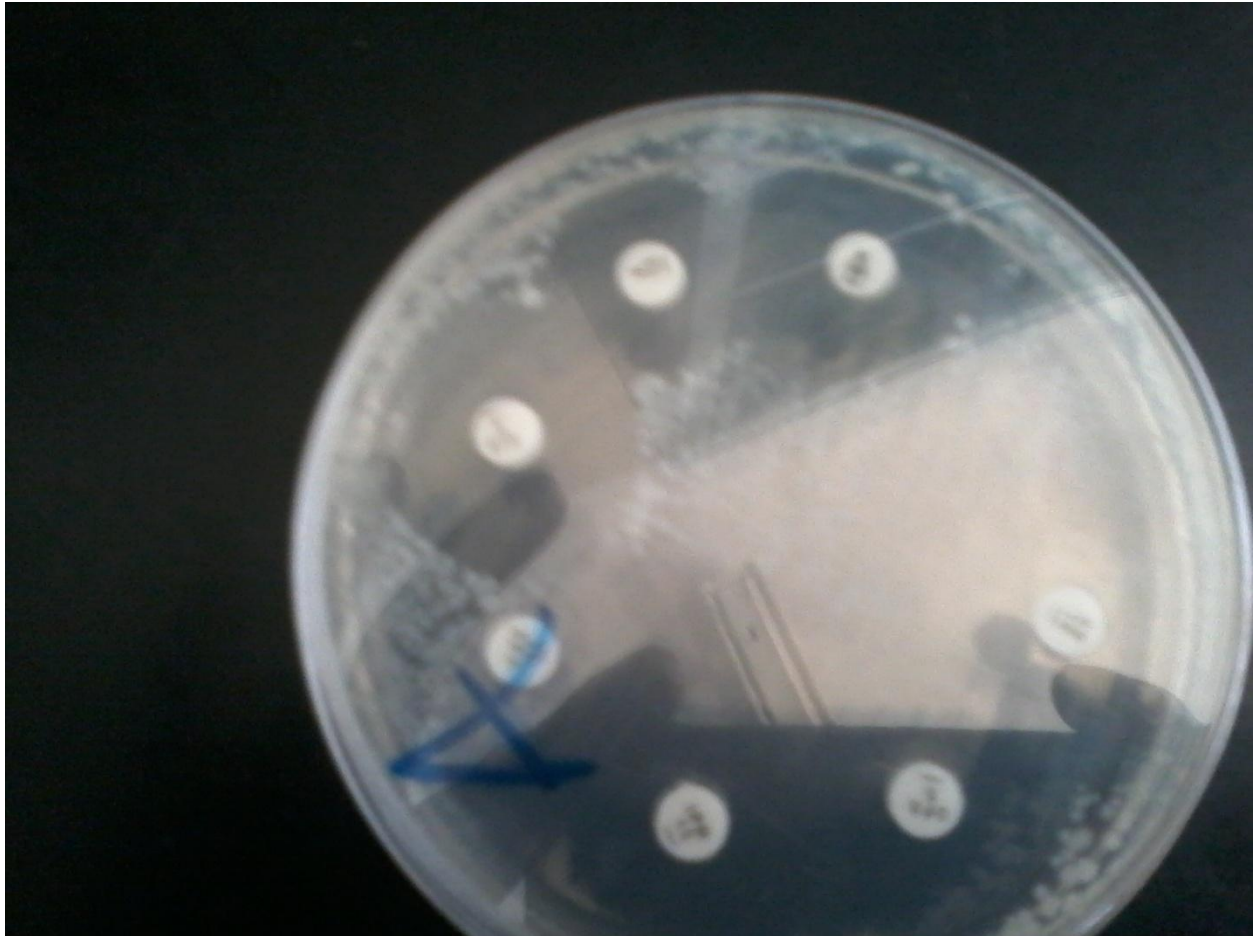


Plate 1: A clear zone of inhibition on Mueller-Hinton Agar by one of the antibiotic disc

Appendix 3

Growth of *Salmonella* on Plates



Appendix 4

Antimicrobial susceptibility of isolates

| Sample No | CRO | AMC | C | DO | VA | K | CN | IPM | CAZ | CIP | E | OFX | ENR |
|-----------|-----|-----|---|----|----|---|----|-----|-----|-----|---|-----|-----|
| 160 | S | R | R | R | R | S | R | S | S | S | R | S | S |
| 36 | S | R | R | R | R | S | S | S | S | S | R | S | S |
| 225 | S | R | S | R | R | S | S | S | S | S | R | S | S |
| 161 | S | R | R | R | R | R | I | S | S | S | R | S | I |
| 60 | S | R | R | R | R | S | I | S | S | S | R | R | R |
| A | S | R | R | R | R | S | S | S | R | S | S | S | I |
| 122 | S | R | R | R | R | S | S | S | S | S | R | S | S |
| 47 | S | R | R | R | R | R | I | S | S | S | R | S | S |
| 289 | S | R | S | R | R | S | S | S | S | S | R | S | S |
| C | S | R | R | S | R | R | S | S | R | S | R | S | S |
| 127 | S | R | R | R | R | R | I | S | S | S | R | S | I |
| 275 | S | R | S | R | R | S | S | S | S | S | R | S | S |
| 267 | S | R | R | I | R | S | S | S | S | S | R | S | S |
| 57 | S | R | R | R | R | S | R | S | S | I | R | S | S |
| 176 | S | R | R | R | R | R | I | S | S | S | R | S | S |
| 67 | S | R | S | I | R | S | S | S | S | S | S | S | S |

KEY

S= Susceptible

R=Resistant

I=Intermediate

CRO: Ceftriaxone, AMC: Amoxicillin-clavulanic acid, C: Chloramphenicol, DO: Doxycycline, VA: Vancomycin, K: Kanamycin, CN:Gentamicin, IPM: Imipenem, CAZ: Ceftazidime, CIP: Ciprofloxacin, E: Erythromycin, OFX: Ofloxacin, ENR: Enrofloxacin.

Appendix 5

Conventional biochemical results

| Sample No | TSI | C | U | I | M | H ₂ S | MR | VP |
|-----------|---------------------------|---|---|---|---|------------------|----|----|
| 225 | AK/A+H ₂ S | + | - | - | + | - | + | - |
| 160 | AK/A+H ₂ S | + | - | - | + | - | + | - |
| 176 | AK+A | + | - | - | + | - | + | - |
| 267 | AK/A+H ₂ S | + | - | - | + | + | + | - |
| 289 | AK/A+H ₂ S | + | + | - | + | - | + | - |
| 156 | AK/A+H ₂ S | + | - | - | + | + | + | - |
| 191 | AK/A+H ₂ S | + | + | + | + | - | + | - |
| 122 | AK/A+H ₂ S | + | + | + | + | + | + | - |
| 127 | AK/A+H ₂ S | + | - | + | + | - | + | - |
| 275 | AK/A+H ₂ S | + | + | - | + | - | + | - |
| 148 | AK/A+GAS | + | - | - | + | + | + | - |
| 230 | AK/A+H ₂ S | + | - | - | + | - | + | - |
| 231 | AK/A+H ₂ S | + | - | - | + | + | + | - |
| C | AK+A | + | + | - | + | + | + | - |
| 132 | AK/A+H ₂ S+GAS | + | - | - | + | + | + | - |
| 67 | AK+A | + | - | - | + | - | + | - |
| 74 | AK/A+GAS | + | + | - | + | - | + | - |
| 47 | AK/A+GAS | + | - | + | + | - | + | - |
| 57 | AK/A+H ₂ S | + | - | + | + | - | + | - |
| 36 | AK+A | + | - | + | + | + | + | - |
| 60 | AK+A | + | + | + | + | + | + | - |

Key

AK = Alkaline, A= Acid (Yellow) += Positive, - = Negative
 I = Indole. M= Motility, H₂S= Hydrogen Sulphide,
 SIM= Sulphur Indole Motility, TSI= Triple Sugar Iron

Appendix 6



TIME FRAME

| TASKS TO BE PERFORMED | NOV. 2014 | DEC. 2014 | JAN. 2015 | FEB. 2015 | MARCH. 2015 |
|--|--------------|--------------|--------------|--------------|----------------|
| 1. Finalize research proposal and submit for clearance | | | | | |
| 2. Design questionnaires and make copies | | | | | |
| 5. After clearance collect samples and data | | | | | |
| 6. Analyze samples and enter data | | | | | |
| 7. Analyze data and write report | | | | | |
| 8. Feedback, discuss and disseminate final research findings | | | | | |
| 9. Submit final thesis | | | | | |

Appendix 7
Bench work



Appendix 8
Ethical Clearance

| | | |
|--|------------------|--------------|
|  | | |
| ZAMFARA STATE OF NIGERIA MINISTRY OF HEALTH ADDRESS: J.B. Yakubu Secretariate, Gusau - Zamfara State. ☎:063-203772, 200906, 200896 | | |
| Our Ref: | Your Ref: | Date: |
| DR. SHUAIBU SAMUEL, | | 10/02/2015 |
| NIGERIA FIELD EPIDEMIOLOGY LABORATORY TRAINING PROGRAMM (NFELTP) ABUJA | | |
| <u>LETTER OF APPROVAL FOR RESEARCH TITLED "OCCURRENCE AND ANTIMICROBIAL SUSCEPTIBILITY OF SALMONELLA AMOND LIFE BIRD MARKET IN ZAMFARA STATE"</u> | | |
| With reference to your letter no NFLTP/CG/AO/109/14 DATED 6 TH February, 2015, on the need to carry out a research study on the above subject matter, | | |
| I am directed to write and convey the ministry approval to conduct the research no the "occurrence and antimicrobial susceptibility of salmonella among life bird market in Zamfara state" | | |
| Thank you | | |
|  NAJIBU M. NALADO STAFF OFFICER FOR; HON. COMMISSIONER | | |