PREVALENCE AND CHARACTERIZATION OF *SALMONELLA* SPECIES FROM ORGANS AND FAECES OF CHICKENS SOLD AT RETAIL MARKETS IN ZARIA, NIGERIA

BY

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DEPARTMENT OF VETERINARY PUBLIC HEALTH AND PREVENTIVE MEDICINE, AHMADU BELLO UNIVERSITY, ZARIA

OCTOBER, 2012

DECLARATION

I declare that this Thesis entitled ‘’Prevalence and characterization of *Salmonella* spp. from organs and faeces of chickens sold at retail markets in Zaria, Nigeria’’, has been performed by me in the Department of Veterinary Public Health and Preventive Medicine, Ahmadu Bello University, Zaria under the supervision of Dr. E.C. Okolocha, Dr. J. Kabir and Prof. I. Ajogi. 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 for another degree or diploma at any University.

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CERTIFICATION

This thesis titled "**PREVALENCE AND CHARACTERIZATION OF *SAMONELLA* SPECIES FROM ORGANS AND FAECES OF CHICKENS SOLD AT RETAIL MARKETS IN ZARIA, NIGERIA"** meets the regulations governing the award of the degree of Master of Science of Ahmadu Bello University, Zaria, and is approved for its contribution to knowledge and literary presentation.

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ABSTRACT

Chickens and chicken products such as eggs are abundant in the markets (Samaru, Zaria City, Sabon Gari, Tudun Wada and Dan Magaji) of Zaria. The purpose of this study was to determine the prevalence of *Salmonella* species from organs and faeces of chickens, the extent of tissue distribution of *Salmonella* species, the most common serotypes that invade and colonize the tissues of chickens in Zaria and the susceptibilities of the isolated *Salmonella* serotypes to commonly used antibiotics. Four hundred samples from 100 apparently healthy slaughtered adult local chickens in Zaria were examined for the presence of *Salmonella*. *Salmonella* were isolated and identified according to the techniques recommended by the World Health Organization: pre-enrichment, selective enrichment, selective plating, biochemical testing and serotyping. A total of 17 *Salmonella* isolates consisting of 10 different serotypes were identified. There was an overall *Salmonella* prevalence of 15%, and the isolates were distributed as follows, caecal content (12), liver (3), caecum (1) and gallbladder (1).The predominant serovars were S. Jedburgh (5), *S.* Typhimurium – DT29 (2), *S.* Derby (2) and *S. enterica subsp. enterica* gr. E (2). *S.* Typhimurium – NT (1), *S.* Agoueve (1), *S.* Takoradi (1), *S.* Ealing (1), *S.* Stanleyville (1) and *S.* Koenigstuhl (1) were also isolated. The 2 *S.* Derby, 1 *S. enterica subsp. enterica* gr. E and *S.* Stanleyville were resistant (23.53 %) to sulfamethoxazole, sulphonamide, kanamycin, streptomycin, tetracycline and chloramphenicol. Thirteen isolates (76.57 %) were sensitive to the panel of antibiotics used. There was a significant association ($p = 0.000$, $p < 0.05$) between organs and infection, and markets and infection ($p = 0.003$, $p < 0.05$). Chickens sold at retail markets in Zaria were infected with *Salmonella* organisms. *Salmonella* species were distributed in caecal contents, caecum, liver and gall bladder. The most common serotype was *S.* Jedburgh. Multidrug resistance patterns were observed in four isolates. The national and local health authorities should

enforce the food hygiene regulations to reduce the spread of zoonotic and multidrug resistant *Salmonella* to humans. Public enlightenment programmes on the modes of transmission of *Salmonella* should be conducted by Human and Veterinary Public Health services. Further studies should be conducted to know the extent of distribution of *Salmonella* spp. in different areas of Kaduna State and the country at large.

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ABBREVIATIONS

AOAC:	Association of Official Analytical Chemists
APHIS:	Animal and Plant Health Inspection Services
BGA:	Brilliant Green Agar
BGS:	Brilliant Green Sulfa
BPW:	Buffered Peptone Water
BSA:	Bismuth Sulphite Agar
Camavet:	Centrale d'approvisionnement en matériels et produits vétérinaires
CD:	Cluster of Differentiation
CDC:	Centres for Disease Control and Prevention
DNA:	Deoxyribonucleic Acid
DT:	Definitive Phage Type
ELISA:	Enzyme Linked Immunosorbent Assay
EU:	European Union
FAO:	Food and Agriculture Organization
FDA:	Food and Drug Administration
GPHIN:	Global Public Health Intelligence Network
GSD:	Groupements de défense Sanitaire
HIV:	Human Immunodeficiency Virus
INPPAZ:	Pan America Institute for Food Protection and Zoonoses
Labocel:	Laboratoire central de l'Elevage
LDR:	Ligation Detection Reaction
LPS:	Lipopolysaccharide
NAHMS:	National Animal Health Monitoring System
NARMS:	National Antimicrobial Resistance Monitoring System

NCCLS:	National Committee for Clinical Laboratory Standards
NNDSS:	National Notifiable Diseases Surveillance System
OIE:	Office International des Epizooties
PCR:	Polymerase Chain Reaction
PIM:	Pulmonary Intravascular Macrophages
SPI1:	*Salmonella* Pathogenicity Island 1
TTSS:	Type III Secretion System
UK:	United Kingdom
US:	United States
USA:	United States of America
USDA:	United States Department of Agriculture
VTH:	Veterinary Teaching Hospital
WHO:	World Health Organization

CHAPTER 1

INTRODUCTION

1.1 BACKGROUND OF THE STUDY

The incidence of food borne diseases in humans has increased considerably worldwide in the last few years. Poultry products have been repeatedly implicated in food borne infections (Hafez, 2005). Poultry can harbour different food borne pathogens. Many reports in recent years have shown that *Salmonella* and *Campylobacter* spp are the most common causes of human food borne bacterial diseases linked to poultry (Hafez, 2005). The main carriers of *Salmonella* Typhimurium (> 80 %) and of *Salmonella* Enteritidis (> 90 %) are different kinds of poultry and other domestic animals (Koehler, 1992). About 1.4 million cases of salmonellosis occur annually in the USA, and 40,000 are culture-confirmed cases reported to the Centres for Disease Control and Prevention (Mead *et al.*, 1999). In the European Union (EU), the number of human cases uploaded on the Internet was greater than 100,000 in 1997 (O' Brien and de Valk, 2003).

The genus *Salmonella* is a typical member of the family *Enterobacteriaceae* and consists of Gram negative, non spore-forming bacilli (Popoff *et al.*, 2003). According to the latest nomenclature, which reflects recent advances in taxonomy the genus *Salmonella* consists of only two major species: *S. enterica* and *S. bongori* (Grimont and Weill, 2007). *S. enterica* is divided into six subspecies: *enterica*, *salamae*, *arizonae*, *diarizonae*, *houtenae* and *indica*. Within *S. enterica* subsp. I (*S. enterica* subsp. *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 (Uzzau *et al.*, 2000). Serotypes in other subspecies are usually isolated from cold-blooded animals and the environment but rarely from humans (Humbert, 1994; Uzzau *et*

al., 2000). Bacteria constituting the genus contains three different types of antigens: the somatic O, flagellar H and capsular Vi antigens which are used to differentiate among more than 2, 500 serologically distinct types of *Salmonella* (Popoff *et al.*, 2003). *Salmonella* causes gastroenteritis in both man and animals, and is one of the major foodborne pathogens of significant public health concern in both developed and developing countries (Hafez, 2005; Philippe *et al.*, 2005).

Salmonella has been recognised as causes of intestinal disease for many years, and methods of control are well established. Despite these, *Salmonella* remains the primary cause of reported food poisoning worldwide and recent years have seen massive outbreaks (Mead *et al.*, 1999).

The most common vehicles for transmission of food-borne salmonellosis are meat, meat products, eggs, and egg products that are contaminated as a direct result of animal infection or faecal contamination during processing (Rabsch *et al.*, 2001). Many *Salmonella* types have been isolated from poultry and egg products (Dack, 1963). Barnes in 1944, considered the domestic fowl as "the main animal reservoir of organisms affecting man" (Dack, 1963).

The problem of non-typhoidal *Salmonella* in Africa is very serious, but has generally been overshadowed by the 'big three': malaria, human immunodeficiency virus and tuberculosis (MacLennan, 2008). In Nigeria, of the poultry diseases diagnosed in Kaduna for the period of 1981 to 1985, fowl typhoid accounted for 8.0% (Salami *et al.*, 1989b). Kabir *et al.* (2007) conducted a study on salmonellosis in Lagos. They isolated *Salmonella* Typhi, *Salmonella* Enteritidis and *Salmonella* Paratyphi from blood of

patients showing pyrexia for up to 5 days, and *Salmonella* Enteritidis, *Salmonella* Arizonae and *Salmonella* Paratyphi from faeces of patients having diarrhoea for two or more days. Okwori *et al.* (2007) obtained 37.9 % positive results from a serological survey of *Salmonella* Gallinarum antibodies in chickens around Jos, Plateau State, Nigeria.

1.2 STATEMENT OF RESEARCH PROBLEM

Chickens and chicken products are abundant in the markets of Zaria. Many house holders in Zaria are in contact with chickens and their products. Abdu *et al.* (1985) found that in Zaria, the prevalence of salmonellosis was 1.1%. A serological investigation using sera obtained from chickens in Zaria and Kaduna revealed that 28 % were positive for *Salmonella* (Salami *et al.*, 1989a). Mbuko *et al.* (2009) conducted a five-year retrospective study (2003 - 2007) in the Avian Unit of the Veterinary Teaching Hospital (VTH), Ahmadu Bello University, Zaria, Nigeria and found that 18.4 % cases were diagnosed positive for fowl typhoid.

Salmonellosis and *Salmonella* infections in poultry are distributed world-wide and result in severe economic losses when no effort is made to control them (Hafez, 2005). The most important aspect, however, is the effect of *Salmonella*-contaminated eggs, poultry meat and meat products on public health. Adult birds serve mostly as intestinal or internal organ carriers over longer period with little or no evidence of infection (Hafez, 2005). Infection of poultry with *Salmonella* spp. become latent in most cases and remains subclinical. Therefore, there is a potential danger for human health when products are not sufficiently heated (Schluter, 1992).

Multiple-antibiotic resistant *S.* Typhimurium definitive phage type (DT) 104 emerged during the last decade as a global health problem because of its involvement in diseases in animals and humans (Poppe *et al.*, 1998; Threlfall *et al.*, 2000; Cloeckaert and Schwarz, 2001). Strains are generally resistant to ampicillin (Ap), chloramphenicol (Cm), florfenicol (Ff), streptomycin (Sm), spectinomycin (Sp), sulphonamides (Su) and tetracyclines (Tc). Genes associated with these resistance properties have been found to be chromosomally encoded (Threlfall *et al.*, 1994). In the United Kingdom substantial increases in resistance to fluoroquinolones in *Salmonella* Hadar and *Salmonella* Virchow, and in multiresistant *Salmonella* Typhimurium DT104 followed the licensing for veterinary use of enrofloxacin in 1993 and danofloxacin in 1996 was reported by Threlfall *et al.* (1997). In Germany, an increase in the incidence of strains that are resistant to nalidixic acid was observed after the licensing of enrofloxacin (Malorny *et al.,* 2003). Extended spectrum (E-S) cephalosporins are the drugs of choice for children because they cannot be treated with FQ. *Salmonella* and *E. coli* exhibiting resistance to E-S cephalosporins are an emergent problem worldwide. Before 1996, resistance to E-S cephalosporins was rarely reported among *Salmonella* spp. In 2000, the emergence of domestically acquired infections by E-S cephalosporin-resistant *Salmonella* carrying a plasmid-mediated CMY-2 AmpC β-lactamase was reported by the National Antimicrobial Resistance Monitoring System (NARMS) in the USA (Dunne *et al.*, 2000).

1.3 JUSTIFICATION OF THE STUDY

Most of the prevalence rates obtained in Zaria were proportional morbidity rates (Abdu *et al*., 1985; Salami *et al*., 1989b; Mbuko *et al*., 2009). Records on the tissue distribution of *Salmonella* species in organs and products from chickens are not available. Most of the studies were conducted on *Salmonella* Gallinarum (Salami *et al.,* 1989b; Okwori *et al*.,

2007; Mbuko *et al*. 2009). And the multiple-antibiotic resistant strains of *Salmonella* are reported worldwide (Poppe *et al.*, 1998; Threlfall *et al.*, 2000; Cloeckaert and Schwarz, 2001).

It is, therefore, important to study the prevalence of *Salmonella* species in organs and products from chickens sold at retail markets in Zaria; to know their tissue distribution; the most common serotypes that colonize and invade chicken tissues and the anti-microbial profile of those serotypes.

1.4 AIM

The aim of this study was to determine the prevalence of *Salmonella* in chickens sold at retail markets in Zaria, Nigeria.

1.5 OBJECTIVES

1. To determine the prevalence of *Salmonella* species in internal organs and faeces of chickens sold at five retail markets in Zaria, Nigeria.
2. To determine the tissue distribution of *Salmonella* species in chicken sold at retail markets in Zaria, Nigeria,
3. To determine the most common serotypes that invade and colonize the tissues of chickens in Zaria, Nigeria.
4. To determine the susceptibilities of the isolated *Salmonella* serotypes to commonly used antibiotics.

1.6 RESEARCH QUESTIONS

1. Are chickens sold at retail markets in Zaria infected with *Salmonella* species?
2. What is the tissue distribution of *salmonella* species in chickens sold at retail markets in Zaria?
3. What are the most common *Salmonella* serotypes that invade and colonize the tissues of chickens in Zaria?
4. Are the isolated *Salmonella* serotypes susceptible to commonly used antibiotics?

CHAPTER 2

LITERATURE REVIEW

2.1 HISTORICAL BACKGROUND OF *SALMONELLA*

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 area (Winslow, 1952). The typhoid bacillus was described in tissue in 1880 and isolated by Gaffky in1884 (Morgan, 1965). Salmon and Smith in 1885 isolated *Salmonella* Choleraesuis (*B*. *cholerae suum*, *Bact*. *suipestifer*, the Hog cholera bacillus) from hogs suffering cholera, which was thought to be the cause of the disease (Dack, 1963). Gartner in 1888 isolated *Salmonella* Enteritidis from a man who died from eating a raw meat of a diseased cow (Dack, 1963). De Nobele in 1889 isolated *Salmonella* Typhimurium from a food poisoning outbreak (Dack, 1963).

2.2 DEFINITION AND CLASSIFICATION OF *SALMONELLA*

Salmonella are gram negative, facultatively anaerobic, non-spore forming, rod shaped bacteria that are motile (with a few exceptions) by means of peritrichous flagella. They are catalase positive, oxidase negative, and chemo-organotrophic with the ability to metabolize nutrients by both respiratory and fermentative pathways (Doyle and Beuchat, 2007; Safe Food, 2009). This pathogen grows on citrate, forming acetate, formate, and carbon dioxide as products, and catabolizing D-glucose with the production of acid and gas (Doyle and Beuchat, 2007; Bott, 2009). In addition, *Salmonella* readily generate hydrogen sulfide gas in both triple sugar iron and lysine agar media (Labbe and Gracia, 2001; Todar, 2008).

The genus *Salmonella* is a typical member of the family *Enterobacteriaceae* and consists of Gram negative, non spore-forming bacilli (Popoff *et al.*, 2003). The DNA of this genus is most closely related to other genera of the *Enterobacteriaceae* family: *Escherichia*, *Shigella*, and *Citrobacter*. (Todar, 2008; *Salmonella*.org. 2009).

Bacteria constituting the genus contain three different types of antigen: somatic (O) or cell wall antigens; flagellar (H) antigens; and capsular antigens (Popoff *et al.*, 2003; Todar, 2008; Safe Poultry, 2009). Somatic (O) or Cell Wall Antigens - This type includes lipopolysaccharide-protein chains on the external surface of the bacterial outer membrane. They are heat stable, alcohol resistant, and hydrophilic which enables the bacteria to form stable suspensions in saline solutions (Todar, 2008). In *S. enterica*, 46 types of O antigens are present, which differ in sugar composition and in linkages between sugars and O units (Curd *et al.*, 1997). Flagellar (H) Antigens represent the determinant groups in the flagellar protein. They are heat and alcohol-labile proteins, and individual *Salmonella* strains may produce one (monophasic) or two (diphasic) sets of flagellar antigens. Most serovars alternately express two sets of antigens, i.e., phase 1 and phase 2 Antigens (Doyle and Beuchat, 2007). Surface (Envelope) Antigen is commonly observed in other genera of enteric bacteria (e.g., *Escherichia coli* and *Klebsiella*) and may be found in some *Salmonella* serovars. A well known surface antigen, the (Vi) antigen, is heat labile occurring in only three *Salmonella* serovars: Typhi, Paratyphi C, and Dublin (Todar, 2008).

The agglutinating properties of the somatic O, flagellar H and capsular (Vi) antigens are used to differentiate among more than 2, 500 serologically distinct types of *Salmonella* (Popoff *et al.*, 2003). The genus *Salmonella* consists of only two species, *S. bongori* and

S. enterica, with the latter being divided into six subspecies: *enterica*, *salamae*, *arizonae*, *diarizonae*, *houtenae*, *indica*. Within *S. enterica* subsp. I (*S. enterica* subsp. *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 (Uzzau *et al.*, 2000). Serotypes in other subspecies are usually isolated from cold-blooded animals and the environment but rarely from humans (Humbert, 1994; Uzzau *et al.*, 2000). The serotypes of *Salmonella* may be grouped depending on the host range such as:

Those restricted to human hosts: This group includes *S.* Typhi*, S.* Paratyphi types A, B, C and *S.* Sendai which are pathogenic only in humans (Uzzau *et al.*, 2000).

Those restricted to animal hosts: *S.* Abortusovis: causes disease in ovine, *S.* Gallinarum: causes disease in poultry, *S.* Typhisuis: causes disease in swine, *S.* Abortusequi: causes disease in equine (Uzzau *et al.*, 2000).

Those adapted to animal hosts: This group includes two common strains, *S.* Dublin with cattle as natural host, also causes disease in human and ovine. *S.* Choleraesuis with swine as natural host and also causes disease in human.

Ubiquitous (or un-restricted) serotypes: examples are *S.* Typhimurium and *S.* Enteritidis (Uzzau *et al.*, 2000).

S. enterica serovars: *S. enterica* serovars cause intestinal diseases and represent more than 99.5 percent of the *Salmonella* serotypes isolated from humans and other warm-blooded animals.

The three main serovars of *S. enterica* are:

S. enterica serovar Typhi (*S.* Typhi): This bacterium is the causative agent of typhoid fever. It is not widespread in the U.S. but is very common in developing countries. It can only infect humans and no other host has been identified.

S. enterica serovar Typhimurium (*S.* Typhimurium): This *Salmonella* serovar is one of the most common causes of food-borne illnesses in the U.S. It causes a typhoid-like disease in mice and does not cause as severe a disease in humans as *S.* Typhi.

S. enterica serovar Enteritidis (*S.* Enteritidis): This bacterium is the most common cause of food-borne illness in the U.S. It usually infects chicken flocks, spreads from hen to hen, and rapidly transmits through the food chain causing the disease in humans. This food-borne disease is almost identical to that of *S.* Typhimurium which is very closely related (Todar, 2008; *Salmonella*.org., 2009).

2.3 VIRULENCE CHARACTERISTICS OF *SALMONELLA*

2.3.1 Adherence

The first host barrier for many invading pathogens is usually a mucosal surface, such as the gut or respiratory tract. Since epithelial cell turnover is around 48 hours in these environments, the bacterium must attach and replicate sufficiently to avoid being swept away.

Therefore, many have evolved motile or attachment elements like flagella and pili/fimbriae (Sauer *et al.*, 2000) to cross the barrier and invade. Simple attachment is mediated through a receptor on the host cell surface, and an adhesin on the bacterial one.

2.3.2 Internalization

A systemic infection requires bacterial penetration of the intestinal epithelium, a process which occurs primarily through the M cells of Peyer's patches (Jones *et al.*, 1994). The invasiveness of *Salmonella* is attributed to a type III secretion system, TTSS-1, encoded by *Salmonella* pathogenicity island 1 (SPI1) (Galan and Curtiss, 1989; Lostroh and Lee 2001; Zhou and Galan, 2001). BarA/SirA orthologs are present throughout the proteobacteria and control secondary metabolism and virulence functions. However, only in *S. enterica* serovar Typhimurium has a systematic effort been made to identify the entire SirA regulon (Ahmer *et al.*, 1999; Goodier and Ahmer, 2001).

With the use of genetic techniques and tissue culture several laboratories have identified *Salmonella* genetic loci that are necessary for invasion (Finlay *et al.*, 1988; Galan and Curtiss, 1991). Galan and Curtiss (1989) identified four *Salmonella* genes (*inv*A, -B, -C, and -D) that allow *Salmonella* Typhimurium to enter cultured epithelial cells. Virulent strains of *Salmonella* carrying defined mutations in these genes were shown to have higher 50% lethal doses than their parent strains when administered orally to mice. A more recent study (Galan and Curtiss, 1991) showed that the *inv*A, -B, -C, and -D genes are present and functional in most (if not all) *Salmonella* serovars. Christine *et al.* (1992) reported the molecular characterization of *inv*E, an invasion gene that is involved in the triggering of intracellular events that lead to bacterial entry into cultured epithelial cells.

2.3.3 Biofilm Formation

Biofilms are a collection of microorganisms surrounded by the slime they secrete, attached to either an inert or living surface (Paula, 2003). More than 99 percent of all bacteria live in biofilm communities. Some are beneficial. Sewage treatment plants, for

instance, rely on biofilms to remove contaminants from water (Paula, 2003). But biofilms can also cause problems by corroding pipes, clogging water filters, causing rejection of medical implants, and harbouring bacteria that contaminate drinking water (Paula, 2003).

Different species live cheek-by-jowl in slime cities, helping each other to exploit food supplies and to resist antibiotics through neighborly interactions. Toxic waste produced by one species might be hungrily devoured by its neighbor. And by pooling their biochemical resources to build a communal slime city, several species of bacteria, each armed with different enzymes, can break down food supplies that no single species could digest alone. The biofilms are permeated at all levels by a network of channels through which water, bacterial garbage, nutrients, enzymes, metabolites and oxygen travel to and fro. Gradients of chemicals and ions between microzones provide the power to shunt the substances around the biofilm (Coghlan, 1996). Once the microorganisms have attached, they must be capable of withstanding normal disinfection processes. Biofilm bacteria display a resistance to biocides that may be considered stunning (LeChevallier, 1988).

When bacteria are in a film, they are very resistant to biocides. In fact, they often produce more exopolymers after biocide treatment to protect themselves (Borenstein, 1994). In addition to their direct role in infection pathogenesis, biofilms also provide important environmental reservoirs for pathogenic bacteria. Many characteristics of biofilms, such as resistance to antimicrobials and increased opportunities for genetic exchange, could increase the pathogenic potential of a biofilm community while promoting survival of the organisms in the environment (Matthew and Pradeep, 2003).

Another interesting example of hyperbiofilm-forming phenotypes is the rdar phenotype (for red, dry, and rough) of *Salmonella* Typhimurium and *E. coli* (Romling *et al.*, 1998a).This phenotype is characterized by colonies on solid medium that bind the dye Congo red and exhibit a wrinkly, spreading phenotype relative to the smooth wild type colonies (Romling *et al.*, 1998a; Romling *et al.*, 1998b).

Biofilm formation on abiotic surfaces is enhanced by this phenotype, as well as adherence to eukaryotic serum proteins such as fibronectin (Austin *et al.*, 1998; Olsen *et al.*, 1998; Romling *et al.*, 1998a; Prigent-Combaret, 2000). The rdar phenotype results from expression of the *agf* genes (*csg* genes in *E. coli*), which encode thin aggregative fimbriae (curli) and production of the secreted polysaccharide cellulose (Hammar *et al.*, 1995; Romling *et al.*, 1998a; Zogaj *et al.*, 2001). The regulation of this phenotype is complex, depending upon environmental factors such as nutrient depletion, low-oxygen tension, low osmolarity, and temperature (Romling *et al.*, 1998a; Romling *et al.*, 1998b; Gerstel and Romling, 2001).

The rdar phenotype can also occur constitutively in an environment-independent manner (Collinson *et al.*, 1991). This can result from mutations in the promoter of the *agfD* gene, which encodes a regulatory protein shown to control expression of the rdar phenotype (Romling *et al.*, 1998a). The role of curli and the rdar phenotype in the pathogenesis of these organisms is unclear; however, it has been suggested that this phenotype may allow persistence of the organism in environmental biofilms outside an animal host (Sakellaris *et al.*, 2000; Zogaj *et al.*, 2001; Uhlich *et al.*, 2002).

2.3.4 Quorum Sensing

In bacteria, the regulation of gene expression in response to changes in cell density is called quorum sensing. Quorum sensing bacteria produce, release and respond to hormone-like molecules (autoinducers) that accumulate in the *Salmonella* external environment as the cell population grows .*Salmonella* Typhimurium 14028 and *Salmonella* Typhimurium TL2 produce autoinducer AI 2. The responsible gene for AI 2 production is *Lux*$S_{S.t.}$ homologous to *Lux*$S_{V.h.}$ and *Lux*SE.c. (Bassler, 1999; Surette *et al.*, 1999).

The species of bacteria that contain *luxS* genes include *E. coli*, *S.* Typhimurium, *Salmonella* Typhi, *Salmonella* Paratyphi, *Haemophilus influenzae*, *Helicobacter pylori*, *B. subtilis*, *Borrelia burgdorferi*, *Neisseria meningitidis*, *Yersinia pestis*, *Campylobacter jejuni*, *Vibrio cholerae*, *Mycobacterium tuberculosis*, *Enterococcus faecalis*, *S. pneumoniae*, *Streptococcus pyogenes*, *S. aureus*, *Clostridium perfringens*, *Clostridium difficile*, and *Klebsiella pneumoniae* (Bassler, 1999; Surette *et al.*, 1999).

2.3.5 Siderophores

The host environment is ideal for bacteria in every way except for one; iron is plentiful, but tightly bound in haem, ferritin, transferrin, or lactoferrin. It can be the limiting factor in infections (Thulasiraman *et al.*, 1998). Therefore, some pathogens have evolved virulence factors that mediate the release of host iron for parasitic consumption (Thulasiraman *et al.*, 1998). Examples include enterochelin from *Escherichia* and *Salmonella* spp, which scavenges bound iron from the host via high binding constants (Thulasiraman *et al.*, 1998). Experiments involving a deletion of the seven enterochelin genes from *Salmonella* have shown that the pathogens lose their virulence when injected

into mouse models. Thus, siderophores are essential for virulence (Thulasiraman *et al.*, 1998).

2.3.6 Endotoxins

Possessed solely by Gram negative organisms; endotoxin or lipopolysaccharide (LPS) activates the host complement pathway, and is a potent inducer of inflammation. It is an outer membrane chemical moiety consisting of three sections: a toxic lipid (Lipid A) anchored in the outer membrane, an immunogenic polysaccharide core, and an antigenic O-linked series of oligosaccharides at the extracellular surface. It is considered a part of Gram negative bacterial pathology. Species as diverse as *Salmonella* Typhimurium and *Neisseria meningitidis* express LPS on the cell surfaces (El-Samalouti *et al*., 2000).

Endotoxins are toxic to most mammals, and can be lethal if encountered in high concentration. Specifically, release of LPS into the host circulation promotes binding by a certain protein, dubbed "LPS-binding complex". This interacts with CD14 receptors on a variety of monocytes and macrophages, triggering inflammatory cytokine release, and activation of the complement and coagulation cascades (Wisconsin, 2000).

Physiological distress involving pyrogenicity and mitogenicity eventually leads to blood sepsis and death. However, low levels of LPS used, for example, as an adjuvant favourably increases the host's microbial resistance, and induces T-cells to produce more antiviral-enhancing interferon (Goodier and Londei, 2000). The Lipid A component of LPS is a strong biological enhancer, and can boost the immune system (Goodier and Londei, 2000).

2.4 EFFECT OF GLOBAL CLIMATE CHANGE ON *SALMONELLA* SURVIVAL

There are a few characteristics of pathogens that may predispose them to being more sensitive to the impacts of global climate change. For example, those food borne pathogens that cause disease at very low doses (enteric viruses, parasitic protozoa, *Shigella* spp., enterohemorrhagic *E. coli* strains) and/or have notable environmental persistence (enteric viruses and parasitic protozoa) will likely to be of great concern, particularly after adverse weather events. Those pathogens with documented stress tolerance responses (temperature, pH), such as enterohemorrhagic *E. coli* and *Salmonella* are likely to compete better in the event of climate change (FAO, 2008).

2.5 EFFECT OF HIV/AIDS ON *SALMONELLA* INFECTION

The HIV-associated damage to cell mediated immunity is profound in later stages of AIDS, with frequent opportunistic infections and increased susceptibility to intracellular microorganisms such as *Salmonella* spp., *Mycobacterium avium* and *Bartonella* spp. (Davy, 2002).

2.6 MOST COMMON *SALMONELLA* ISOLATES

Lammerding *et al.* (1988) found that *S.* Typhimurium was the predominant serotype isolated from chicken carcasses in Canada. Bokanyi *et al.* (1990) found that the most frequently isolated serotypes were *S.* Hadar, *S.* Heidelberg and *S.* Johannesburg. Moll and Hildebrandt (1991) isolated mainly *S.* Virchow, *S.* Typhimurium and *S.* Indiana from deep-frozen chicken giblets and offals, and from fresh livers in Berlin, Germany. Overall 21% of samples collected by Roberts (1991) in United Kingdom contained *S.* Enteritidis phage type 4. Mutluer *et al.* (1992) found that the main isolates from chicken products

from retail markets of Ankara in Turkey were *S.* Enteritidis*, S.* Typhimurium and *S.* Agona.

2.7 HOST SPECIES

Ubiquitous serotypes, such as *S.* Enteritidis or *S.* Typhimurium, which affect both man and animals, generally cause gastrointestinal infections 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 (Cowden *et al.*, 1989). *Salmonella* spp. can be found widely on farms of many types, including those for beef and dairy cattle, swine farrowing and finishing facilities, and poultry farms. Rodriguez *et al.* (2006) found that 4.7% of all samples collected from farm animals were positive for *Salmonella,* with the majority of positive findings occurring on swine farms (57%), followed by dairy farms (18%), poultry farms (16%), and beef farms (9%). *Salmonella* was present on 18 diverse farm types in 5 states and was found in soil, bedding litter, feces, and feeds (Rodriguez *et al.*, 2006). The *Salmonella* isolates came from all materials examined on the farms.

2.8 NATURAL RESERVOIRS

Salmonella is a zoonotic pathogen and is widespread in humans and animals worldwide. Animals and their environment are the most important reservoirs of *Salmonella* beside feed factories, food processing plants, and communal hygienic biotopes (Koehler, 1992).

The serovars of *Salmonella* can be found predominantly in one particular host, can be ubiquitous or can have an unknown habitat. For example, *S.* Typhi and Paratyphi are strictly human pathogens while all other non-typhoid *Salmonella* species (*Salmonella*

enterica) are ubiquitous in the environment and reside in the intestinal tracts of animals (Todar, 2008).

The pathogen has large and varied animal reservoirs; the most common are chickens, turkeys, pigs, and cows. Many domestic animals (e.g. ducks, dogs, cats, pet turtles), birds, reptiles, flies, and rats are also potential sources (Vermont Department of Health, 2005). Several authors have suggested that *S.* Enteritidis was introduced into poultry flocks by rodents where it is endemic since in the distant past it was used as a rodenticide (Henzler and Opitz, 1992; Friedman *et al.*, 1996).

Imported birds and animals may also serve to introduce different *Salmonella* species to the local area resulting in an increase in its prevalence (Avian Biotechnology International, 2009). *Salmonella* exists in human and animal carrier states. Approximately 3 percent of persons infected with *S.* Typhi and less than 1 percent of non-typhoidal cases become chronic carriers of *Salmonella*. The carrier state may last from many weeks to years; however, many humans and animals just serve as reservoirs and passive carriers of *Salmonella* without showing symptoms of the disease (FAO and WHO, 2002; Hatta and Smits, 2007). *Salmonella* species are often found in aquatic environments: sewage, freshwater, marine coastal water, and groundwater. Contaminated water is the most common source of *S.* Typhi. The pathogen can survive for long periods in natural waters, and the persistence of epidemic *Salmonella* strains (e.g. *S.* Typhi, *S.* Typhimurium) is of great concern to public health (Baudart *et al.*, 2000).

2.9 DISTRIBUTION ACROSS THE WORLD

By the 1980s, *S.* Enteritidis O9, 12: g, m had emerged as a major concern for food safety in Europe and the Americas. By 1990 in the USA and by 1993 in Europe, it was the most frequently reported *Salmonella* serotype (Rabsch *et al.*, 2001; Bouvet *et al.*, 2001; Cogan and Humphrey, 2003).

In England and Wales, there were 200 reported human cases in 1966, which rose to 10,000 in 1981, and peaked at 33, 000 in 1997 (more than 70% of human cases of salmonellosis) (Ward *et al.*, 2000; Cogan and Humphrey, 2003). Despite a subsequent decline in its incidence, *S.* Enteritidis continues to be the most frequently isolated *Salmonella* serotype in the United Kingdom with 16, 465 cases in 2001 (Cogan and Humphrey, 2003).

In France, *S.* Enteritidis has become the most commonly isolated serotype in 1993. The incidence of *S.* Enteritidis human isolates increased exponentially from 1987 to peak at 6, 500 in 1994 and 1997 and subsequently declined to 4, 500 cases in 1999 (Bouvet *et al.*, 2001). Similar trends have also been reported from other countries in South America and Europe (Rabsch *et al.*, 2001; Cogan and Humphrey, 2003).

2.10 SEASONALITY AND TEMPERATURE EFFECTS ON SALMONELLOSIS

The crucial National Animal Health Monitoring System (NAHMS) animal surveys were conducted in February through July and March through September, which is when fecal shedding appears to be at its highest (USDA-APHIS, 2001; Huston *et al.*, 2002).

An eastern regional gradient has been observed in Australia for notifications of salmonellosis, i.e. rates of notification increase with decreasing latitude and hence, increasing average yearly temperature (Hall *et al.,* 2002). Seasonality of fecal shedding is critical to understanding the flow of *Salmonella* through the food chain. There is a correlation between shedding in animals and human outbreaks. Shedding by food animals can approach zero during the winter months and reaches its peak in summer and early fall (McEvoy *et al.*, 2003; Fossler *et al.*, 2005), especially in cattle and swine, and human outbreaks also peak during this period. Increases in disease notifications, particularly salmonellosis (D'Souza *et al.*, 2008) and to a lesser extent campylobacteriosis (Kovats *et al.*, 2005) are frequently preceded by weeks of elevated ambient temperature. Interestingly, it has been shown recently that the human *Salmonella* incidence peaks 13 days after the peak in ambient temperature (Naumova *et al.*, 2007). However, other researchers have found that the highest incidence of *Salmonella* on farms occurs during the late fall (October–December) instead of the summer (Rodriguez *et al.*, 2006). Although a physical correlation to temperature exists, it must be noted that the internal temperature of the gut is fairly consistent, so that temperature is not the sole source of the observed seasonality. Other potential factors for seasonality of pathogen shedding include thyroid hormones and melatonin levels; however, the linkage to these factors is still preliminary (Edrington *et al.*, 2006; Edrington *et al.,* 2007).

2.11 PREVALENCE OF THE DISEASE IN CHICKENS

The main carriers of *Salmonella* Typhimurium (> 80 %) and of *Salmonella* Enteritidis (>90 %) are different kinds of poultry and other domestic animals (Koehler, 1992). In Nigeria, Abdu *et al.* (1985) studied cases of diseases diagnosed in chickens at the Avian Clinic of Ahmadu Bello University, Zaria and found that the prevalence of

salmonellosis was 1.1%. A serological investigation using 598 sera obtained from chickens in Zaria and Kaduna, Nigeria revealed that 167 (28 %) were positive for *Salmonella* (Salami *et al.*, 1989a). Of the poultry diseases diagnosed in Kaduna for the period of 1981 to 1985, fowl typhoid accounted for 8.0% (Salami *et al.*, 1989b). Okwori *et al.* (2007) obtained 37.9 % of positive results from a serological survey of *Salmonella* Gallinarum antibodies in chickens around Jos, Plateau State, Nigeria. Mbuko *et al.* (2009) conducted a five-year retrospective study (2003 - 2007) on the prevalence of fowl typhoid and other poultry diseases diagnosed at the Avian Unit of the Veterinary Teaching Hospital (VTH), Ahmadu Bello University, Zaria, Kaduna State, Nigeria. In a total of 700 cases of poultry diseases documented, 129 (18.4%) cases were diagnosed positive for fowl typhoid.

Improvement in processing procedures and sanitary methods within processing plants have allowed for general microbiological improvements in overall broiler carcass quality through the sequential stages of processing (Lillard, 1989a). However, the incidence of *Salmonella* on broiler carcasses has been shown to increase with successive stages of processing, possibly due to the ability of *Salmonella* to firmly attach to poultry tissues (Lillard, 1989b).

Roberts (1991) reported that the incidence of *Salmonella* in U.K. was 42% in chilled and 54% in frozen chickens. Moll and Hildebrandt (1991) isolated *Salmonella* from 53% of 133 retail samples of deep-frozen chicken giblets and offals on the Berlin market in Germany. They also collected 100 fresh chicken livers from a slaughterhouse and found that 93% were positive.

Lammerding *et al.* (1988) isolated *Salmonella* from 60.9% of chicken carcasses from federally inspected slaughter establishments in Canada. Bokanyi *et al.* (1990) isolated 16 serotypes of *Salmonella* from 43% of 142 ready-to-cook broilers or parts.

Mutluer *et al.* (1992) isolated *Salmonella* from 27.5% of 200 samples including whole carcass, wing, breast, leg and giblet of chickens collected from retail markets in Ankara in Turkey.

2.12 PUBLIC HEALTH IMPORTANCE OF *SALMONELLA*

Infection of healthy adults with non-typhoidal *Salmonella* usually results in self-limiting gastroenteritis that does not require antibiotic therapy, but severe clinical consequences can include septicaemia, arthritis, meningitis, and pneumonia (Saphra and Winter, 1957; Cohen *et al.*, 1987). Infection of poultry with *Salmonella* spp. become latent in most cases and remains unwatched. Therefore there is a potential danger for human health when products are not sufficiently heated (Schluter *et al.,* 1992). However, the very young, elderly, and immunocompromised are at increased risk of complications and death, even if the infecting dose is low (Hohmann, 2001).

In the European Union (EU), the number of human cases reported to Internet was greater than 100, 000 in 1997 (O'Brien and de Valk, 2003). In recent years, the incidence of salmonellosis has shown a sustained decrease across the EU (73, 000 cases in 2001) and also in the USA since 1996 (O'Brien and de Valk, 2003). It has been estimated that approximately 1.4 million non-typhoidal *Salmonella* infections occur in the United States annually, resulting in approximately15, 000 hospitalizations and 400 deaths (Voetsch *et al.*, 2004).

The incidence of food-borne diseases in humans has increased considerably worldwide in the last few years (Hafez, 2005). Poultry products have been repeatedly implicated in food borne infections (Hafez, 2005). Poultry can harbour different food borne pathogens. Many reports during recent years have shown that *Salmonella* and *Campylobacter* spp. are the most common causes of human food borne bacterial diseases linked to poultry (Hafez, 2005). The most important aspect, however, is the effect of *Salmonella* contaminated eggs, poultry meat and a meat products on public health (Hafez, 2005). The problem of non-typhoidal *Salmonella* in Africa in humans is very serious, but has generally been overshadowed by the 'big three': malaria, human immunodeficiency virus and tuberculosis (MacLennan, 2008).

2.13 MODES OF TRANSMISSION OF *SALMONELLA*

People and animals can be exposed to bacterial zoonotic pathogens through a variety of routes (Glaser *et al.*, 1994).

2.13.1 Faecal-Oral

The most common mode of transmission of *Salmonella* strains is through ingestion of contaminated food or water. Many zoonotic bacterial infections are food or water-borne in origin and pose a particular risk to the immunocompromised (Gerba *et al.*, 1996). Probably the most likely setting for zoonotic disease transmission is in or around the household, where everyday activities such as food preparation, pet care, and gardening can potentially result in zoonotic disease transmission to the immunocompromised. For instance, food borne pathogens, which are typically transmitted via the fecal-oral route, can contaminate meat, meat products and other food products (Glaser *et al.*, 1994; Tauxe,

1997). The most common vehicles for transmission of food-borne salmonellosis are meat, meat products, eggs, and egg products that are contaminated as a direct result of animal infection or animal and human fecal contamination during processing or harvesting (Rabsch *et al.*, 2001; Davis, 2009).

Animal foods that are not thoroughly cooked, and fruits and vegetables that are not thoroughly washed are also found to be vehicles of transmission (USDA, 2006). *S.* Typhi and *S.* Paratyphi are mainly transmitted by fecally contaminated water. For example, the fertilization of crops with untreated sludge or sewage effluents transmits the pathogen. In addition, shellfish harvested from contaminated waters are also a common source of *Salmonella* (Health Canada, 2002; FreeMD, 2008).

2.13.2 Faecal-Nasal

Alternatively, *Salmonella* serotypes capable of dissemination in a particular host may utilize alveolar macrophages and pulmonary intravascular macrophages (PIM) for translocation (Fedorka-Cray *et al.*, 1995). Calves, sheep, goats, and pigs, but not man or small rodents, possess PIM densities and clearance capacity in the lung parenchyma similar to that of human and murine Kupffer cells in the liver (Winkler, 1988). Pigs rooting behaviour and the ovine and bovine grazing allow *salmonella* in the environment an easy access to the nasal cavity and thus to the lungs.

Salmonella serotypes (i.e. Dublin, Abortusovis, Choleraesuis, and Typhimurium) able to produce systemic infection in these animals might have developed specific mechanisms to take advantage of both the intestinal and the pulmonary route of entry and dissemination. It is worth noting that salmonella infection of these hosts is often

characterized by pneumonia and that bovine-adapted Dublin may cause pneumonia as a major sign of infection in sheep (Jack, 1971; Le Chevalier *et al.*, 1985; Turk *et al.*, 1992; Trueman *et al.*, 1996).

2.13.3 Person-to-Person

This mode of transmission occurs when infected persons do not thoroughly wash their hands after using the toilet. For example, infected food handlers and health care providers may contaminate food during preparation, or while feeding a patient, if their hands have not been washed thoroughly (Vermont Department of Health, 2005). The epidemiology of typhoid fever primarily involves person-to-person spread because the associated organisms lack an animal reservoir. In contrast, non-typhoidal *Salmonella* have enormous animal reservoirs and transmit through other modes.

2.13.4 Animal-to-Animal

Contact with newly acquired farm animals, the use of contaminated food and water sources, and pastureland at the farm facilitates this type of transmission. The stress and overcrowded conditions associated with the transport of animals to the slaughterhouse increase the shed and spread of bacteria (Exploring Global Issues in Veterinary Medicine-Brazil, 2002). In addition, cross infection at the abattoir leads to widespread occurrence of *Salmonella* in animals and subsequently in meats. The infected animals on the farm may be only 0.5 percent, but this may increase to 35 percent after 2 to 5 days in the abattoir (Microbiologyprocedure, 2009).

2.13.5 Animal-to-Human

Salmonella can be acquired directly from pets (e.g. cats and dogs), reptiles, and birds. The faeces of pets, especially those with diarrhea, contain *Salmonella* and humans can become infected if they do not wash their hands after contact with pets or pet faeces (CDC, 2008). The pets may suffer Salmonellosis as a reverse zoonosis, with infection transmitted from human-to-pet and subsequently back to other humans. *Salmonella* can also be found in healthy dogs and cats at rates of up to 36 percent and 18 percent, respectively (Wisconsin, 2004). Reptiles such as turtles, lizards, and snakes harbor *Salmonella* and an estimated 6 percent of all sporadic human cases of *Salmonella* infection are associated with reptiles (Wisconsin, 2004).Problems/RAJI/SALMONELLA TESTS/A Focus on Salmonella -- Updated Version.htm - 52 Many chicks and young birds carry the pathogen. The bacteria are shed in the environment from infected birds through nasal or ocular secretions, fecal materials, and feather dust; and, are transmitted to humans most frequently through the inhalation of contaminated air (Avian Biotechnology International, 2009).

2.13.6 Transmission through Poultry Production Systems

Poultry is considered one of the most important *Salmonella* reservoirs and a prominent asymptomatic *Salmonella* carrier in the human food chain (Park *et al.*, 2008). A few percent of birds entering the poultry plant may harbor *Salmonella* in their intestinal tract, but after processing 30 to 35 percent may be infected. As a result, *Salmonella* are commonly found on egg shells, and in the contents of clean fresh eggs. Even, a high percentage of processed products such as egg powder are contaminated with *Salmonella* (Microbiologyprocedure, 2009).Problems/RAJI/SALMONELLA TESTS/A Focus on Salmonella -- Updated Version.htm - 41

2.13.6.1 Modes of Transmission through Poultry Plants

Vertical Transmission

The transovarian transmission of the pathogen from hens to eggs. It rapidly increases the *Salmonella* prevalence and allows cross-contamination in poultry flocks (Davies and Wray, 1994; Park *et al.*, 2008).

Horizontal Transmission

This is through direct contact of uninfected birds with infected birds, or indirect contact through contaminated drinking water and feed (Davies and Wray, 1994; Park *et al.*, 2008).

2.13.6.2 Common Routes of Transmission

Feed

Feed is an important source of horizontal transmission if contaminated by faeces, feathers, or airborne *Salmonella*. The feed ingredients and dust in the feed can be a main source of *Salmonella* contamination (Jones and Richardson, 2004). The high risk ingredients are meat and bone meal including imported meat meal (Mead, 2005).

Egg borne Sources

Eggs and egg products are important sources of vertical transmission. *S.* Enteritidis is the primary pathogen that frequently causes egg associated human illnesses (CDC, 2005). The pathogen is also carried in the large intestine of the adult-laying hens and is shed in their feces leading to contamination of the egg shell after the egg is laid. In addition,

contamination may also occur by horizontal transmission or by direct contamination of reproductive organs with *S.* Enteritidis before the egg is laid. This causes the laying of contaminated eggs and the hatching of infected chicks (Park *et al.*, 2008).

Other Sources

Numerous potential sources of *Salmonella* exist in the commercial laying house environment. Egg belts, egg collectors, ventilation fans, and flush water have been identified as sources of contamination. The presence of mice also increases the spread of the pathogen (Park *et al.*, 2008).

2.14 MAJOR SOURCES OF CONTAMINATION OF POULTRY BY-PRODUCTS

Much of the current research regarding the source of *Salmonella* contamination has focused on cecal and intestinal content contamination (Corrier *et al.*, 1990). Indeed the caeca have long been considered the primary source of *Salmonella* within the chicken (Fanelli *et al.*, 1971; Snoeyenbos *et al.*, 1982). Hargis *et al.* (1995) demonstrated that the crop may serve as a source of carcass contamination with *Salmonella* within some processing plants. An unabsorbed yolk sac contaminated with *Salmonella* may also serve as reservoir for subsequent pathogen recolonization of the intestine or contamination of the abdominal cavity if ruptured during processing (Cox *et al.*, 2005).

2.15 MECHANISM OF INFECTION

Salmonella infection most commonly begins with the ingestion of contaminated food or water and can cause illness ranging from gastroenteritis to typhoid fever (Owens and Warren, 2008). The severity of illness in infected individuals is primarily determined by

the virulence mechanisms of the infecting strain. Pathogenic *Salmonella* serotypes possess several virulence factors which include:

i. Acid Tolerance: ability to survive the pH of the stomach
ii. Cell Adhesion and Invasion: ability to attach and invade cells
iii. Defense Mechanisms: ability to defend against host's response
iv. Intracellular Replication: ability to replicate intracellularly
v. Toxin Elaboration: ability to produce toxins (Ohl and Miller, 2001).

After ingestion, *Salmonella* bacteria exhibit an adaptive acid tolerance response that enables them to survive their passage through the acidic pH environment of the stomach. When they reach the intestine, they attach to specialized epithelial cells (M cells) of the Peyer's patches by means of fimbriae (Zapor and Dooley, 2009). This results in the bacterial colonization of the lower intestine (ileum and cecum). *Salmonella* bacteria then invade host cells by a process known as bacterial-mediated endocytosis which is distinct from receptor-mediated endocytosis, a process by which many other pathogens enter host cells (Ohl and Miller, 2001).

This process induces host cell membrane ruffling and is dependent on the rearrangement of the host cell cytoskeleton, and involves an increase in cellular inositol phosphate and calcium levels. The membrane ruffles non-specifically engulf the bacteria and internalize them into the cell by macropinocytosis (Finlay, 2006). This is an example of signal transduction where molecules secreted by the invasive microorganism lead to profound structural changes in the targeted host cells (Labbe and Gracia, 2001). Attachment and invasion are under distinct genetic control and involve multiple genes in both chromosomes and plasmids (Doyle and Beuchat, 2007). Depending on the invading strain

and associated virulence factors, the infection process after invasion commonly takes place in the following two ways:

1. Gastroenteritis
2. Typhoid Fever

2.16 PATHOGENESIS OF GASTROENTERITIS (NON-TYPHOIDAL STRAINS)

In addition to the invasion of the intestinal epithelial barrier, *S.* Enteriditis and *S.* Typhimurium induce a secretory response in the intestinal epithelium and initiate recruitment and transmigration of neutrophils into the intestinal lumen (Ohl and Miller, 2001). This causes epithelial cells to synthesize and release several proinflammatory cytokines, including interleukin-1 (IL-1), interleukin-6 (IL-6), interleukin-8 (IL-8), which evoke an acute inflammatory response leading to intestinal fluid secretion and diarrhea (Giannella, 1979; Klimpel *et al*., 1995). Depending on the serotype and the effectiveness of the host defense, some bacteria may also spread to the liver, spleen, gallbladder, or central nervous system. However, the non-typhoid serovars are killed promptly in extraintestinal sites due to lack of human-specific virulence factors. Therefore, the most common non-typhoid *Salmonella* infection, gastroenteritis, remains confined to the intestine (Owens and Warren, 2008).

2.17 PATHOGENESIS OF TYPHOID FEVER (TYPHOIDAL STRAINS)

Following invasion of the intestinal epithelium, *S.* Typhi and *S.* Paratyphi enter macrophages by macropinocytosis, and subsequently activate virulence mechanisms that evade microbicidal functions of the macrophages. This permits pathogen survival and replication in the intracellular environment (Ohl and Miller, 2001). The infected macrophages (with bacteria inside them) spread to the mesenteric lymph nodes and

throughout the body via systemic circulation. They are then taken up by the reticuloendothelial system which confine and control the spread of the organism. However, the Typhi serotype may be released into the blood stream and other organs including the spleen, liver, gallbladder, kidneys and central nervous system resulting in bacteraemia and clinical symptoms of typhoid (Owens and Warren, 2008). *S.* Paratyphi can also cause enteric fever but severity of disease is less than that caused by *S.* Typhi (MedicineNet.com., 2009).

2.18 SALMONELLOSIS

Infection of healthy adults with non-typhoidal *Salmonella* usually results in self-limiting gastroenteritis that does not require antibiotic therapy, but severe clinical consequences can include septicaemia, arthritis, meningitis, and pneumonia (Saphra and Winter, 1957; Cohen *et al.*, 1987). A recent study of extra-intestinal non-typhoidal *Salmonella* infections in infants (< one year) and children up to 15 years of age showed that immunocompromised states resulting from therapy for malignancies, Corticosteroid use, and severe malnutrition as a consequence of congenital heart disease or chronic pulmonary disease were associated with increased mortality rates (Sirinavin *et al.*, 1999). A multi-state survey of consumer food-handling practices found that the elderly (> 60 years old) were more likely to report consumption of undercooked eggs than any other risky food handling behavior, and reported more frequent consumption of undercooked eggs than did persons < 60 years old (Alterkruse *et al.*, 1999). Non-immunological host risk factors include gastric hypoacidity in infants, pernicious anaemia, and sickle cell anaemia. Underlying immunosuppression should be ruled out in patients presented with non-typhoidal *Salmonella* bacteraemia in the absence of gastroenteritis (Brown and Eykyn, 2000). However, the very young, elderly, and immunocompromised are at

increased risk of complications and death, even if the infecting dose is low (Hohmann, 2001). It has been estimated that approximately 1.4 million non-typhoidal *Salmonella* infections occur in the United States annually, resulting in approximately15, 000 hospitalizations and 400 deaths (Voetsch *et al.*, 2004).

The disease can affect all species of domestic animals; young animals and pregnant animals are the most susceptible. Enteric disease, often presenting as bloody or profuse watery diarrhoea with pyrexia, is the commonest clinical manifestation, but a wide range of clinical signs, which include acute septicaemia, abortion, arthritis, necrosis of extremities and respiratory disease, may be seen (Wray and Wray, 2000). The signs and lesions are not pathognomonic. Many animals, especially poultry and pigs, may also be infected but show no clinical illness (Wray and Wray, 2000). Such animals may be important in relation to the spread of infection between flocks and herds and as causes of human food poisoning. In the latter case, this can occur when these animals enter the food chain thus producing contaminated food products. Wild animals such as badgers and some types of birds may carry specific strains of *Salmonella* (Wray and Wray, 2000).

Infection of poultry with *Salmonella* spp. become latent in most cases and remains unwatched. Therefore there is a potential danger for human health when products are not sufficiently heated (Schluter *et al.,* 1992). The infection and prevalence of salmonellosis in poultry depend on different factors such as: involved *Salmonella* serovar*,* age of birds, infectious dose and route of infection. Bad management, poor ventilation, high stocking density or concurrent disease may contribute to the development of a systemic infection (Hafez, 2005). The incubation period ranges between 2 and 5 days. Symptoms seen in young birds are somnolence, weakness, drooping wings, ruffled feathers and huddling

together near heat source. There are a lot of unpipped and pipped eggs with dead embryos if infection was egg transmitted or occurred in the incubator (Hafez, 2005). Mortality in young birds varies from negligible to 10-20 %, and in severe outbreaks may reach 80 % or higher (Hafez, 2005). Adult birds serve mostly as intestinal or internal organ carriers over longer period with little or no evidence of infection (Hafez, 2005). The large economic losses are caused by high cost of treatment, reduction in egg production in breeder flocks, poor chick quality and high costs for eradication and control measures (Hafez, 2005). The most important aspect, however, is the continuing effect of *Salmonella* contaminated eggs, poultry meat and meat products on public health (Hafez, 2005).

2.19 POST MORTEM FINDINGS

Birds that die in an acute phase of the disease have shown a persistent yolk sac, catarrhal and haemorrhagic enteritis, necrotic foci in liver, spleen and heart muscle. Furthermore, congestion of the liver, kidney, gall bladder and heart muscle are the most constant post-mortem findings (Gast, 2003; Shivaprasad, 2003). Caeca may contain caseous core or sometimes are filled with blood (Gast, 2003; Shivaprasad, 2003). In adult birds lesions most frequently found in the chronic carrier hen are misshapen, pedunculated, discoloured cystic ova. The involved ova usually contain oily and caseous material enclosed in a thickened capsule. The ovary may be haemorrhaged with atrophic discoloured follicles. Ovarian and oviduct dysfunction may lead to abdominal ovulation or impassable oviduct which in turn brings about extensive peritonitis and adhesion of the abdominal viscera (Gast, 2003; Shivaprasad, 2003). In male birds, the testes may be atrophied with thickening of the tunica albuginea and multiple abscesses. Both sexes reveal myocarditis and pericarditis (Gast, 2003; Shivaprasad, 2003).

2.20 DETECTION METHODS FOR *SALMONELLA*

Methods for the isolation and identification of *Salmonella* in food samples are categorized into two types:

1. Conventional Methods
2. Rapid Methods

2.20.1 Conventional Methods

Conventional methods for *Salmonella* detection require up to five days and are based on the cultural, biochemical, and serological properties of *Salmonella* (Brehm- Stecher and Johnson, 1998; Yousef and Carlstrom, 2003). The procedures for conventional methods are available from several regulatory bodies including the Food and Drug administration (FDA), United States Department of Agriculture (USDA), and Association of Official Analytical Chemists (AOAC). Standard conventional methods consist of five distinct steps:

2.20.1.1 Non-Selective Enrichment (Pre-enrichment)

Pre-enrichment in a non-selective broth medium is done for recovery of stressed or sublethally injured *Salmonella* which may arise from exposure to heat, freezing, desiccation, preservatives, high osmotic pressure or wide temperature fluctuations in food processing operations. This step allows for the proliferation of *Salmonella* to detectable levels (WHO, 1994; Health Canada, 2002; Miliotis and Bier, 2003). Buffered peptone water is the medium of choice for this pre-enrichment (WHO, 1994). The most common pre-enrichment broths are lactose and brilliant green (Health Canada, 2002; Miliotis and Bier, 2003).

2.20.1.2 Selective Enrichment

Selective enrichment involves the inoculation of pre-enrichment culture into enrichment broths. Enrichment broths recommended for *Salmonella* include tetrathionate brilliant green (TBG) and selenite cystine (SC). This step facilitates the recovery of *Salmonella* on plating media while inhibiting the growth of competitive microorganisms (Health Canada, 2002; Miliotis and Bier, 2003). Mueller (1923) demonstrated the effectiveness of Tetrathionate Broth for enriching typhoid and paratyphoid bacilli while inhibiting coliform organisms. Tetrathionate Broth was used in studies for the poultry industry (Jones *et al.*, 1991; Barnhart *et al.*, 1991), and in a collaborative study for rapid screening of *Salmonella* in food (Eckner *et al.*, 1994). Tetrathionate Broth Base, abbreviated as TT Broth Base, is specified in standard methods for *Salmonella* testing (Vanderzant and Splittstoesser, 1992). Tetrathionate Broth Base is used in processing faecal cultures for bacteria (Federal Register, 1991).

2.20.1.3 Selective Plating

Selective plating of enrichment cultures onto selective agar media is done for the presumptive identification of *Salmonella* colonies on the basis of distinct biochemical reactions. Plating to highly selective media also allows for the development of discrete *Salmonella* colonies while inhibiting the growth of other bacteria. Standard plating media include brilliant green agar (BGA), brilliant green sulfa (BGS), xylose-lysine-desoxycholate (XLD), and Hektoen enteric (HE) agars. These media indicate acid production from lactose and/or sucrose utilization through color change. Bismuth sulfite agar (BSA) is another highly selective medium on which *Salmonella* typically produce hydrogen sulfide and appear as charcoal black colonies (Labbe and Gracia, 2001).

Numerous media have been developed for the isolation and differentiation of enteric bacteria, most designed to recover a broad spectrum of enteric pathogens (Miller *et al.*, 1992; Koneman, 1997). As a result of this, overgrowth of inconsequential bacteria can be a problem, when recovery of a specific species is desired. This is true of *Salmonella* isolation media where *Proteus, Providencia, and Pseudomonas* can interfere with the desired results (Miller *et al.*, 1992; Koneman, 1997). Xylose-Lysine-Desoxycholate (XLD) media was developed as a selective and differential media for the isolation of gram-negative enteric pathogens. The sodium desoxycholate found in the XLD media is replaced by Tergitol 4 in Xylose-Lysine-Tergitol 4 (XLT-4) Agar. This addition makes XLT-4 more highly selective for *Salmonella* than its predecessor (Miller *et al.*, 1992; Koneman, 1997).

Proteose Peptone No.3 in XLT-4 Agar provides a source of complex nitrogen compounds. Yeast extract is added to supply vitamins and co-factors. Differentiation on this medium is based on xylose, lactose, sucrose, lysine decarboxylation, and hydrogen sulfide production. The pH shifts in the medium due to the fermentation and decarboxylation reactions are visualized by the addition of phenol red. The Tergitol 4 (sodium tetradecyl sulphate) in the XLT-4 Agar inhibits all gram-positive bacteria and molds, and inhibits the growth of numerous gram-negative bacteria including Proteus, Providencia and Pseudomonas species (Miller *et al.*, 1992; Koneman, 1997). This attribute makes XLT-4 Agar excellent for the isolation and detection of non-typhi *Salmonella* (Miller *et al.*, 1992; Koneman, 1997).Problems/RAJI/SALMONELLA TESTS/A Focus on Salmonella -- Updated Version.htm - 37

2.20.1.4. Biochemical Screening

Biochemical screening involves a series of biochemical tests for further confirmation of presumptive *Salmonella* colonies. Typical *Salmonella* colonies have the following characteristics which are used in biochemical screening:

i. Produce acid from glucose but not from lactose or sucrose in triple sugar iron (TSI) agar medium.
ii. Decarboxylate lysine to cadaverine (alkaline product) in lysine iron agar (LIA) medium.
iii. Generate hydrogen sulfide in TSI agar and LIA.
iv. Do not ferment lactose or sucrose in XLD and HE (Yousef and Carlstrom, 2003).

The inability of *Salmonella* to hydrolyze urea, produce indole from tryptophan, and to grow in potassium cyanide broth are also useful in biochemical characterization (Yousef and Carlstrom, 2003).

2.20.1.5 Serological Identification/Confirmation

Serological identification is done for the confirmation of colonies biochemically suspected of being *Salmonella*. The step involves the reaction of somatic (O) and flagellar (H) polyvalent and single-grouping antisera with the suspected isolates. Mixing *Salmonella* cells with H antisera gives a characteristic pattern of agglutination (*Salmonella* are loosely attached to each other by their flagella and can be dissociated by shaking) (Todar, 2008). In contrast, the reaction of *Salmonella* cells with O antisera may not result in agglutination because surface antigens may mask O antigens. Therefore,

thermal solubilization of surface antigens is necessary for serological confirmation of O antigens by agglutination (Health Protection Agency, 2007; Doyle and Beuchat, 2007).

2.20.2 Rapid Methods

Rapid methods for *Salmonella* detection in foods offer detection times as low as 18 hours, and are more convenient and less labor-intensive than conventional methods. Extensive research has resulted in the development of many commercially available rapid methods based on the following techniques and methodologies (Labbe and Gracia, 2001; Riemann and Cliver, 2006):

i. Problems/RAJI/SALMONELLA TESTS/A Focus on Salmonella -- Updated Version.htm - 37*Salmonella* -specific phages
ii. Latex agglutination
iii. Colorimetric and fluorometric enzyme-linked immunosorbent assays (ELISA)
iv. DNA probe hybridization
v. Polymerase chain reaction (PCR)
vi. Premi^{R} Test *Salmonella*

The Premi^{R} Test *Salmonella* system is an important tool for typing the isolates (Wattiau *et al.,* 2008). It uses a methodology called multiplex ligation detection reaction (LDR) to generate a collection of circular DNA molecules that are subsequently PCR amplified by means of a single pair of amplimers (Schouten *et al.*, 2002; Eijk van *et al.*, 2004). The PCR products are next sorted by hybridization to a low-density DNA microarray. Positive hybridization is detected using a biotin label incorporated in one of the PCR primers. A set of genetic markers has been selected with the purpose of yielding unique microarray hybridization profiles to identify and discriminate S. *enterica* serovars (Baner *et al.*, 2003; Andreoli *et al.,* 2004). The test allows single-tube processing, which simplifies the technical work associated with strain typing (Baner *et al.*, 2003; Andreoli *et al.*, 2004).

Although rapid methods require less time than traditional culture procedures, they are currently used as a screening tool only. Standard cultural, serological, and biochemical methods are still in use for definitive identification of *Salmonella* serotypes (Miliotis and Bier, 2003).

2.21. TREATMENT OF *SALMONELLA* INFECTION

Salmonella infections usually resolve in 5 to 7 days and do not require any specific treatment other than oral fluids. Since antibiotic treatments may increase the risk of becoming a carrier, they are not routinely used to treat uncomplicated non-typhoidal *Salmonella* gastroenteritis (CDC, 2008; Zapor and Dooley, 2009). However, in people with weakened immune systems and in cases of typhoid fever, the infection can be severe and fatal; therefore, treatment is essential (Mayoclinic.com, 2008). The treatment options include:

2.21.1 Fluid Replacement

Minerals such as sodium, potassium and calcium that maintain the balance of fluids that are lost in persistent diarrhea and need to be replaced. This can be done either by drinking lots of liquids or receiving fluids intravenously in cases of serious fluid loss. This will help in rehydration and proper functioning of the body (Mayoclinic.com, 2008).

2.21.2 Diet Restrictions

Milk products should be avoided to reduce abdominal pains and stomach-soothing foods such as bananas, rice, applesauce and toast should be substituted for other foods (Mayoclinic.com, 2008).

2.21.3 Antibiotics

These are administered only if the infection spreads from the intestine to other body parts. The duration of antibiotic treatments vary depending on the form of illness, and range from 14 days for enteric fever to six weeks for bacteraemia. The antibiotics include:

Ciprofloxacin (Cipro), Sulfamethoxazole and trimethoprim combination (Bactrim), Ceftriaxone (Rocephin), Amoxicillin (Amoxil) (Mayoclinic.com, 2008).

2.21.4 Antidiarrheals

These medications alleviate diarrhea by slowing down intestinal movements and increasing fluid absorption. These drugs include loperamide, bismuth subsalicylate, and diphenoxylate (Mayoclinic.com, 2008).

2.22 ANTIMICROBIAL RESISTANCE

An inevitable side effect of antibiotic use is the emergence and dissemination of resistant bacteria, not only in pathogenic bacteria but also in the endogenous flora of man and animals. Resistant commensal bacteria of food animals might contaminate, like zoonotic bacteria, meat products, thus reaching the intestinal tract of humans (O'Brien, 2002). Resistance genes against antibiotics that are or have only been used in animals, were soon after their introduction not only found in animal bacteria, but also in the commensal flora of humans, in zoonotic pathogens like *Salmonella*, and in strictly human pathogens, like *Shigella* (Barza, 2002). There is evidence, indeed that resistance determinants can be transferred between unrelated bacteria such as *Bacteroides* on the one hand and

Salmonella and *Escherichia* on the other (O'Brien, 2002). Therefore, not only does the clonal spread of resistant strains occur, but there is also a transfer of resistance genes between human and animal bacteria (O'Brien, 2002). The most common multi-drugs resistant (MDR) *Salmonella* serotypes are: Typhimurium, Heidelberg, and Newport (NARMS, 2002).

Resistance can be caused by a large number of mechanisms, involving decreased antibiotic accumulation, physical modification or destruction of the antibiotics, and alteration of the enzyme target of antibiotic action. Recently, a mechanism of resistance involving the active efflux of antibiotics by multidrug efflux pumps was also elucidated (Lacroix *et al.*, 1996; Poole, 2001; Soto *et al.*, 2003).

Multiple-antibiotic resistant *S.* Typhimurium definitive phage type (DT) 104 emerged during the last decade as a global health problem because of its involvement in diseases in animals and humans (Poppe *et al.*, 1998; Threlfall, 2000; Cloeckaert and Schwarz, 2001). These multiple-antibiotic resistant strains are generally resistant to ampicillin (Ap), chloramphenicol (Cm), florfenicol (Ff), streptomycin (Sm), spectinomycin (Sp), sulphonamides (Su), and tetracyclines (Tc).

Genes associated with these resistance phenotypes have been found to be chromosomally encoded (Threlfall *et al.*, 1994). Additional resistance to trimethoprim (Tm), occasionally seen among *S.* Typhimurium DT104 strains, may be encoded by a non-conjugative but mobilisable plasmid of approximately 4.6 MDa which also encodes resistance to Su (Threlfall *et al.*, 1996). Transferable apramycin resistance has also been described in some DT104 strains (Low *et al.*, 1997).

The ApCmFfStSpSuTc multiple-antibiotic resistance profile of *S.* Typhimurium DT104 is conferred by an antibiotic resistance gene cluster carried by a chromosomal genomic island called *Salmonella* genomic island 1 (SGI1) (Boyd *et al.*, 2000; Boyd *et al.*, 2001). The first reported study of fluoroquinolone resistance in *Campylobacter* from animals was from The Netherlands where water medication with the fluoroquinolone enrofloxacin in the poultry production was followed by an emergence of fluoroquinolone resistant *Campylobacter* species among both poultry and humans (Endtz *et al.,* 1991). Since then several studies worldwide have documented an increase in the occurrence of resistance to fluoroquinolones among *Campylobacter* from food animals and humans following the introduction of fluoroquinolones for the treatment of infections in food animals (reviewed in Engberg *et al.,* 2001; Lucey *et al.,* 2002; Nachamkin *et al.,* 2002; Gaudreau and Gilbert, 2003; Hein *et al.,* 2003).

For *Salmonella* similar trends have been observed. Thus, in Germany, an increase in the incidence of strains that are resistant to nalidixic acid was observed after the licensing of enrofloxacin (Malorny *et al.,* 2003). In France a simultaneous increase in resistance has been observed among isolates from animals and humans, and the same clones observed among the different reservoirs (Heurtin-Le Corre *et al.,* 1999). Also in Spain the occurrence of quinolone resistance among *Salmonella* causing infections in humans increased from less than 0.5% before 1991 to 38.5% in 2003 in one study (Marimon *et al.,* 2004) and from around 6–15% in 1991 to 40–85% in 2001 in another study (Guerri Santos and Rotger, 2004). In the United Kingdom substantial increases in resistance to fluoroquinolones (FQ) in *Salmonella* Hadar and *Salmonella* Virchow, and in multi-resistant *Salmonella* Typhimurium DT104 followed the licensing for veterinary use of

enrofloxacin in 1993 and danofloxacin in 1996 was reported by Threlfall *et al.* (1997). In Taiwan a number of studies have shown the emergence of quinolones resistance in *Salmonella* from pigs and subsequent spread of those isolates to humans (Su *et al.,* 2001; Chiu *et al.,* 2002; Hsueh *et al.,* 2004).

In *Salmonella*, quinolone resistance was initially attributed to point mutations in the *gyrA* gene encoding the A subunit of gyrase, whose complex with DNA is the primary target of quinolones. FQ resistance in *S.* Typhimurium has also been attributed to active efflux mechanisms, and especially to the participation of the AcrAB-TolC efflux system (Giraud *et al.*, 2000; Piddock *et al.*, 2000). Extended spectrum (E-S) cephalosporins are the drugs of choice for children because they cannot be treated with FQ. *Salmonella* and *E. coli* exhibiting resistance to E-S cephalosporins are an emergent problem worldwide. Before 1996, resistance to E-S cephalosporins was rarely reported among *Salmonella sp*. In 2000, the emergence of domestically acquired infections by E-S cephalosporin-resistant *Salmonella* carrying a plasmid-mediated CMY-2 AmpC β-lactamase was reported by the National Antimicrobial Resistance Monitoring System (NARMS) in the USA (Dunne *et al.*, 2000).

It has been recently demonstrated that the *bla*CMY genes in *Salmonella* reside on different large plasmids (Carattoli *et al.*, 2002). The occurrence of CMY-2-mediated cephalosporin resistance in *Salmonella* has been reported in Canada (Allen and Poppe, 2002), Spain (Navarro *et al.,* 2001), Romania (Miriagou *et al.*, 2002), and Taiwan (Yan *et al.*, 2003). In 2002, the CDC investigated an outbreak of multiple-antibiotic resistant *S. newport* infections associated with eating raw or undercooked ground beef (Zansky *et al.*, 2002). This multidrug resistance phenotype included resistance to Sm, sulfamethoxazole,

Tc, Ap, Cm, and decreased susceptibility to E-S cephalosporins. These resistant strains have also been detected in cattle (Rankin *et al.*, 2002). An increase in prevalence of E-S cephalosporin-resistant strains could be in part related to the use in food animals of ceftiofur, which is an E-S cephalosporin approved for use in veterinary medicine (White *et al.*, 2001). Since the *bla*CMY genes confer decreased susceptibility to both ceftiofur and ceftriaxone, the use of ceftiofur has the potential to select for *Salmonella* cross-resistant to ceftriaxone, another E-S cephalosporin used in human medicine. In fact, many plasmids containing *bla*CMY-2 genes have been reported to confer additional resistance to aminoglycosides, phenicols, Tc, and Su. Ceftriaxone-resistant *Salmonella* strains recently isolated from animals in Canada (Allen and Poppe, 2002) and retail foods in the USA (White *et al.*, 2001) have also been reported to be resistant to florfenicol. The *bla*CMY-2-carrying plasmids studied were recently shown to also carry the Ff resistance gene, *floR,* on a genetic structure previously identified in *E. coli* plasmids in Europe (Cloeckaert *et al.*, 2000). These data indicated that the use of different antimicrobial agents, including phenicols, might serve to maintain multiple-antibiotic resistance plasmids on which E-S cephalosporin resistance determinants co-exist with other resistance genes in *Salmonella*. Besides the emergence of multidrug resistance plasmids carrying *bla*CMY-2 and *floR*, other extended-spectrum β-lactamases have been reported in recent years in *Salmonella* (AitMhand *et al.,* 2002; Baraniak *et al.,* 2002; Mulvey *et al.*, 2003).

2.23 CONTROL AND PREVENTION STRATEGIES

Current methods used to control bacterial zoonoses are mainly aimed at reducing the burden of the zoonotic agent in its animal reservoir, or eradicating it, using the classical methods of sanitary or medical prophylaxis.

2.23.1 Sanitary Prophylaxis

Sanitary prophylaxis of zoonoses consists of slaughtering and/or destroying all infected or contaminated animals (stamping-out method) (Blancou *et al.*, 2005). A test-and-slaughter flock (depopulation of farms positive for *Salmonella*) approach may be effective for eliminating *S*. Enteritidis from parent and grandparent breeder flocks and in layer flocks because *S*. Enteritidis can be transmitted through eggs (Callaway *et al.*, 2002; Doyle and Erickson, 2006). Another essential element in the prophylaxis of human salmonellosis is retailer and consumer education, in particular awareness of safe handling and storage of food, kitchen hygiene and proper cooking to limit the risk of infection (OIE, 2010).

2.23.2 Medical Prophylaxis

Medical prophylaxis of zoonoses, based on either parenteral vaccination of animals or on chemoprophylaxis, is usually more expensive than sanitary prophylaxis on the long run and also prevents to achieve the eradication of the pathogen from its animal reservoir, since some vaccinated individuals can remain healthy carriers (Blancou *et al.*, 2005). Hinton (1992) reported that there are a number of strategies that can be adopted to reduce the prevalence of *Salmonella:*

1. The production of *Salmonella* free chicks
2. The treatment of the feed with heat, and in special circumstances irradiation
3. The use of feed additives including short chain organic acids and antibiotics
4. Competitive exclusion and Pre and Probiotics
5. Vaccination.

The ecology of *Salmonella* infection in poultry is complex and it will be necessary to introduce control measures at several points in the chain of production if *Salmonella* food poisoning associated with poultry meat is to be reduced (Hinton, 1992). Inevitably the introduction of more stringent controls will be costly and ultimately the consumer must be prepared to pay through higher prices for the product (Hinton, 1992). The use of compulsory, legally enforceable, control measures is justified for infectious disease agents who have a simple life cycle, e.g. foot and mouth disease virus but they do not prove as effective for microorganisms with complex pathways of dissemination such as *Salmonella*. As a consequence, ultimate control of this type of pathogen will only be achieved when Government, the veterinary profession, the producers, the processors and the retailers are prepared to collaborate in a harmonious and positive manner (Hinton, 1992).

In competitive exclusion, susceptibility to *Salmonella* infection in poultry can sometimes be substantially reduced by spray or oral treatment prior to exposure (ideally in the hatchery) with an anaerobic culture of caecal microflora that inhibits colonisation by *Salmonella* by occupying intestinal colonisation sites, producing acids and other inhibitory substances and stimulating local and mucosal generalised immune responses. This treatment is widely used in some countries but only used as an aid for decontaminating persistently infected farms in other countries. It is also useful to minimise perturbation of intestinal flora following antimicrobial treatment or stress and to potentiate the effect of vaccines given subsequently. As with any treatment, including vaccination or organic acid treatment, that may reduce the prevalence or numbers of *Salmonella* organisms excreted by infected groups of animals, there may be some

interference with monitoring programmes and the sampling and test sensitivity may have to be adjusted to compensate for this (Mead, 2000; Revolledo *et al*., 2006).

Vaccination using inactivated *S*. Gallinarum prevents colonization of poultry by *S*. Enteritidis; however, if using blood tests to determine *Salmonella* populations, vaccinated birds are indistinguishable from those infected by *Salmonella*. Thus, special care must be given when reporting blood results from vaccinated flocks (Callaway *et al*., 2002; Doyle and Erickson, 2006).

Live vaccines have also been used in a number of countries; these include the semi-rough strains, such as 9R for fowl typhoid and HWS51 for *S*. Dublin infections (Mastroeni *et al*., 2001). The most commonly used vaccine is a live vaccine derived from the stable rough strain of *S*. Gallinarum known as '9R' (OIE, 2008). Other attenuated vaccines include auxotrophic and 'metabolic drift' mutants, which are used to prevent *Salmonella* infections in farm animals in Germany and for *S*. Enteritidis and *S*. Typhimurium in poultry in the United Kingdom and other countries. Mutant vaccines attenuated rationally by molecular biological gene-deletion techniques have been developed for poultry and other species; these include aroA mutants and strains with mutations in the genes encoding adenylate cyclase (cya) and the cyclic adenosine monophosphate receptor protein (crp) (Cooper, 1994), which is available in the United States of America. In Europe genetically modified organisms are not normally permitted for use as vaccines (OIE, 2010).

For those traveling to developing nations, vaccines against Typhoid Fever are available to reduce the risk of typhoid fever (CDC, 2008). There are two vaccines currently

recommended by the World Health Organization for the prevention of typhoid (WHO, 2008). These are the live, oral Ty21a vaccine (sold as *Vivotif Berna*) and the injectable Vi capsular polysaccharide vaccine (sold as *Typhim Vi*). Both are between 50 to 80% protective and are recommended for travelers to areas where typhoid is endemic (WHO, 2008). Challenges are identifying unrecognized major sources of *Salmonella* infections; determining the sources of *Salmonella* infections in infants; preventing contamination of vegetables with manure from concentrated animal feeding operations; preventing further emergence of highly resistant strains; controlling *Salmonella* Enteritidis infections through changes in the egg industry and education of food service workers and consumers; and developing effective education methods and materials to prevent reptile-associated salmonellosis (CDC, 2008).

Opportunities are improving detection of dispersed outbreaks through collaborations among PulseNet laboratories and OutbreakNet epidemiologists; training state health department personnel in *Salmonella* serotyping; and encouraging judicious use of antimicrobial agents in veterinary medicine (CDC, 2008).

2.24 SURVEILLANCE SYSTEMS

2.24.1 National or Regional Systems

The organization of regional or national surveillance systems for bacterial zoonoses is based on the analysis and synthesis of information usually collected by official public health or animal health systems (Blancou *et al.*, 2005). Data may be provided to health authorities through partnership and networks organized with the help of medical practitioners, veterinarians, animal health and wildlife specialists or livestock breeders grouped together for the sanitary defense of their livestock (for example, “Groupements

de Défense Sanitaire" (GDS) in France), as developed in many countries by commodities, such as cattle farmer associations, egg producer associations (Blancou *et al.*, 2005). Such networks usually receive some financial aid from their government for setting prevention measures (Blancou *et al.*, 2005).

In United States of America the national surveillance is conducted through the public health laboratories for culture-confirmed cases and through the National Notifiable Diseases Surveillance System (NNDSS) (CDC, 2008). Active laboratory and population-based surveillance is conducted in FoodNet sites (CDC, 2008).

2.24.2 The World System

The value of a worldwide network for zoonoses surveillance can only be the result of that of national networks, since any interference into the zoo-sanitary information of a country is currently impossible without the agreement of this country. The establishment of such worldwide networks has still to overcome several obstacles. However, such systems are important to be set in a more and more interdependent economy and are usually a booster for improving national systems. At the global level, there are a number of recognized surveillance systems, including the World Health Organization (WHO)-Global Outbreak Alert and Response Network (GPHIN).

The surveillance systems for food-borne diseases include Global *Salmonella* Surveillance (Global *Salm*-Surv), the SIRVETA system (diarrhea syndrome surveillance) coordinated by INPPAZ (Pan American Institute for food protection and zoonoses) which include countries from South America and the Caribbean.

For instance, Global *Salm*- Surv is a global network of laboratories and individuals under the WHO coordination involved in surveillance, isolation, identification and antimicrobial resistance testing of *Salmonella*. The methods used by these different surveillance systems may vary from laboratory-based sentinel surveillance to active and intensive epidemiological investigations, with sometimes an overlap in these various systems (Blancou *et al.*, 2005).

CHAPTER 3

MATERIALS AND METHODS

3.1 AREA OF STUDY

The area of study is Zaria which is located within the Northern Guinea Savannah zone of Nigeria in Kaduna State, between latitude 7^0 and 11^0 N, and longitude 7^0 44' E (Saidu *et al.*, 1994). The average rainfall ranges from 1000 to 1250 mm, and the average temperature ranges from 17^0 C to 33^0 C (Saidu *et al*., 1994).

3.2 METHODOLOGY

3.2.1 Determination of the Prevalence of *Salmonella* species from Organs and Faeces from Chicken sold at Retail Markets in Zaria, Nigeria

3.2.1.1 Sampling method

Samples were collected from the five main markets in Zaria where poultry are sold and processed. The markets included Samaru, Sabon Gari, Tudun Wada, Dan Magaji and Zaria City markets. Convenience sampling was used to collect the samples. The sampling was done by consent.

3.2.1.2 Sample Collection and Transportation

Preliminary work before going to the field

Buffered peptone water (BPW) was prepared according to manufacturer's recommendation (BIO RAD, France). The test tubes containing sterile BPW were stored in the fridge at 4^0 C. These test tubes were used for pre-enrichment of liver, gallbladder and caeca. Buffered peptone water was also prepared in flasks. This sterile BPW was used for tissue maceration in stomacher. It was also used as transport medium for organs

(viscera). Tetrathionate broth was prepared according to manufacturer's recommendation (Oxoid, England). It was stored in a fridge at 4^0 C. It was used as selective enrichment medium for liver, gallbladder, caeca and faeces. Tetrathionate broth when prepared can be stored for a month. But when mixed with iodine solution, it was used within few hours (Oxoid, England). Polythene bags were wrapped into aluminium foil and autoclaved at 121^0 C for 15 minutes. They were used for sample collection and organ maceration in stomacher. Empty flasks were covered with aluminium foils and empty test tubes with cotton wool; they were sterilized in the hot air oven at 160^0 C for 1 hour. Scissors and forceps were wrapped in aluminium foil and sterilized in hot air oven at160^0 C for 1 hour. They were used for processing of organs. Pipettes were wrapped in aluminium foil and sterilized in hot air oven at160^0 C for 1 hour. They were used to dispense the tetrathionate broth into test tubes and for the biochemical reagents. This process was repeated before each sample collection to avoid contamination and cross-contamination.

Sample Collection

Pre-sensitization was conducted in each market before sampling. Viscera of 100 apparently healthy adult local chickens were bought from retailers at slaughter points (20 viscera from each market) between June and August 2010. The caecum, liver, gallbladder and faeces were collected from each chicken. All samples were collected under aseptic conditions to prevent contamination or cross-contamination as described by WHO (1994). Sterile sampling materials (i.e. swabs) and disposable gloves were used. Hands were disinfected with 70 % ethyl alcohol before starting the procedure. Organ samples were collected in sterile polythene bags by wearing sterile gloves. Viscera were removed by holding them with sterile forceps and cutting them into tiny pieces with sterile scissors. They were put into the sterile polythene bags. Sterile BPW was aliquoted into

the bags as transport medium. Samples were put into a cooler and immediately transported to the Bacterial Zoonoses Laboratory of the Department of Veterinary Public Health and Preventive Medicine, Ahmadu Bello University Zaria for laboratory analyses.

3.2.1.3 Laboratory Procedures

Organs

Chicken organs bought from retailers, consisting of caeca, livers and gall bladders were processed according to standard methods of isolation of *Salmonella* by Walkman *et al.* (1998). Sections of livers, gallbladders and caeca were cut using sterile scissors and forceps and put into sterile polythene bags. For the gall bladders if there was bile a sterile syringe was used to aspirate it and it was inoculated into a test tube containing 10 ml of sterile BPW for pre-enrichment. Sterile BPW was added and they were macerated using stomacher. One (1) ml of the macerated broth was inoculated into a test tube containing 10mls of sterile BPW for pre-enrichment. The test tubes were incubated at 37^0C for 24 hours as described by WHO (1994). After 24 hours, 1 ml from each culture was inoculated into a test tube containing 10 ml of tetrathionate broth for selective enrichment and incubated at 37^0 C for 24 hours as described WHO (1994). After selective enrichment, the tetrathionate broth was inoculated onto Xylose lysine tergitol 4 (XLT4) plates and incubated at 37^0 C for 24 hours for selective plating. The suspect colonies on XLT4 plates appeared black. These suspect colonies were grown onto XLT4 plates for pure culture. From the pure culture nutrient agar slants were inoculated and incubated at 37^0 C for 24 hours. After growth the nutrient slants were stored in the fridge for further procedures.

Faecal samples

Sterile scissors and forceps were used to cut through the caeca. Sterile swabs were used to collect the faeces from the caeca. Test tubes containing 10 ml of tetrathionate broth each were inoculated for selective plating. After inoculation the test tubes were incubated at 37^0 C for 24 hours as described by WHO (1994). After selective enrichment, the tetrathionate broth was inoculated onto Xylose lysine tergitol 4 (XLT4) plates for selective plating. The plates were incubated at 37^0 C for 24 hours as described by WHO (1994). The suspect colonies on XLT4 plates appeared black. The suspect colonies were grown onto XLT4 plates for pure culture. From the pure culture nutrient agar slants were inoculated and incubated at 37^0 C for 24 hours. After growth the nutrient slants were stored in the fridge for further procedures.

3.2.1.4 Biochemical Tests

The biochemical tests were performed from the isolates stored on nutrient agar slants. Two biochemical tests were performed first to screen the isolates: Triple Sugar Iron (TSI) Test and Urease Test.

Triple Sugar Iron Test

TSI slants were prepared according to manufacturer's recommendation (Merck, Germany). The TSI slants were inoculated by stabbing the butt and streaking the surface of the slants with sterile straight wire. The slants were incubated at 37^0 C for 24 hours. After 24 hours the results were read. The reaction of many *Salmonella* organisms on TSI slant is Alkaline over acid (Pink in slant, Yellow in butt), and gas (crack or bubble in the agar), and hydrogen sulphide (black coloration).

Urease Test

Urea slants were prepared according to manufacturer's recommendation (Merck, Germany). The urea slants were inoculated by streaking the surface of the slants with sterile wire loop. The test tubes were incubated at 37^0 C for 24 hours. A positive result is pink and a negative result is no change in color. The isolates with *Salmonella* characteristics in TSI Test and Urease Test were tested further.

Hydrogen Sulphide Test, Indole Test and Motility Test

They were conducted using SIM medium (Oxoid, England). It was prepared according to manufacturer's recommendation. Test tubes were inoculated using sterile straight wire loop to stab them. Tubes were incubated at 37^0 C for 24 hours.

Citrate Test

Citrate was prepared according to manufacturer's recommendation (BIOTEC, United Kingdom). Citrate slants were inoculated using sterile wire loop to swab the surface. Slants were then incubated at 37^0 C for 24 hours.

Methyl Red (MR) and Voges Proskauer (VP) Tests

MRVP broth was prepared according to manufacturer's recommendation (Oxoid, England). Test tubes were inoculated using sterile wire loop and incubated at 37^0 C for 24 hours. After incubation the broth were divided into two using sterile test tubes. In one test tube MR reagent was added; in the second test tube VP reagent 1 and VP reagent 2 were added.

3.2.2 Determination of the Tissue Distribution of *Salmonella* Species from Chickens Sold at Retail Markets in Zaria

Frequencies of occurrence of *Salmonella* in the various tissue samples were obtained. The proportional distribution rates were calculated by dividing the number of *Salmonella* isolated from each tissue by the total number of *Salmonella* isolated.

3.2.3 Determination of the Most Common *Salmonella* Serotypes that Invade and Colonize the Tissues from Chickens in Zaria

Suspected *Salmonella* isolates were freeze-dried and sent to the OIE, Italian Reference Laboratory for Salmonellosis (Instituto Zooprofilattico Sperimentale delle Venezie, Padova, Italy) for serotyping as recommended by WHO (1994), following the Kauffmann-White scheme.

3.2.4. Determination of the Sensitivity and the Resistance of the Isolated *Salmonella* Serotypes to commonly used Antibiotics

This was carried out at the OIE, Italian Reference Laboratory for Salmonellosis. It was conducted using the Kirby-Bauer disk diffusion method (Bauer *et al*., 1966). The organisms were tested against a panel of 16 antibiotics namely colistin (10 µg), sulfamethoxazole + trimethoprim (23.75 µg + 1.75 µg), Kanamycin (30 µg), gentamicin (10 µg), Cefotaxime (30 µg), amoxicillin + Clavulonic acid (20 µg + 10 µg), Ceftazidime (30 µg), nalidixic acid (30 µg), tetracycline (30 µg), ampicillin (10 µg), streptomycin (10 µg), sulphonamide (0.25 µg), chloramphenicol (30 µg), Cephalothin (30 µg), enrofloxacin (5 µg), ciprofloxacin (5 µg) were tested. Each isolate was suspended in sterile saline solution to a 0.5 McFarland standard. The diluted bacterial suspension was transferred onto Müller Hinton agar plates using sterile swabs. The plates were seeded

uniformly by rubbing the swabs against the entire agar surface. Each antimicrobial impregnated disk was applied onto the surface of the inoculated plate by using sterile stick dispenser. The plates were incubated at 37^0 C for 18 hours. Interpretation of the growth inhibition zones and classification of isolates as susceptible, intermediate and resistant was done following guide lines of the National Committee for Clinical Laboratory Standards (NCCLS, 2004).

3.3 STATISTICAL ANALYSES

Fisher's Exact Test was used to test the association between organs and infection, and between markets and infection. SPSS Version 16.0 was used.

CHAPTER 4

RESULTS

4.1. THE PREVALENCE OF *SALMONELLA* SPECIES FROM ORGANS AND FAECES OF CHICKENS.

A total of 17 *Salmonella* isolates were obtained from four hundred samples from 100 apparently healthy slaughtered adult local chickens. The prevalence was obtained by dividing the number of positive chickens by the number of chickens tested multiplied by 100. The prevalence of *Salmonella* in each market is shown in Table 4.1. An overall prevalence of 15 % was observed for all the markets. The prevalence per market was Samaru (26.7 %), Sabon Gari (40 %) and Dan Magaji (33.3 %). There is a significant association ($p = 0.003$, $p < 0.05$) between markets and infection (Appendix VIII). A pure culture of *Salmonella* is shown in Appendix I. Biochemical reactions of *Salmonella* on TSI and Urea are shown in Appendices II and III. Results of the biochemical tests are shown in Appendices IVA, IVB and V.

Table 4.1: Isolation rate of *Salmonella* species from chickens sold at retail markets in Zaria.

Market	No. of chicken tested	No. of positive chickens (%)	No. of *Salmonella* isolates
Zaria	20	0 (0.0)	0
Samaru	20	4 (26.7)	6
Sabon Gari	20	6 (40)	6
Tudun Wada	20	0 (0.0)	0
Dan Magaji	20	5 (33.3)	5
Total	**100**	**15 (100)**	**17**

4.2. THE DISTRIBUTION OF *SALMONELLA* SPECIES IN CHICKENS BASED ON SAMPLE TYPE

The proportional distribution rates of the isolates were: caecal content (70.5 %), liver (17.5 %), caecum (6 %) and gall bladder (6 %). This is shown in Table 4.2. There is a significant association ($p = 0.000$, $p < 0.05$) between organs and infection (Appendix VII).

Table 4.2: Frequency of *Salmonella* isolates according to tissue types

Sample type	No. of samples collected	No. +ve samples (%)	No. of *Salmonella* isolates
Gall bladder	100	1 (6.0)	1
Caecum	100	1 (6.0)	1
Liver	100	3 (17.5)	3
Caecal contents	100	12 (70.5)	12
Total	**400**	**17 (100)**	**17**

Chicken N. 5 from Samaru yielded 3 different isolates.

4.3. *SALMONELLA* SEROTYPES ISOLATED FROM CHICKENS IN ZARIA.

Ten different serotypes of *Salmonella* were identified among the seventeen isolates. Serotypes of *Salmonella* isolated from organs and faeces of chickens are shown in Table 4.3 and in Appendix VI. Isolates were obtained from three markets: Samaru, Dan Magaji and Tudun Wada.

Table 4.3: *Salmonella* serotypes isolated from organs and faeces of chickens slaughtered at retail markets in Zaria.

Isolate code	Location	Organ	*Salmonella* serotypes
SI5	Samaru	Intestine	*S.* Typhimurium-DT29
SG5	Samaru	Gall bladder	*S.* Agoueve
SL5	Samaru	Liver	*S.* Typhimurium-NT
SL2	Samaru	Liver	*S.* Takoradi
SL15	Samaru	Liver	*S.* Typhimurium-DT29
SF9	Samaru	Faeces	*S.* Ealing
DF1	Dan Magaji	Faeces	*S.* Jedburgh
DF3	Dan Magaji	Faeces	*S.* Jedburgh
DF7	Dan Magaji	Faeces	*S.* Jedburgh
DF10	Dan Magaji	Faeces	*S.* Jedburgh
DF20	Dan Magaji	Faeces	*S.* Jedburgh
BF4	Sabon Gari	Faeces	*S.* Derby
BF9	Sabon Gari	Faeces	*S. enterica subsp. enterica* gr. E
BF10	Sabon Gari	Faeces	*S. enterica subsp. enterica* gr. E
BF11	Sabon Gari	Faeces	*S.* Stanleyville
BF12	Sabon Gari	Faeces	*S.* Derby
BF18	Sabon Gari	Faeces	*S.* Koenigstuhl

Faeces = caecal content

Intestine = caecum

4.4. THE SENSITIVITY AND THE RESISTANCE OF THE ISOLATED *SALMONELLA* SEROTYPES TO COMMONLY USED ANTIBIOTICS

The following antimicrobial agents were used for antibiotic susceptibility testing: colistin (CL), sulfamethoxazole + trimethoprim (SXT), kanamycin (K), gentamycin (GM), cefotaxime (CTX), amox/clavulonic acid (AMC), ceftazidime (CAZ), nalidixic acid (NA), tetracycline (Te), ampicillin (AM), streptomycin (S), sulfonamide (SSS), cephalothin (CF), chloramphenicol (C), enrofloxacin (Enro), ciprofloxacin (Cip). The sensitivity and the resistance of the isolated *Salmonella* serotypes are shown in Appendix IX. The drug resistance pattern is shown in Table 4.4. Four isolates (23.53 %) were resistant to sulfamethoxazole + trimethoprim, sulfonamide, kanamycin, streptomycin, chloramphenicol and tetracycline. Thirteen isolates (76.47 %) were sensitive to all the antibiotics used.

Table 4.4: Antibiogram of *Salmonella* isolates

Serotype	Resistance Pattern
S. Derby	SXT, K, Te, S, SSS, C
S. enterica subsp. enterica gr. E	SXT, K, Te, S, SSS, C
S. Derby	SXT, K, Te, S, SSS, C
S. Stanleyville	SXT, K, Te, S, SSS, C

SXT= sulfamethoxazole + trimethoprim; K= kanamycin; Te= tetracycline;

S= streptomycin; SSS= sulfonamide; C= chloramphenicol.

CHAPTER 5

DISCUSSION

In this study 15 % of the apparently healthy adult local chickens sold and slaughtered at retail markets in Zaria were positive for *Salmonella*. This shows that chickens harbour *Salmonella* organisms. This implies that apparently healthy adult local chickens are carriers of *Salmonella* in Zaria. They may constitute an important source for spread in the environment. Koehler (1992) reported that the main carriers of *Salmonella* Typhimurium (> 80 %) and of *Salmonella* Enteritidis (> 90 %) are different kinds of poultry and other domestic animals. Hafez (2005) also reported that adult birds serve mostly as intestinal or internal organ carriers over longer periods with little or no evidence of infection. Abdu *et al.* (1985) studied cases of diseases diagnosed in chickens at the Avian Clinic of Ahmadu Bello University, Zaria and found that the prevalence of salmonellosis was 1.1 %. Salami *et al.* (1989a) obtained a prevalence rate of 28 % for salmonellosis in a serological investigation conducted using sera of chickens in Zaria and Kaduna, Nigeria. Mbuko *et al.* (2009) conducted a five-year retrospective study (2003-2007) on the prevalence of fowl typhoid and poultry diseases diagnosed at the avian unit of the veterinary teaching hospital, Ahmadu Bello University, Zaria, Nigeria, and found that 18.4 % of the cases were positive for fowl typhoid.

Salmonella spp. were isolated from intestine (caecum), liver, gallbladder and faeces of apparently healthy adult local chickens sold at retail markets in Zaria. This shows that *Salmonella* organisms are distributed in many organs. Caecal contents had the highest number of isolates (12 out of 17). This may be due to the fact that microbes are known to form biofilms in the intestinal tract (Paula, 2003; Matthew and Pradeep, 2003). Paula (2003) reported that more than 99 percent of all bacteria live in biofilm communities.

Biofilms provide important environmental reservoirs for pathogenic bacteria (Matthew and Pradeep, 2003). Earlier reports by Fanelli *et al.* (1971) and Snoeyenbos *et al.* (1982) considered caeca as the primary source of *Salmonella* within the chicken.

Ten (10) different serotypes were isolated from apparently healthy slaughtered adult local chickens sold at retail market in Zaria: *S.* Jedburgh (5), *S.* Typhimurium – DT29 (2), *S.* Derby (2), *S. enteric subsp. enterica* gr. E (2), *S.* Typhimurium – NT (1), *S.* Agoueve (1), *S.* Takoradi (1), *S.* Ealing (1), *S.* Stanleyville (1) and *S.* Koenigstuhl (1). Of the seventeen isolates, 12 were from the faeces, 3 from liver and 1 each from the caecum and the gallbladder. No isolate of *S.* Gallinarum was obtained though it was reported to have a high prevalence of 8 % and 18.4 % in Zaria as reported by Salami *et al.* (1989b) and Mbuko *et al.* (2009) respectively. May be this is due to the fact that tetrathionate broth was used as selective enrichment medium, and it may have inhibitory effect on some strains of *S.* Gallinarum biovar Gallinarum and *S.* Gallinarum biovar Pullorum (OIE, 2008). Okwori *et al.* (2007) obtained 37.9 % positive results from a serological survey of *Salmonella* Gallinarum antibodies in chickens around Jos, Plateau State, Nigeria. The *Salmonella* serotypes isolated from apparently healthy adult local chickens are of public health importance, since all serotypes are potentially zoonotic. Patrick *et al*. (1959) demonstrated antibodies against *S.* Typhimurium –DT29, *S.* Typhimurium – NT, *S.* Derby, *S.* Stanleyville and *S.* Takoradi in sera of healthy adults at Ibadan. *S.* Agoueve, *S.* Derby, *S.* Ealing, *S.* Jedburgh, *S.* Stanleyville, *S.* Takoradi, *S.* Typhimurium have been isolated from human cases reported to CDC from 1996 to 2006 (NSSS, 2006).

Multiple resistances were observed in four isolates: *S.* Derby (BF4), *S.* Derby (BF12), *S. enterica subsp. enterica* gr. E (BF9) and *S.* Stanleyville (BF11). They were all resistant to

sulfamethoxazole + trimethoprim (SXT), sulfonamide (SSS), kanamycin (K), streptomycin (S), tetracycline (Te) and chloramphenicol (C). Multiple-antibiotic resistant *S.* Typhimurium definitive phage type (DT) 104 emerged during the last decade as a global health problem because of its involvement in diseases in animals and humans (Poppe *et al.*, 1998; Threlfall *et al.*, 2000; Cloeckaert and Schwarz, 2001). Strains are generally resistant to ampicillin (Ap), chloramphenicol (Cm), florfenicol (Ff), streptomycin (Sm), spectinomycin (Sp), sulphonamides (Su), and tetracyclines (Tc). Genes associated with these resistance phenotypes have been found to be chromosomally encoded (Threlfall *et al.*, 1994). Some isolates had intermediate susceptibility to nalidixic acid, enrofloxacin, streptomycin and sulphonamide. All the 17 isolates were sensitive to colistin, gentamicin, amoxicillin/clavulonic acid, ampicillin, ceftazidime, cefotaxime, cephalothin and ciprofloxacin. Ibrahim *et al.* (2005) isolated 38 *S*. Typhi, 13 *S*. Paratyphi and 59 non-typhoidal *Salmonella* spp. from blood of patients diagnosed with typhoid fever in Zaria, northern Nigeria. The multiple antibiotic resistance indexing showed that 90.9 % of the isolates had multiple drug resistance index > 0.2.

The data obtained in this study have expanded the knowledge concerning the carrier state of adult chickens, and the presence of zoonotic *Salmonella* serotypes in apparently healthy adult local chickens sold at retail markets in Zaria, Nigeria.

CHAPTER 6

SUMMARY, CONCLUSION, RECOMMENDATIONS

A total of 17 isolates of *Salmonella* were obtained from organs and faeces of chickens sold and slaughtered at retail markets in Zaria, Nigeria. An overall prevalence of 15 % was observed for all the markets. The prevalence per market was Samaru (26.7 %), Sabon Gari (40 %) and Dan Magaji (33.3 %).

The distribution of the isolates was: caecal contents (70.5 %), liver (17.5 %), caecum (6 %) and gall bladder (6 %).

Ten different serotypes of *Salmonella* were identified among the seventeen isolates (*S.* Jedburgh, *S.* Typhimurium – DT29, *S.* Derby, *S. enterica subsp. enterica* gr. E, *S.* Typhimurium – NT, *S.* Agoueve, *S.* Takoradi, *S.* Ealing, *S.* Stanleyville and *S.* Koenigstuhl).

Multiple resistance patterns were observed in four isolates: *S.* Derby (BF4), *S.* Derby (BF12), *S. enterica subsp. enterica* gr. E (BF9) and *S.* Stanleyville (BF11). All the 17 isolates were sensitive to colistin, gentamicin, amoxicillin/clavulonic acid, ampicillin, ceftazidime, cefotaxime, cephalothin, nalidixic acid, enrofloxacin and ciprofloxacin.

Chickens sold at retail markets in Zaria were infected with *Salmonella* organisms. *Salmonella* species were distributed in caecal contents, caecum, liver and gall bladder. The most common serotype was *S.* Jedburgh. Multidrug resistance patterns were observed in four isolates.

Recommendations:

1. The national and local health authorities should enforce the food hygiene regulations to reduce the spread of zoonotic and multidrug resistant *Salmonella* to humans.
2. Public enlightenment programmes on the modes of transmission of *Salmonella* should be conducted by Human and Veterinary Public Health services.
3. Further studies should be conducted to know the extent of distribution of *Salmonella* spp. in different areas of Kaduna State and the country at large.

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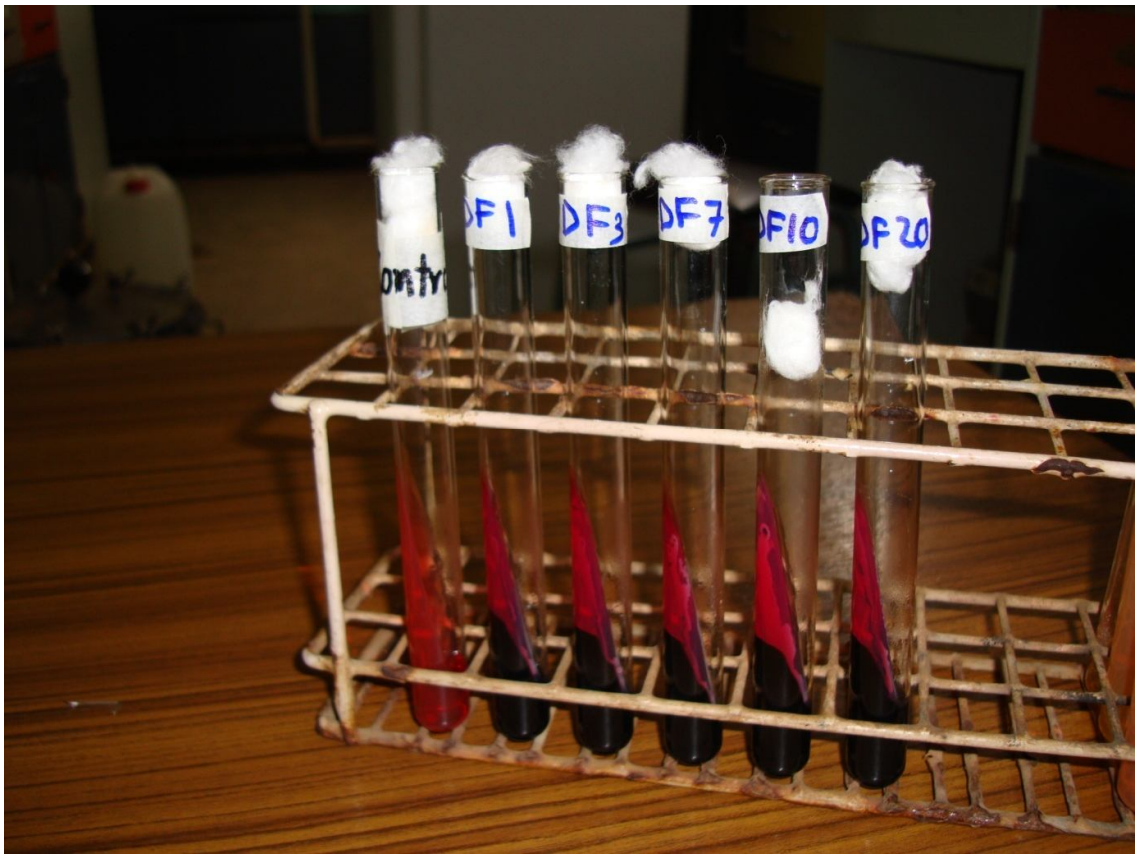
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APPENDIX I



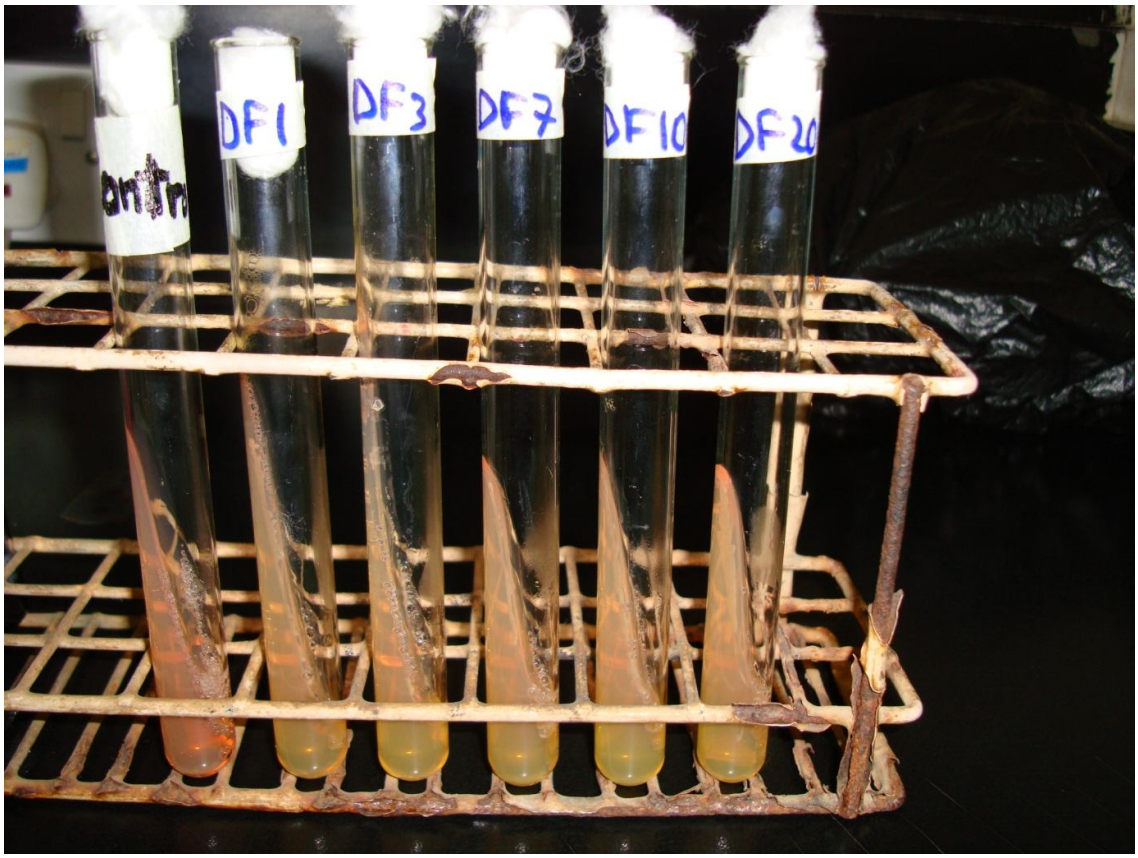
Pure culture of *Salmonella* on XLT4 plate.

APPENDIX II



Triple Sugar Iron Test (Left control; after control: Alkaline/Acid + H_2S +gas).

APPENDIX III



Urease Test (Left control; after control Urease -).

APPENDIX IVA

Biochemical tests for screening the isolates from XLT4 plates

ID	ISOLATE CODE	TSI	UREASE
1	SI5	AL/Y + H2S + GAS	_
2	SG5	AL/Y + H2S + GAS	_
3	SL5	AL/Y + H2S	_
4	SL2	AL/Y + H2S + GAS	_
5	SL15	AL/Y + H2S + GAS	_
6	SF1	AL/Y + H2S + GAS	+
7	SF9	AL/Y + H2S + GAS	_
8	SF10	AL/Y + H2S + GAS	+
9	SF11	AL/Y + H2S + GAS	+
10	TL18	AL/Y + H2S + GAS	+
11	TG9	AL/Y + H2S + GAS	+
12	TF3	AL/Y + H2S + GAS	+
13	TF13	AL/Y + H2S + GAS	+
14	TI1	AL/Y + H2S + GAS	+
15	TI13	AL/Y + H2S + GAS	+
16	TI20	AL/Y + H2S + GAS	+
17	DF1	AL/Y + H2S + GAS	_
18	DF3	AL/Y + H2S + GAS	_
19	DF7	AL/Y + H2S + GAS	_
20	DF10	AL/Y + H2S + GAS	_
21	DF20	AL/Y + H2S + GAS	_

S= Samaru; T= Tudun Wada; D= Dan Magaji; H2S= Hydrogen Sulphide
AL= Alkaline; Y= Yellow (Acid); TSI= Triple Sugar Iron; I= Intestine; L= Liver; G= Gall Bladder; F= Faeces

APPENDIX IVB

Biochemical tests for screening the isolates from XLT4 plates

ID	ISOLATE CODE	TSI	UREASE
22	BF4	AL/Y + H2S + GAS	_
23	BF6	AL/Y + H2S + GAS	+
24	BF9	AL/Y + H2S + GAS	_
25	BF10	AL/Y + H2S + GAS	_
26	BF11	AL/Y + H2S + GAS	_
27	BF12	AL/Y + H2S + GAS	_
28	BF18	AL/Y + H2S + GAS	_
29	BI12	AL/Y + H2S + GAS	+
30	BI15	AL/Y + H2S + GAS	+
31	BI19	AL/Y + H2S + GAS	+
32	BL1	AL/Y + H2S + GAS	+
33	BL3	AL/Y + H2S + GAS	+
34	BL5	AL/Y + H2S + GAS	+
35	BL10	AL/Y + H2S + GAS	+
36	BL18	AL/Y + H2S + GAS	+
37	BL20	AL/Y + H2S + GAS	+
38	BG6	AL/Y + H2S + GAS	+
39	BG19	AL/Y + H2S + GAS	+

B= Sabon Gari; I= Intestine; L= Liver; G= Gall Bladder
AL= Alkaline; Y= Yellow (Acid); H2S= Hydrogen Sulphide
TSI= Triple Sugar Iron
+= Positive; _= Negative

APPENDIX V

Results of Biochemical Tests

ID	ISOLATE CODE	TSI	UREASE	SIM H2S	SIM I	SIM M	MR	VP	CITRATE
1	SI5	AL/Y + H_2S + GAS	_	+	_	+	+	_	+
2	SG5	AL/Y + H2S + GAS	_	+	_	+	+	_	+
3	SL5	AL/Y +H2S	_	+	_	+	+	_	+
4	SL2	AL/Y + H2S + GAS	_	+	_	+	+	_	+
5	SL15	AL/Y + H2S + GAS	_	+	_	+	+	_	+
6	SF9	AL/Y + H2S + GAS	_	+	_	+	+	_	+
7	DF1	AL/Y + H2S + GAS	_	+	_	+	+	_	+
8	DF3	AL/Y + H2S + GAS	_	+	_	+	+	_	+
9	DF7	AL/Y + H2S + GAS	_	+	_	+	+	_	+
10	DF10	AL/Y + H2S + GAS	_	+	_	+	+	_	+
11	DF20	AL/Y + H2S + GAS	_	+	_	+	+	_	+
12	BF4	AL/Y + H2S + GAS	_	+	_	+	+	_	+
13	BF9	AL/Y + H2S + GAS	_	+	_	+	+	_	+
14	BF10	AL/Y + H2S + GAS	_	+	_	+	+	_	+
15	BF11	AL/Y + H2S + GAS	_	+	_	+	+	_	+
16	BF12	AL/Y + H2S + GAS	_	+	_	+	+	_	+
17	BF18	AL/Y + H2S + GAS	_	+	_	+	+	_	+

S=Samaru; B=Sabon Gari; D=DanMagaji;

I=Intestine; G=Gall Bladder; L=Liver; F=Faeces

AL=Akaline; Y=Yellow (Acid); +=Positive; _=Negative

I= Indole; M= Motility; H_2S= Hydrogen Sulphide; SIM= Sulphur Indole Motility; TSI= Triple Sugar Iron

APPENDIX VI

Serotype distribution of *Salmonella* from organs and faeces of chickens sold at retail markets in Zaria.

Serotype	Liver	Gall bladder	Intestine	Faeces	Total
S. Typhimurium - DT29	1		1		**2**
S. Typhimurium - NT	1				**1**
S. Takoradi	1				**1**
S. Agoueve		1			**1**
S. Ealing				1	**1**
S. Jedburgh				5	**5**
S. Derby				2	**2**
S. ent. subsp. ent. gr. E				2	**2**
S. Stanleyville				1	**1**
S Koenigstuhl				1	**1**
TOTAL	**3**	**1**	**1**	**12**	**17**

APPENDIX VII

Fisher's Exact Test for Association between Organs and Infection.

Type of Sample * Salmonella Status Crosstabulation

Count

		Salmonella Status		
		Negative	Positive	Total
Type of Sample	Faeces	88	12	100
	Gall bladder	99	1	100
	Intestine	99	1	100
	Liver	97	3	100
Total		383	17	400

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)
Pearson Chi-Square	20.335[a]	3	.000	.000
Likelihood Ratio	17.914	3	.000	.001
Fisher's Exact Test	16.089			.000
N of Valid Cases	400			

a. 4 cells (50.0%) have expected count less than 5. The minimum expected count is 4.25.

APPENDIX VIII

Fisher's Exact Test for Association between Markets and Infection.

Market * Salmonella Status Crosstabulation

Count

		Salmonella Status		
		Negative	Positive	Total
Market	Dan Magaji	75	5	80
	Sabon Gari	74	6	80
	Samaru	74	6	80
	Tudun Wada	80	0	80
	Zaria City	80	0	80
Total		383	17	400

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)
Pearson Chi-Square	12.041[a]	4	.017	.017
Likelihood Ratio	17.998	4	.001	.002
Fisher's Exact Test	13.472			.003
N of Valid Cases	400			

a. 5 cells (50.0%) have expected count less than 5. The minimum expected count is 3.40.

Appendix IX: The sensitivity and the resistance of the isolated *Salmonella* serotypes to commonly used antibiotics

Serotype	CL	SXT	K	GM	CTX	AMC	CAZ	NA	Te	AM	S	SSS
***S.*Typhimurium-DT29**	S	S	S	S	S	S	S	S	S	S	S	S
***S.* Agoueve**	S	S	S	S	S	S	S	S	S	S	S	I
***S.* Typhimurium-NT**	S	S	S	S	S	S	S	S	S	S	I	I
***S.* Takoradi**	S	S	S	S	S	S	S	S	S	S	I	I
***S.*Typhimurium-DT29**	S	S	S	S	S	S	S	S	S	S	I	I
***S.* Ealing**	S	S	S	S	S	S	S	S	S	S	S	S
***S.* Jedburgh**	S	S	S	S	S	S	S	S	S	S	S	S
***S.* Jedburgh**	S	S	S	S	S	S	S	S	S	S	S	S
***S.* Jedburgh**	S	S	S	S	S	S	S	S	S	S	S	S
***S.* Jedburgh**	S	S	S	S	S	S	S	S	S	S	S	S
***S.* Jedburgh**	S	S	S	S	S	S	S	S	S	S	S	S
***S.* Derby**	S	R	R	S	S	S	S	I	R	S	R	R
***S. enterica enterica* gr. E**	S	R	R	S	S	S	S	I	R	S	R	R
***S. enterica enterica* gr. E**	S	S	S	S	S	S	S	S	S	S	S	S
***S.* Stanleyville**	S	R	R	S	S	S	S	S	R	S	R	R
***S.* Derby**	S	R	R	S	S	S	S	I	R	S	R	R
***S.* Koenigstuhl**	S	S	S	S	S	S	S	S	S	S	I	I

S= Susceptible
I= Intermediate
R= Resistance