PREVALENCE AND ANTIBIOGRAM OF *SALMONELLA* SPECIES IN CATTLE CARCASSES SLAUGHTER FROM ZANGO ABATTOIR ZARIA, NIGERIA

BY

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SEPTEMBER, 2016

DECLARATION

I declare that the work in this dissertation entitled "Prevalence and Antibiogram of *Salmonella* Species in Cattle Carcasses Slaughtered in Zango Abattoir Zaria, Nigeria", has been carried out by me in the Department of Veterinary Public Health and Preventive Medicine under the supervision of Dr. M. Bello and Dr. N. B. Adamu. The information derived from the literature has been duly acknowledged in the text and in list of references provided. The work has neither in part nor whole been submitted anywhere for the award of a similar degree or any other degree, diploma or certificate.

Hauwa Yusuf DAYA	_____	_____
Name of Student	Signature	Date

CERTIFICATION

This dissertation entitled "PREVALENCE AND ANTIBIOGRAM OF *SALMONELLA* SPECIES IN CATTLE CARCASSES SLAUGHTER FROM ZANGO ABATTOIR ZARIA, NIGERIA." by Hauwa Yusuf DAYA meets the regulation governing the award of Master of Science in the Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This project is dedicated to my husband, Dr. D. Laku and my daughter, Aishatu Dauda Laku

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All praises and glory be to Almighty Allah who gave me the courage, patience and strength to carry out this work. Peace and blessings of Allah be upon the last Messenger Muhammad (SAW).

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ABSTRACT

Cross sectional study was conducted in Zango abattoir Zaria to determine the prevalence of *Salmonella* species in carcasses. A total of 200 pooled swab samples were collected from neck, brisket, flank and rump of cattle carcasses from February to March 2015. Ninety eight (49%) out of the two hundred (200) samples showed characteristics of *Salmonella* on *Salmonellla Shigella* Agar. Conventional biochemical tests were conducted on the 98 isolates, where 48 (48.9%) showed characteristics of *Salmonella species*. The 48 isolates were further subjected to Microbact 12E GNB identification system. The results showed that 19 (39.9%) were *Salmonella arizonae*, 3 (6.25%) were *Salmonella* Cholerasuis, and 2 (4.16%) were *unclassified specie*s. Other organisms detected included *Serrattia liquefaciens* 5 (10.4%), *Escherichia coli 3*(6.25%), *Klebsiella pneumoniae* 4 (8.33%), *Edwardsiella tarda* 2(4.16%), *Citrobacter diversus* 2(4.16%) and *Hafnia alvei*, *Escherichia vulneris, Enterobacter gergoviae, Acinetobacter baumanni, Acinetobacter iwoffii, Enterobacter agglomerans, Klebsiella rhinoscleromatis* and *Klebsiella ozaenae* 1 (2.08%) each. Twenty five (25) out of the 48 isolates did not meet the cut- off point of 75% for species identification. Agglutination test using Rapid Latex Agglutination was also performed, where 10 (55.55%) out of the 18 isolates agglutinated while 8 (44.44%) did not. Antibiotic susceptibility of these isolates using disk diffusion method revealed 100% susceptibility to gentamicin and varying percentages of susceptibility to the rest of the antibiotics. These were Ciprofloxacin (90%), Amikacin (70%), Chloramphenicol (60%), Nitrofurantoin (40%), Trimethoprim (40%), Nalidixic acid (30ug), Kenamycin (40%), Tetracycline (30%), Streptomycin (20%), Imipenem (20%) and Aoxicillin + Clavulanic acid (30%). All the isolates showed multiple drug resistance with the least showing resistance to 2 antibiotics and the highest to 7 antibiotics. In conclussion, prevalence of 5% of *Salmonella* species in cattle carcasses was recorded in this study with the isolates showing 100% suseptibilty to Gentamicin and 90% to Ciprofloxacin. This study demonstrated heavy contamination of carcasses with broad spectrum microbes that are of zoonotic importance and the use of Gentamicin and Ciprofloxacin should be with caution for their high potential antibiotic resistance to most of the zoonotic microbes.

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ABBREVIATIONS

β	Beta
^{o}C	Degrees Celsius
%	Percent
<	Less than
>	Greater than
µg	microgram
µm	micrometre
AIDS	Acquired Immune Deficiency Syndrome
AK	Amikacin
AMC	Amoxicillin - Clavulanic Acid
AML	Amoxicillin
AU-IBAR	African Union Inter-African Bureau for Animal Resources
BAM	Bacterial Analytical Manual
BG	Brilliant Green Agar
BIS	Bismuth Sulphite
BPW	Buffered Peptone Water
C	Chloramphenicol

CFU	Colony Forming Unit
CIP	Ciprofloxacin
CLSI	Clinical and Laboratory Standards Institute
Cm^2	Centimetre square
CN	Gentamicin
CDC	Centres for Disease Control and Prevention
EDTA	Ethylenediaminetetraacetic Acid
EU	European Union
F	Nitrofurantoin
FAO	Food and Agriculture Organisation
FDA	Food and Drug Administration
H	Flagella Antigen
HACCP	Hazard Analysis and Critical Control Point
HIV	Human Immuno-deficiency Virus
H_2S	Hydrosulphide
IC	Immuncompromised
IPM	Imipenem

K	Kenamycin
Kg	Kilogram
KOH	Potassium Hydroxide
KW	Kauffman White
Lab	Laboratory
LPs	Lipopolysaccharide
Mg	Milligram
MKTTn	Muller Kauffman Tetrathionate- Novobiocin Broth
Ml	Millilitre
MR-VP	Methyl Red – Voges Proskauer
NA	Nalidixic Acid
Nacl	Sodium chloride
NPC	National Population Commission
NTS	Non typhoidal *Salmonella*
O	Somatic Antigen
OIE	Office International des Epizooties
ONPG	Ortho-Nitrophenyl-β-galactoside

PAHO	Pan American Health Organisation
PCR	Polymerase Chain Reaction
RVS	Rappaport Vasiliadis Soy broth
S	Streptomycin
S	*Salmonella*
SC	Selenite Cystine broth
SIM	Sulphur- indole Mortility
Spp	Species
SSA	*Salmonella Shigella* Agar
Subspp	Subspecie
TE	Tetracycline
TSA	Tryptic Soy Agar
TSI	Triple Sugar Iron
TT	Tetrathionate Broth
USA	United States of America
USDA	United States Department of Agriculture
Vi	Capsular Antigen

W	Trimethoprim
WHO	World Health Organisation
XLD	Xylose Lysine Decarboxylase Agar

CHAPTER ONE

INTRODUCTION

1.1Background

Microbial food safety is an increasing public health concern worldwide (Kabir, 2010). Foodborne diseases often follow the consumption of contaminated food-stuff especially from animal products such as meat from infected animals or carcasses contaminated with pathogenic bacteria such as *Salmonella* species., *Staphylococcus aureus, Listeria monocytogenes, Campylobacter species* and *Escherichia coli* O157: H7 (Nouichi and Hamdi, 2009). Meat is a highly perishable food. Increase in demand for meat without the infrastructure for proper sanitary handling may lead to transfer of pathogenic organisms from the animals to the consumers (Mrema *et al*., 2006). Furthermore, food safety issues have recently gained considerable attention from the public and represent an important concern for the veterinary profession. Problems related to food contamination, however, are not unique to humans, as animals are also susceptible to a large number of food-borne infections (LeJeune and Hancock, 2001). *Salmonella* is one of the most important pathogenic genera implicated in bacterial foodborne outbreaks and diseases (Cetinkaya *et al*., 2008). *Salmonella* are gram negative bacteria that belong to the Family Enterobacteriaceae genus *Salmonella*. Members of the genus *Salmonella* are often referred to as the "universal pathogens", *Salmonella* pathogens are recovered from almost all vertebrates and some insects (Cetinkaya *et al.*, 2008). Because of their widespread presence in the environment, some scientists consider *Salmonella* to be primarily environmental organisms that are pathogenic to animals (Blackburn, 1993). They cause most of the foodpoisoning. Most *Salmonella* species are resident in the intestinal tracts of humans and

animals including birds. *Salmonella* strains that can persist for years in the environment withstanding periods of stress and nutrient depletion have been reported (Foley and Lynne, 2008). Humans are usually infected by ingesting food contaminated with animal feces. Contaminated foods are often of animal origin. *Salmonella* contamination may occur at abattoir from the excretion of symptomless animals, contaminated abattoir equipment, floor, and personnel (Foley and Lynne, 2008). The pathogen can get access to the meat at any stage during butchering. Cross contamination of carcasses and meat products could continue during subsequent handling, processing, preparation and distribution (Cetinkaya *et al*., 2008). Understanding the prevalence and distribution of *Salmonella* species in food animals and determining management strategies associated with lower prevalence are keys to decreasing the risk of high pathogen loads at harvest (Foley and Lynne, 2008). In recent years, the health concern all over the world is the occurrence of antibiotic resistant strains of a number of pathogenic bacteria including *Salmonella* in foods (Cetinkaya *et al*., 2008). The presence of antimicrobial resistance determinants have the potential to adversely affect human health by causing illness that is more difficult to treat because of the resistance profile of the organism (Foley and Lynne,2008). Globally, *Salmonella* accounts for over one-half of all food-borne diseases (Curtiss *et al*., 1993).

1.2 Statement of Research Problem

In Nigeria, the presence of food-borne organisms has been demonstrated in the abattoirs (Addo and Diallo, 1982). Foodborne salmonellosis in humans has been in existence since 19th Century, of which the first case was reported to be caused by the ingestion of

contaminated bovine meat (Barrow, 1993). It is considered one of the most problematic zoonosis in terms of public health all over the world.

Worldwide occurrence of salmonellosis is calculated in 1.3 billion cases and 3 million deaths (Thong *et al*, 1995). Salmonellosis causes significant morbidity and mortality in both humans and animals and has a substantial global socioeconomic impact (Tassios *et al*., 1997). There are 16 million annual cases of typhoid fever, 1.3 billion cases of gastroenteritis and 3 million deaths worldwide due to *Salmonella* (Bhunia, 2008). Mortality due to *Salmonella* infections is mainly a health problem in developing countries including Nigeria.

The extensive use of the first line drugs has led to the development of multiple drug resistance at a level which could pose a serious problem in the near future (Getenet, 2008).

1.3 Justification of the Study

Cattle is among the commonly raised domestic animals in sub- Sahara especially the northern Nigeria. Commonly called "beef" the muscle obtained from the cattle constitutes a major source of animal protein for humans globally (Lawan *et a*l., 2013). However, because of the very low standards of operations in the abattoirs, low levels of biosecurity as well as poor methods of meat processing in Nigeria (Lawan *et al.*, 2013), cattle carcasses have become a pivotal source of enteric infectious diseases like salmonellosis, listeriosis and compylobateriosis posing significant threat to human population.

Salmonella species have been reported to be the leading cause of acute gastroenteritis in several countries and salmonellosis remains an important public health problem world

wide, particularly in developing countries like Nigeria (Rotimi *et al.,* 2008).. In a study that assessed the incidence of *Salmonella* within the premises of abattoirs and on processed carcasses in Nigeria, an incidence of 25.9% was reported (Addo and Diallo, 1982). The works of Kwaga *et al.* (1984 and 1985) isolated *Salmonella* from cattle rectal swabs with a recovery rate of 1.5% and from lymph nodes and raw beef samples with an isolation rate of 13.7% and 8.2% respectively from Zaria. Similarly, the occurrence of *Salmonella* was recently demonstrated in raw and processed meat products in Zaria by Tafida *et al.* (2013) with prevalence of 2.43% in raw meat. Previous studies have shown *Salmonella* to be one of the major problems in the world like the work of Obaro *et al.* (2016) who reported *Salmonella* as the commonest cause of bacteremia in humans and accounts for 24 – 59.8% of bacterrmia in children. The situation is agrevated by the ever increasing rate of antimicrobial resistant strains (Zelalem *et al.,* 2011).

Although, some works have been done on occurrence of *Salmonella* in different food products in Zaria, there is the need to periodically check the status of the organisms in food products and abattoirs especially those that are not hygienically properly maintained are generally too exposed, indicative of a seemingly favorable milieu for transmission of *Salmonella* through meat. Thus, this work was designed to determine the current status of *Salmonella* organisms in beef carcasses at Zango abattoir, Zaria.

1.4 Aim of the Study

To determine the prevalence and antibiotic susceptibility of *Salmonella* isolates from cattle carcasses at Zango Abattoir of Zaria Metropolis.

1.5 Objectives of the Study

1. To isolate and identify and confirm *Salmonella species* from cattle carcasses using conventional biochemical methods, Microbact 12E identification system and Latex agglutination (Oxoid).
2. To determine the antimicrobial susceptibility of *Salmonella* isolates.

CHAPTER TWO

LITERATURE REVIEW

2.1 Common present in Meat and Meat Products

Microorganisms of relevance with regards to meat hygiene include helminthes, moulds, bacteria and viruses. Within these groups, bacteria play the most important role. Parasites are of insignificant value in meat that has passed meat inspection, or where efficient internal parasite control programmes or measures are in place. The most frequently identified bacterial pathogen associated with the consumption of beef products are *Salmonella* spp, *Compylobacter spp, Stahylococcus aureus, Escherichia coli, Listeria monocytogenes, Clostridium perfringes, Yersinia enterocolitica, Bacillus cereus* and *Vibrio parahaemolyticus* (Biswas *et al.,* 2011). *Compylobacter spp, Salmonella spp* and *Escherichia coli* are often present in fresh meat and poultry products (Zhao *et al.,* 2001).

2.2 Sources of Beef Contamination

Unless the animals are infected, the meats of freshly slaughtered animals are generally sterile. The presence of microorganisms on post slaughtered carcasses is due to contamination occurring immediately before, during and after slaughter (Gill, 1998). The microbial contamination of carcasses occur mainly during processing and manipulation and also during skinning, evisceration, processing at abattoir and retailer establishments (Gill, 1998). The main sources of meat contamination are from animal/carcass source, on farm factor, transport factor, abattoir and butcher facilities, parasites, wild animals, meat van, retail meat outlet and workers.

2.3 *Salmonella*

2.3.1 History of *Salmonella*

The first bacterium in the genus *Salmonella* was identified towards the end of the 19th century.The genus *Salmonella* started to be classified in 1925 with the use of serological methods.

In the current classification of the Bergey's manual, all *Salmonella* serotypes belong to one oftwo species: *Salmonella bongori*, which has at least 10 extremely rare serotypes; and*Salmonella enterica,* which is phenotypically and genotypically divided into six subspecies*enterica, salamae, arizonae, diarizonae, houtenae* and *indica*, differentiated by their biochemical behavior, mainly in terms of sugar and amino acid metabolism (Forshell and Wierup, 2006).

In the current nomenclature, the name of the serovar begins with an uppercase letter, but itis never written in italics. For example in subspecies enterica: *Salmonella enterica* subspecies *enterica* serovar Typhimurium. The short form would be *Salmonella* ser. Typhimurium or *Salmonella* Typhimurium. Typification of *Salmonella* spp. serovars is based on the antigens found in bacterial cells, somatic (O), flagellar (H) and capsular (Vi) (Selander *et al*., 1996). Vi antigen is associated withvirulence, and is only expressed by serovars Typhi, Paratyphi C and Dublin (Rycroft, 2000; Grimont *et al.*, 2000). H antigen is thermolabile, whereas O and Vi are thermoresistant, and not destroyed by heating at 100°C for two hours (Franco and Landgraf, 1996). The combination ofthe antigens O, H1 (flagellar, phase 1) and H2 (flagellar, phase 2) determine the antigenic formula of a serovar. All the 2,610 serovars recognized by Kauffman-White scheme (Grimont andWeill,

2007) today are considered to be potentially pathogenic to man but only 200 serovars are more frequently related with human disease (Baird-Parker, 1990).

2.3.2 Classification and nomenclature

Historically *Salmonella* had been named based on the original places of isolation such as *Salmonella* London and *Salmonella* Indiana. This nomenclature system was replaced by the classification based on the susceptibility of isolates to different selected bacteriophages which is also known as phage typing.

Epidemiologic classification of *Salmonella* is based on the host preferences. The first group includes host-restricted serotypes that infect only humans such as *S.* Typhi. The second group includes host-adapted serotypes which are associated with one host species but can cause disease in other hosts serotypes such as *S.* Pullorum in avian. The third group includes the remaining serotypes. Typically, *Salmonella* Enteritidis, *Salmonella* Typhimurium and *Salmonella* Heidelbergare the three most frequent serotypes recovered from humans each year (Gray and Fedorka-Cray, 2002 and Boyen *et al*., 2008).

The genus consists of two species: the first is *S. enterica* which is divided into six subspecies (Figure 1): *S. enterica* subsp. *enterica*, *S. enterica* subsp. *salamae*, *S. enterica* subsp. *arizonae*, *S. enterica* subsp. *diarizonae*, *S. enterica* subsp. *houtenae* and *S. enterica* subsp. *indica*; and the second is *S. bongori* (formerly called *S. enterica* subsp. *bongori*) (WHO, 2003c). *Salmonella enterica* subspecie I is mainly isolated from warm-blooded animals and accounts for more than 99% of clinical isolates whereas remaining subspecies and *S. bongori* are mainly isolated from cold-blooded animals and account for less than 1% of clinical isolates. As an example, the Kauffmann species *Salmonella* Typhimurium is now designated as *Salmonella enterica* subspecies I serotype Typhimurium. Under the

modern nomenclature system, the subspecies information is often omitted and culture is called *S. enterica* serotype Typhimurium and in subsequent appearance, it is written as *S.* Typhimurium. This system of nomenclature is used nowadays to bring uniformity in reporting (Andrews and Baumler, 2005 and Parry, 2006).

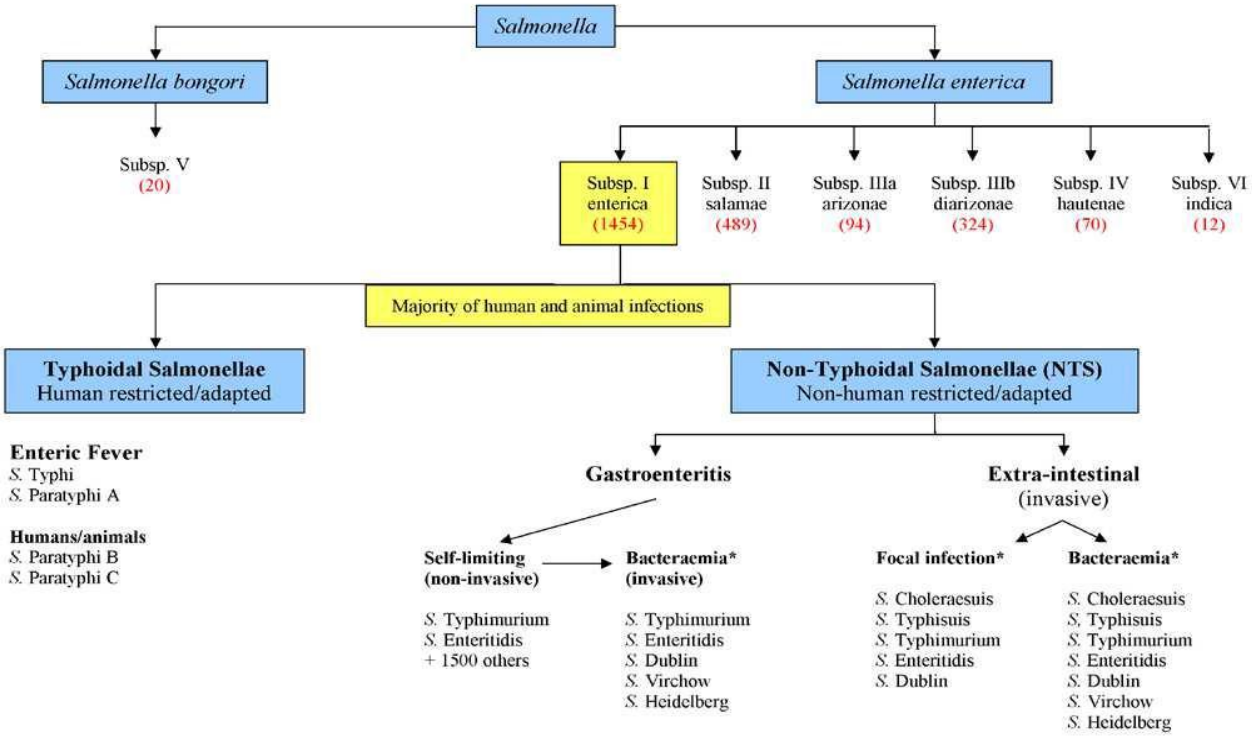

Figure 2.1: Classification of the genus *Salmonella*

Source: Langridge *et al*., (2008).

Note: Numbers in brackets indicate the total number of serotypes included in each subspecies.

* Common serotypes are listed but other serotypes may cause bacteraemia or focal infection; subsp = subspecies.

2.3.3 General characteristics of *Salmonella*

Salmonella make up a large genus of gram-negative bacilli within the family *Enterobacteriaceae* and it constitute a genus of more than 2300 serotypes that are highly adapted for growth in both humans and animals and that cause a wide spectrum of disease. The growth of *S.* Typhi and *S.* Paratyphi is restricted to human hosts, in whom these organisms cause enteric (typhoid) fever. The remainder of *Salmonella* serotypes, referred to as non-typhoidal *Salmonella*, can colonize the gastrointestinal tracts of a broad range of animals, including mammals, reptiles, birds, and insects. More than 200 of these serotypes are pathogenic to humans, in whom they often cause gastroenteritis and can also be associated with localized infections and/or bacteremia (Fuaci and Jameson, 2005). Members of the genus *Salmonella* are ubiquitous pathogens found in humans and livestock, wild animals, reptiles, birds, insects (Getenet, 2008) and can multiply under various environmental conditions outside the living hosts (Pui *et al*., 2011). *Salmonella* are gram-negative, non-spore forming, facultative anaerobic bacilli, and 2 to 3 by 0.4 to 0.6 μm in size (Getenet, 2008). They do not require sodium chloride for growth, but can grow in the presence of 0.4 to 4%of the sodium chloride. Most *Salmonella* serotypes grow at temperature range of 5 to 47°C with optimum temperature of 35 to 37°C but some can grow at temperature as low as 2 to 4°C or as high as 54°C. They are sensitive to heat and often killed at temperature of 70°C or above. *Salmonella* grow in a pH range of 4 to 9 with the optimum between 6.5 and 7.5. They require high water activity between 0.99 and 0.94 (pure water aw=1.0) yet can survive at water activity less than 0.2 such as in dried foods. Complete inhibition of growth occurs at temperatures less than 7°C, pH less than 3.8 or water activity less than 0.94 (Pui *et al*., 2011). Like other members of the family

Enterobacteriaceae, they produce acid on glucose fermentation; reduce nitrates to nitrite, and don't produce cytochrome oxidase. In addition; all *Salmonella* except *S.* Gallinarum-Pullorum are motile by means of peritrichous flagella, and all but *S.* Typhi produce gas (H2S) on sugar fermentation (Fuaci and Jameson, 2005 and Getenet, 2008). *Salmonella* are non- capsulated except *S.* Typhi, *S.* Paratyphi C and some strain of *S.* Dublin (Getenet, 2008).

The members of the genus *Salmonella* cause disease commonly referred to as salmonellosis although the term paratyphoid may be used for example swine paratyphoid (Kinde *et al.*, 1996). The disease has been recognized in all countries, but appears to be more prevalent in areas with intensive system of animal production (Leminor and Popaff, 1987). Importation of animal feeds in large quantities to countries with intensive system of animal production has resulted in wide spread international outbreaks of Salmonellosis in both animals and humans (Clarke and Gylen, 1993).

2.3.4 Transmission of *Salmonella*

Transmission andspread of *Salmonella* occurs by vertical and horizontal routes, or both. Vertical transmission occurs in poultry when *Salmonella* infects follicles in the ovary or the developing eggs become infected in the oviduct. Horizontal occurs on farms by direct contact between infected and uninfected environments. One of the commonest sources of horizontal transmission is feed that contains *Salmonella*. Fertilizers and feeds containing animal products are sometimes contaminated. The organisms occur most frequently in fish meal, bone meal and meat meal (Patel, 1997). Contaminated milk and milk products are

also important sources of *Salmonella* infection, particularly in calves (Mead, 2000). Many other animals that are often latently infected without showing clinical diseases are important in spreading the infection to other animals. These animals excrete *Salmonella* intermittently in their faces and form a large reservoir and source of contamination to healthy animals, humans and the environment. *Salmonella* are readily transferred from animals to animals, animals to humans, humans to animals and humans to humans.

2.3.5 Distribution of *Salmonella*

Salmonellosis is caused by a large group of bacteria, which is widespread in nature, do not recognize international borders, and only few serovars show host specificity (OIE, 2002). The disease has been identified in all countries, but it is most prevalent in countries with intensive animal production system particularly of poultry and pigs (Mead, 2000). In 1995, WHO conducted survey involving 69 member countries that used *Salmonella* serotyping as a part of public health surveillance, and found *Salmonella* Enteritidis was the most common isolate in 35 countries followed by *Salmonella* Typhi in 12 countries and *Salmonella* Typhimuriumin 8 countries (OIE, 2012).

2.3.6 Reservoirs of *Salmonella*

Salmonella lives in the intestines of humans and animals, but do not live well outside the digestive tract (CDC, 2009). It is known that man, farm animals, birds and reptiles especially turtles are the most common animal reservoirs (Karla, 2007). However, the bacteria can survive in water for Weeks and soil Years if the environmental conductions (P^H, temperature, humidity etc) are favorable (Kenneth, 2008). Usually infected animals feces contaminate human food and are transmitted to humans when they eat them (CDC, 2009). Unfortunately, contamination of food is not noticeable so it is always very important

to keep good hygiene especially during cooking (CDC, 2009). The most common contaminations are found in food such as beef, poultry eggs, and dairy foods (milk, mayonnaise, creamed foods etc) (CDC 2009), in addition, fruits and vegetable can also be contaminated. Also seafoods like Oysters and mussels near a sewage plant might concentrate *Salmonella* (from the water) as well, but the bacteria cannot multiply or colonise in the shellfish (Kenneth, 2008).

2.3.7 Carrier states and susceptibility

Stool cultures remain positive for four to five weeks after infection. Morbidity and mortality associated with salmonellosis are highest among the elderly, infants, and immune compromised individuals, including those with hemoglobinopathies and those infected with HIV or with pathogens that cause blockade of the reticuloendothelial system (patients with bartonellosis, malaria, schistosomiasis, or histoplasmosis) (Fuaci and Jameson, 2005).

Conditions that decrease stomach acidity like an age of less than one year, antacid ingestion, or achlorhydric disease or conditions that decrease intestinal integrity (inflammatory bowel disease, history of gastrointestinal surgery, or alteration of the intestinal flora by antibiotic administration) increase susceptibility to *Salmonella* infection (Fuaci and Jameson, 2005).

2.3.8 Epidemiology of *Salmonella*

Members of the genus *Salmonella* are oftenreferred to as the "universal pathogens", *Salmonella* pathogens are recovered from almost all vertebrates and some insects. *Salmonella* inhabit the intestinal tract of a wide range of animals such as mammals, birds, fish and reptiles. Their excretions contaminate water, food and environments, human sewage and any material exposed to fecal contamination. Because of their widespread

presence in the environment, some scientists consider *Salmonella* to be primarily environmental organisms that are pathogenic to humans and animals (Blackburn, 1993).

2.3.9 Host defense

Various host defenses are important in resisting intestinal colonization and invasion by *Salmonella*. Such host defenses against *Salmonella* include gastric factors (gastric acidity, rate of gastric emptying), intestinal factors (intestinal motility, normal intestinal flora, mucos, secretory antibiodies, genetic resistance to invasion) and non-specific factors (nutrition, hysozgines, gut reticuendothelial cell) (Giannela *et al*., 1975). Normal gastric acidity (Ph < 3.5) is lethal to *Salmonella.* In healthy individuals, the number of ingested *Salmonella* is reduced in the stomach, so that fewer or no organisms enter the intestine (Finlay, *et al*., 1989). Normal small intestinal motility also protects the bowel by sweeping ingested *Salmonella* through quickly. The normal intestinal microflora protects against *Salmonella*, probably through anacrobes, which liberate short – chain fatty acids that are thought to be toxic to *Salmonella* (Finlay *et al*., 1989). Alteration of the anaerobic intestinal flora by antibiotics renders the host more susceptible to salmonellosis. Secretary or mucosal antibodies also protect the intestines against *Salmonella*. Animal strains genetically resistant to intestinal invasion by *Salmonella* have been described. When these host defenses are absent or blunted, the host becomes more susceptible to salmonellosis example in AIDS (Finlay *et al*. 1989). Relapses are common. The role of host defense in salmonellosis is extremely important.

2.3.12 *Salmonella* Infections in animals

Salmonella have a wide variety of domestic and wild animal hosts. The infection may or may not be clinically apparent. In the subclinical form, the animal may have a latent infection and harbor the pathogen in its lymph nodes, or it may be a carrier and eliminate the agent in its fecal material briefly, intermittently, or persistently. In domestic animals, there are several well-known clinical enteritis due to species-adapted serotypes, such as *S.* Pullorum or *S.* Abortusequi. Other clinically apparent or in apparent infections are caused by serotypes with multiple hosts (PAHO, 2001).

The principal causes of clinical salmonellosis in cattle are serotype Dublin and *S.* Typhimurium. Other serotypes can sometimes be isolated from sick animals. Salmonellosis in adult cattle occurs sporadically, but in calves it usually acquires epizootic proportions. The disease generally occurs when stress factors are involved. Serotype Dublin, adapted to cattle, has a focal geographic distribution. In America, outbreaks have been confirmed in the western United States, Venezuela, Brazil, and Argentina. It also occurs in Europe and South Africa. In adult cattle, the disease begins with high fever and the appearance of blood clots in the feces, followed by profuse diarrhea, and then a drop in body temperature to normal. Signs of abdominal pain are very pronounced. The disease may be fatal within a few days or the animal may recover, in which case it often becomes a carrier and new cases appear. Calves are more susceptible than adults, and in them the infection gives rise to true epidemic outbreaks, often with high mortality. Septicemia and death are frequent in newborns. The carrier state is less frequent among young animals and occurs primarily in adult cattle. The infection is almost always spread by the feces of a cow that is shedding the agent, but it may also originate from milk (PAHO, 2001).

Swine are host to numerous *Salmonella* serotypes and are the principal reservoir of *S.* Choleraesuis. Serotypes that attack swine include *S.* Enteritidis, *S.* Typhimurium, and *S.* Dublin. *S.* Choleraesuisis very invasive and causes septicemia; it may be isolated from the blood or from any organ. Swine are particularly susceptible and experience epidemic outbreaks between 2 and 4 months of age, but the infection also appears in mature animals, almost always as isolated cases. The most frequent symptoms are fever and diarrhea. The infection usually originates from a carrier pig or contaminated food. Infection by other serotypes may sometimes give rise to serious outbreaks of salmonellosis with high mortality. Because of the frequency with which swine are infected with different types of *Salmonella,* pork products have often been a source of human infection (PAHO, 2001).

Cases of clinical salmonellosis in sheep and goats are infrequent. The most common serotype found in gastroenteritis cases is *S.* Typhimurium, but many other serotypes have also been isolated. Serotype *S.* Abortus ovis, which causes abortions in the last two months of pregnancy and gastroenteritis in sheep and goats, seems to be restricted to Europe and the Middle East (PAHO, 2001). Horses are also susceptible to *Salmonella,* particularly *S.* Typhimurium. *Salmonella* enteritis occurs in these animals, sometimes causing high mortality. Calves suffer from acute enteritis with diarrhea and fever; dehydration may be rapid. Nosocomial transmission has been seen in hospitalized horses (Bauerfeind *et al.*, 1992).

In recent years, a high prevalence of infection caused by numerous serotypes has been confirmed in cats and dogs. These animals may be asymptomatic carriers or may suffer from gastroenteritic salmonellosis with varying degrees of severity. Dogs can contract the infection by eating the feces of other dogs, other domestic or peridomestic animals or man.

Dogs and cats can also be infected by contaminated food. In addition, dogs can transmit the disease to man. Treatment for these animals consists mainly of fluid and electrolyte replacement (PAHO, 2001).

Two serotypes, *S.* Pullorum and *S.* Gallinarum, are adapted to domestic fowl. They are not very pathogenic to man, although cases of salmonellosis caused by these serotypes have been described in children. Many other serotypes are frequently isolated from domestic poultry; for that reason, these animals are considered one of the principal reservoirs of *Salmonella*e. Pullorum disease, caused by serotype *S.* Pullorum, and fowl typhoid, caused by *S.* Gallinarum, produce serious economic losses on poultry farms if not adequately controlled. Both diseases are distributed worldwide and give rise to outbreaks with high morbidity and mortality. Pullorum disease appears during the first 2 weeks of life and causes high mortality. The agent is transmitted vertically as well as horizontally. Carrier birds lay infected eggs that contaminate incubators and hatcheries. Fowl typhoid occurs mainly in adult birds and is transmitted by the fecal matter of carrier fowl. On an affected poultry farm, recuperating birds and apparently healthy birds are reservoirs of infection. *Salmonella* un-adapted to fowl also infect them frequently. Nearly all the serotypes that attack man infect fowl as well. Some of these serotypes are isolated from healthy birds. The infection in adult birds is generally asymptomatic, but during the first few weeks of life, its clinical picture is similar to pullorum disease (loss of appetite, nervous symptoms, and blockage of the cloaca with diarrheal fecal matter). The highest mortality occurs during the first two weeks of life. Most losses occur between six and ten days after hatching (PAHO, 2001).

Rodents become infected with the serotypes prevalent in the environment in which they live. Rodents found in and around food processing plants can be an important source of human infection. Of 974 free-living wild animals examined in Panama, 3.4% were found to be infected, principally by serotype *S.* Enteritidis and, less frequently, by *S. arizonae* (*Arizona hinshawii*) and *Edwardsiella*. The highest rate of infection (11.8%) was found among the 195 marsupials examined. Outbreaks of salmonellosis among wild animals held in captivity in zoos or on pelt farms are not unusual. *Salmonella* infection in cold-blooded animals has merited special attention. An infection rate of 37% was found in 311 reptiles examined live or necropsied at the National Zoo in Washington, D.C. The highest rate of infection was observed in snakes (55%) and the lowest in turtles (3%). The *Salmonella* isolated were 24 different serotypes formerly classified under the common name of *S.* Enteritidis, 1 strain of *S.* Choleraesuis, and 39 of *S. arizonae*. No disease in their hosts was attributed to these bacteria, but they may act together with other agents to cause opportunistic infections (PAHO, 2001).

2.3.13 *Salmonella* infections in humans

Salmonellla infections in humans can range from a self-limited gastroenteritis usually associated with non-typhoidal *Salmonella* (NTS) to typhoid fever with complications such as a fatal intestinal perforation (OIÉ, 2000). Non-typhoidal *Salmonella* is one of the principal cause of food poisoning worldwide with an estimated annual incidence of 1.3 billion cases and 3 million deaths each year (Torpdahl *et al*., 2007). Outbreaks of salmonellosis have been reported for decades, but within the past 25 years, the disease has

increased in incidence in many continents. The disease appears to be most prevalent in areas of intensive animal husbandry (OIÉ, 2000).

The incubation period in people is variable but is usually between 12 and 36 hours. The typical presenting symptom is diarrhea but this may be accompanied by nausea and abdominal pain, although vomiting is not usual. There may also be a headache and fever. While the infection is normally self-limiting and does not require antibiotic treatment, occasionally, with more invasive *Salmonella* such as *S.* Virchow, bacteremia can occur. The infection is rarely fatal in people (Gracey *et al*., 1999).

Salmonellosis is most commonly caused by *S. enterica* subsp. Typhimurium or *S. enterica* subsp. Enteritidis. Secondly, *S. enterica* subsp. Typhi and *S. enterica* subsp. Paratyphi are the causes of typhoid fever or paratyphoid fever, respectively. *Salmonella* can replicate both inside the vacuoles of host cells and in the external environment. *Salmonella* are the second most common pathogens isolated from humans with gastroenteric disease in developed countries (Buncic, 2006).

Salmonella Typhimurium and *S.* Enteritidis occur in the gastro intestinal tract of animals, including livestock. The disease is self-limiting, but can be severe in young, elderly or otherwise IC (immunecompromised) people. *Salmonella* invade epithelial cells in the ileum and proliferate in the lamina propria and profuse watery diarrhea results. Some isolates produce a heat-labile enterotoxin, which initiates diarrhoea. Sequelae include post- enteritis reactive arthritis and reiter‟s syndrome and systemic infection can result. Individuals can develop carrier status of up to 6 months in duration. The infectious dose varies, from only a few CFU to >105 CFU, so growth of the pathogen in foods has not been a factor in all cases of foodborne salmonellosis, but appears to have been in some.

Foods known to have been vehicles of salmonellosis include poultry, eggs, and meat, milk, chocolate, coconut and frog legs. However, any faecally contaminated food can be implicated (Buncic, 2006). *Salmonella* Typhi and *S. enterica* subsp. Paratyphi cause the systemic diseases, typhoid fever and paratyphoid fever, respectively. These pathogens occur in human feces, and are spread via human feces to the environment and to foods. Person-to-person transmission is common. The disease symptoms of typhoid and paratyphoid fevers are dissimilar to those of enteric salmonellosis (Buncic, 2006).

Salmonella penetrate the intestinal epithelium, possibly proliferating in macrophages and polymorphs, pass into mesenteric lymph nodes, liver or spleen then cause septicemia. Peritonitis and subsequent death can occur. Ulceration of the ileum can occur if organisms multiply in the bile of the gall bladder and cause re-infection. Any food could be a vehicle of infection if contaminated with human feces. Foods known to have been vehicles of typhoid fever include raw milk, shellfish and meat. However, typhoid fever is predominantly spread by water contaminated with human feces (Buncic, 2006).

2.3.14 Zoonosis and food safety

The prevalence of *Salmonella* infection in both humans and domestic animals has increased markedly in recent years. This increase may be explained by rapid intensification of animal production in developed countries which has resulted in marked changes in food distribution and eating habits of the human population.

A number of foods of animal origin habor *Salmonella* (pork, beef and poultry meat, egg and milk) (EU 1992). Humans mostly contract salmonellosis through the consumption of

improperly cooked meat or raw foods of animal origin contaminated with feces or by handling infected animals (EU1992). *Salmonella* infections of cattle and sheep as well as those of pets such as dogs and cats have the potential to cause disease in humans by direct contact with the infected animals. *Salmonella* Typhimurium and *Salmonella* Enteritidis are the most common causes of salmonellosis in many countries. Although *Salmonella* Enteritidis is mainly associated with poultry and other animals, meat products and other unpasteurized milk (Patel *et al*., 1997). Human to human transmission is uncommon in developed countries, but can occur in institutions such as baby care units, hospitals and residential homes for the elderly (Feng, 1996)

Salmonella frequently cause mild food poisoning in humans, which resolves without treatment in most cases, but occasionally *Salmonella* can cause severe septicemic disease which requires prompt and vigorous treatment (Selander, 1996).

2.3.15 Diagnosis of *Salmonella*

Diagnosis of *Salmonella* requires bacteriological isolation of the organisms from appropriate clinical specimens. Lab identification of the genus *Salmonella* is done by biochemical tests, the serologic type is confirmed by serological testing (Tietjen and Fung, 1995). Feces, blood or other specimens should be plated on several non-selective and selective agar media (blood, macConkey, eosin-methylene blue, bismuth sulfite, SSA and brilliant green agar) as well as into enrichment both such as selenite or tetrathonate. Any growth in enrichment broth is subsequently subculture on to the various agars. The biochemical reactions of suspicious colonies are then determined on triple sugar ion and

lysine – iron agar, (Wray and Linklater, 2000). The biochemical identification of *Salmonella* has been simplified by systems that permit rapid testing of 10-20 different biochemical parameters simultaneously (Wray & Linklater, 2000). The presumptive biochemical identification of *Salmonella* then can be confirmed by antigenic analysis of O and H antigens using polyvalent and specific antisera (Wallis, 2004). Fortunately approximately 95% of all clinical isolates can be identified with the available group A-E typing antisera. The isolates can then be sent to reference or central laboratory for more comprehensive serologic testing and confirmation (Wallis, 2004).

2.3.16 Detection and isolation of *Salmonella*

Culture based methods are still the most widely used detection techniques and remain the gold standard for the detection of *Salmonella* due to their selectivity and sensitivity. Depending on the approach, standard culture methods typically requires 5-7 days to obtain a result as they rely on the ability of *Salmonella* to multiply to visible colonies , which can then be characterized by performing additional biochemical and serological tests. Due totheir wide spread use, numerous and valid bacteriological media (selective enrichment broth and selective agar plates) are applied to best monitor for *Salmonella* in food and food ingredients .The media may contain inhibitors in other to stop or delay the growth of non target organisms or particular substrate that only the target bacteria can degrade or that confer a particular color to the growing colonies (Manafi, 2000).

Culture methods typically involved the enrichment of a portion of the specimen to recover sublethaly injured cells due to heat, cold, acid or osmotic shock (Sandel *et al* 2003, Gracious and Mckilip, 2004) in a non-selective pre-enrichment media such as Buffered peptone water (BPW), and to increase the number of target cells as these are generally not

uniformly distributed in foods, typically occur in low numbers and may be present in a mixed microbial populations. Next, primary enrichment cultures aretypically inoculated onto secondary enrichement broths such as selenite cystine broth (SC) rappaport vasiliadis soy broth (RVS) , tetrathionate broth (TT), or muller Kauffman tetrationate-novobiocin broth(MKTTn) and incubated at elevatedtemperature (39^{o}C or 42^{o} C for 18-24hours) before being struck onto selective agar such as xylose lysine decarboxylase agar (XLD agar) bismuth sulphite (BIS) ,Brilliant Green agar (BG) with or without the addition of sulfadiazine or sulfapyrine (BGS) Modified semi solid rappaport vasiliadis (MGRV), *Salmonella Shigella* agar (SSA) or hekton enteric agar . There are several published standard methods utilising combinations of media such as the current ISO horizontal methods ISO 6579, 2002 (updated in 2007) for the detection of *Salmonella* including *Salmonella* Typhi and Paratyphi applicable to products intended for human consumption and feeding of animals and to environmental samples in the areas of food production and food handling. Similar standard methods have been published elsewhere, most notably in the FDA bacteriological Analytic manual (BAM).

Immunological methods include Rapid Agglutination Assays (Cheesbrough and Donnelly, 1996), Lateral flow Immunoassay (Cox, 1988) and Enzyme - Linked Immunosorbent Assays (C0x, 1988).

The molecular methods include Polymerase Chain Reaction(PCR) (Chein *et al*., 1976), Real Time PCR (Espy *et al.*,2006), Multipplex PCR (Chamberlain *et al*., 1988), Reverse Transcriptase PCR (Masters *et al.*, 1994), Nucleic Acid Hybridization (Chan *et al*., 1988).

2.3.17 Antimicrobial susceptibility tests and resistance profile

There are three test methods (disk diffusion, broth dilution and agar dilution). Antimicrobial susceptibility testing methods that consistently provide reproducible and repeatable results when followed correctly (CLSI, 2008).

2.3.17.1 Disk diffusion

- Disk diffusion refers to the diffusion of an antimicrobial agent of a specified concentration from disks, tablets or strips, into the solid culture medium that has been seeded with the selected inoculum isolated in a pure culture. Disk diffusion is based on the determination of an inhibition zone proportional to the bacterial susceptibility to the antimicrobial present in the disk. The diffusion of the antimicrobial agent into the seeded culture media results in a gradient of the antimicrobial. When the concentration of the antimicrobial becomes so diluted that it can no longer inhibit the growth of the test bacterium, the zone of inhibition is demarcated. The diameter of this zone of inhibition around the antimicrobial disk is related to minimum inhibitory concentration (MIC) for that particular bacterium/antimicrobial combination; the zone of inhibition correlates inversely with the MIC of the test bacterium. Generally, the larger the zone of inhibition, the lower the concentration of antimicrobial required to inhibit the growth of the organisms. However, this depends on the concentration of antibiotic in the disk and its infusibility (OIE, 2012).

Disk diffusion is straightforward to perform, reproducible, and does not require expensive equipment. Its main advantages are: low cost, ease in modifying test antimicrobial disks when required, can be used as a screening test against large numbers of isolates, can

identify a subset of isolates for further testing by other methods, such as determination of MICs. Manual measurement of zones of inhibition may be time-consuming. Automated zone-reading devices are available that can be integrated with laboratory reporting and data-handling systems. The disks should be distributed evenly so that the zones of inhibition around antimicrobial discs in the disc diffusion test do not overlap to such a degree that the zone of inhibition cannot be determined. Generally this can be accomplished if the discs are no closer than 24 mm from centre to centre, though this is dependent on disk concentration and the ability of the antimicrobial to diffuse in agar (OIE, 2012).

2.3.17.2 Broth and agar dilution methods

The aim of the broth and agar dilution methods is to determine the lowest concentration of the assayed antimicrobial that inhibits the visible growth of the bacterium being tested (MIC, usually expressed in μg/ml or mg/litre). However, the MIC does not always represent an absolute value. The "true" MIC is a point between the lowest test concentration that inhibits the growth of the bacterium and the next lower test concentration. The MIC determinations performed using a dilution series may be considered to have an inherent variation of one dilution. Antimicrobial ranges should encompass both the interpretive criteria (susceptible, intermediate and resistant) for a specific bacterium/antibiotic combination and appropriate quality control reference organisms. Antimicrobial susceptibility dilution methods appear to be more reproducible and quantitative than agar disk diffusion. However, antibiotics are usually tested in doubling dilutions, which can produce inexact MIC data. The selection of an AST methodology may be based on the following factors: ease of performance, flexibility, adaptability to automated or semi-automated systems, cost, reproducibility, reliability,

accuracy, the organisms and the antimicrobials of interest in that particular OIE Member, availability of suitable validation data for the range of organisms to be susceptibility tested (OIE, 2012).

2.3.18 Resistance to antimicrobial agents

Antimicrobial resistance is an increasing global problem and emerging antimicrobial resistance has become a public health issue worldwide (Kaye *et al*., 2002). The problem of such resistant organisms evolving and being transmitted through the food chain from cattle and sheep is becoming increasingly important throughout the world (Glynn *et al*., 1998). A variety of foods and environmental sources harbor bacteria that are resistant to one or more antimicrobial drugs used in humans or veterinary medicine and in food animal production (Bager and Helminth, 2001). The resistance may be caused by a wide range of biochemical and physiological mechanism. The mechanism by which an organism exhibit resistance to antimicrobials include drug inactivation or modification, alteration of target site, alteration in the metabolic path ways, and reduced drug accumulation (Katzung, 2004). The most prevalent gram-negative pathogens such as *Escherichia coli*, *Salmonella enteritica* and *Klebsiella pneumoniae* cause a variety of diseases in humans and animals and a strong correlation between antibiotic used in the treatment of these diseases and antibiotic resistance development has been observed for the past half century. This is especially apparent with the B-lectam class of antibiotics and their related inactivating enzymes are the B-tactamses (Davies and Davies, 2010). The genes of B-lactamase enzymes are probably the most international in distribution: random mutation of the genes encoding the enzymes have given rise to modified catalysts with increasingly extended spectra of

resistance (Gnaidkowski, 2008). The Molecular mechanisms of resistance to antibiotics have beenstudied extensively and have involved investigations of the genetics and biochemistry ofmany different facet of bacterial cells function (Allen *et al*., 2010). The resistance processes arewidely distributed in the microbial kingdom and have been well described for a variety of commensal (Marshall *et al*., 2009) and pathogens most can be dessiminated by one or more distinct gene transfer mechanisms.

Resistance in bacteria has also been seen to occur due to gene transmission. Essentially any of the accessory genetic elements found in bacteria are capable of acquiring resistant genes and promoting their transmission, the type of element involved varies with genus of the pathogen. Gene transmissionby conjugation has been studied extensively in the laboratoryand in microcorms approximating environmental conditions and the frequencies of the transfer events often varies significantly (Sorensen *et al*., 2005)

Antimicrobial resistance in *Salmonella*is of public health importance. Although most infections are limitedto the gastrointestinal tract and also do not acquire antimicrobial therapy, occasionally infections spread to the blood stream, an appropriate antimicrobial therapy can be life saving (Tauxe, 1991). Antimicrobial resistance to the agents of choice compromises such therapy. Of great importance is the observation that treatment with antimicrobial agents can actually promote *Salmonella* infections in both human and animals, particularly if the infecting strain is resistant to the agents being used (Chalker and Blaser, 1988).

2.3.19 Treatment

Treatment for the salmonellosis is controversial since the diseases are often self limiting and some studies show no difference in outcomes between antibiotics treated and untreated patient populations. Many doctors recommend no antibiotics and cite evidence that antibiotics may prolong carrier state. Other physicians disagree and treat with antibiotics for up to 10-14 days. Most physicians treat patients with antibiotics if the patients are immune suppressed (example patients with AIDS, cancer and elderly). If *Salmonella* are isolated from patients, the bacteria should be studied to determine if they are resistant to certain antibiotics so that the physicians can choose the most effective treatment .This needs to be done so that patients will be treated with antibiotics that are effective against the species of *Salmonella* isolated from the patient.

Most patients recover from salmonellosis without getting treatment other than oral fluids, however, it is important to seek and notify a medical doctor in case of a possible public outbreak (CDC, 2009). Patients with fever can be treated with cetaminophen or ibuprofen (CDC, 2009). Dehydrated patients can be treated with electrolyte drinks like Gatorade and pedialyte or intravenous fluids for extreme case (CDC, 2009). For severe infections, hospitalization and possible antibiotics therapy may be needed (CDC, 2009). Common antibiotics used for complications of salmonellosis are Amphicilin, Chloraquinolones, Gentamicin, Ciprofloxacin, Trimethoprim-sulfamethoxozole, Chloramphenicol and Amoxicilline (CDC, 2009). For children with serious infections, third generations cephalosporins are widely used but this is given by injection .Infected infants should continue to breastfeed from their mothers in other to get electrolyte solution (CDC, 2009).

However, anti diarrheal medication is not usually given to any patient because it may prolong the infection (CDC, 2009).

Intravenous and orals fluid and electrolyte therapy increase the survival rate in cattle. Intravenous hypertonic saline (4mg/kg of 7% NaCl) combined with oral rehydration with water or hypertonic sodium-containing fluid via a stomach tube has proved particularly usefull in cattle.

Occasionally, surgery is required to treat a site of focal infection. For example the infected gallbladder is removed in some patients who are carriers. Infected animals remain carriers after treatment.

2.3.20 Prevention and control

Salmonella is a notifiable disease in many countries which have national scheme to controlling the disease in animals and is subject to compulsory control measures.

A number oflive inactivated vaccines, which may protect against clinical disease if given atleast 2 weeks before exposure to *Salmonella* organisms have been described. The vaccines areeffective against intestinal colonization in pigs, chickens and calves with host nonspecific serovars (AU-IBAR, 2011). *Salmonella* organisms for use in vaccines production are usually inactivated using heating or formalin (EU, 1992).More recently,molecular biology techniques have been used to manufacture mutant vaccines which are now available in USA, Germany and other countries for use in poultry, pigs and other species of domestic animals (Feng, 1996). Recent advances in the development of adjuvants such as microspheres, which are potent immunostimulants (Morein *et al.,* 1996)

may lead to the production of next generation of *Salmonella* subunit vaccines. Meanwhile, the use of autogenous vaccines may be a cheaper alternative for countries with limited resources than purchase a commercially available vaccines of dubious efficacy (OIE, 2000).

The major strategy for the control of *Salmonella* should include the following measures, cleaning the production chain from the top, feed hygiene, competitive exclusion, vaccination education programmes, hygienic measures and eradication. Although difficult, eradication of *Salmonella* may be possible by complete depopulation or by treatment of whole herd or flock combined with disinfection. Biosecurity measures such as rodents control, fly control and housing livestock's on wildbirds proof farms may help prevent the introduction of *Salmonella* to healthy herds and flocks (Pomeroy *et al*, 1989). The importance of protecting feed stores from contamination by wild droppings in particular has been recognized. (Tietjen and Fung, 1995). Isolation of infected animals, the use of *Salmonella* free stock, clean feed and water are also important in the control and prevention of salmonellosis.

Salmonellosis control is generally difficult and costly, but progress can be made by introducing effective monitoring programmes, all in all out production where possible, effective cleaning and disinfection and rodent controlling post weaning accommodation to ensure that the breeding pyramid remains free of important zoonotic infectious agents such as *Salmonella* Typhimurium DT 104 (OIE, 2012). Investigation of feeding methods that may reduce colonization of intestines by *Salmonella* is required as well as the investigation of presence of *Salmonella* in clinically healthy animals which is at present unknown (OIE, 2012).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Materials

Salmonella Shigella agar, nutrient agar, polyvalent antisera, Triple sugar iron agar, Methyl Red-Voges Proskauer, Simon citrate, Urea agar, Sulphur Indole Motility Agar, Sterile swab sticks, distilled water, cotton wool, conical flask, inoculating wire loops, polythene bags, aluminium foil paper, ice cooler, hand gloves, permanent marker, ruler, petri dishes, scissors, electronic weighing balance, autoclave, incubator, bunsen burner, bijou bottles, universal bottles, disinfectant, detergents, Microbact 12E GNB kit, antibiotic multiple disc dispenser, measuring cylinder, *Salmonella* Latex agglutination test kit, Test tubes and Selenite "F" broth.

The antibiotics used for this study were in various concentrations. They included: Amikacin (30 µg), Nalidixic acid (30µg), Kenamycin (30µg), Streptomycin (10µg), Chloramphenical (30µg), Ciprofloxacin (5µg), Gentamicin (10µg), Tetracycline (30µg), Imipenem (10µg), Nitrofurantoin (300µg), Trimethoprim (5µg), and Amoxicillin + Clavulanic acidd (30µg). These impregnated discs were all obtained from Oxoid UK. These antimicrobial agents were selected based on their indication in the treatment of Salmonllosis and ease of accessibility for both veterinary and human practices.

3.2 Study Area

This study was conducted in Zaria Metropolis of Kaduna State; an ancient city famed as centre of learning. This renowned city is located in the Northern Guinea Savannah zone of Nigeria with a total area of about 300km square and a population of 408,198 (NPC, 2006). Animals reared in Zaria include cattle, sheep, goats, poultry and pigs among others animals. Zango abattoir was established in 1978 with an initial slaughter capacity of 200 cattle and 500 small ruminants per day (Bello *et al*, 2008). However, the slaughter capacity of the abattoir has now reduced to an average of 40- 50 cattle per day. Other animals slaughtered in the abattoir included camel, sheep and goats. The standard facilities of the abattoir such as the lairage, hoisting materials, water supply system, drainages and disposal systems have been dilapidated leading to unhygienic operations inthe abattoir.

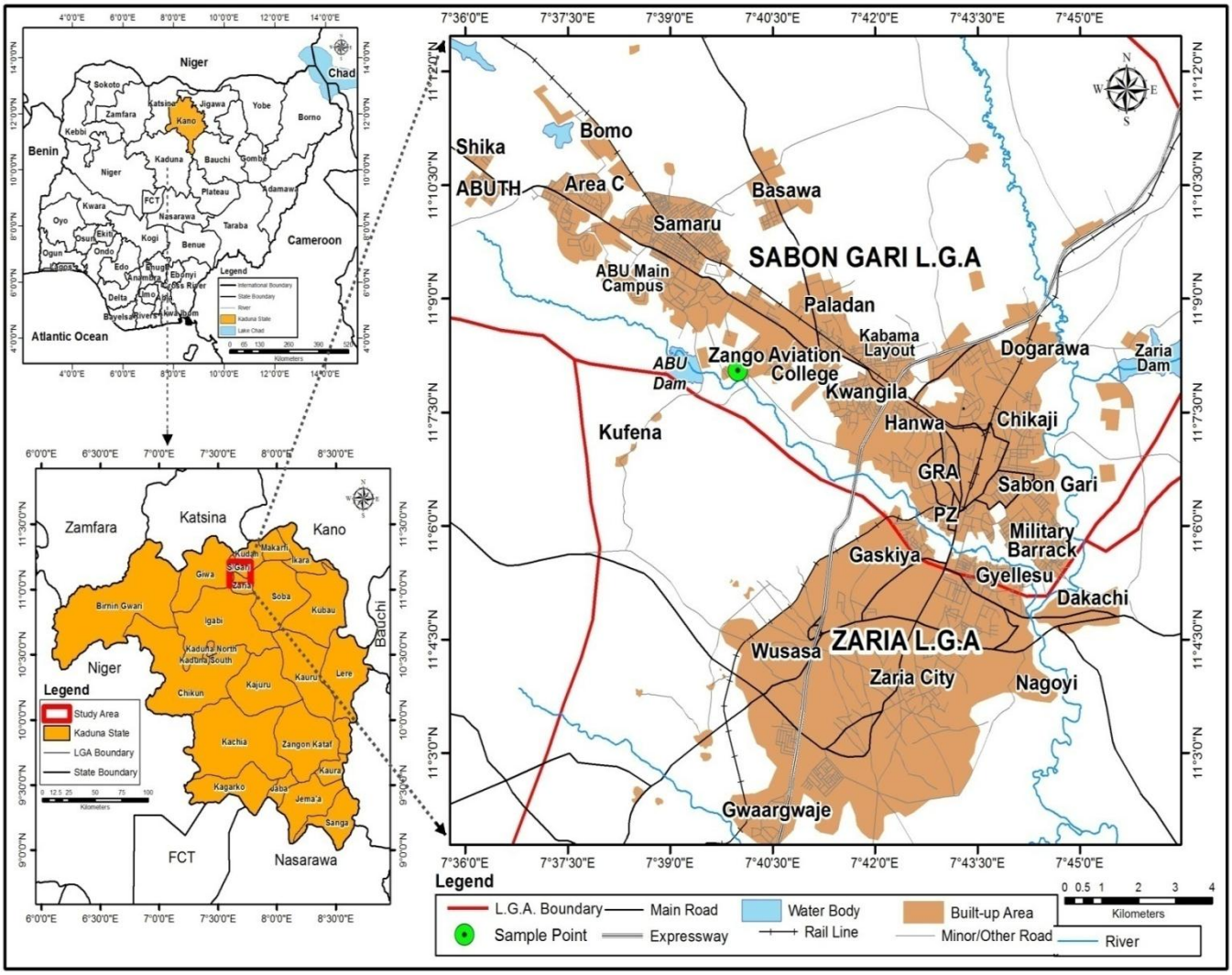

Fig 3.1 Map of Kaduna State showing the location of Zango abattoir (Sample Point) in Zaria Metropolisof Kaduna State, Nigeria

Source: Modified from the Administrative Map of Zaria & Sabon Gari LGAs (2015)

3.3 Study Design

A cross sectional study was carried out to determine the prevalence of *Salmonella* in cattle carcasses slaughtered in Zango abattoir Zaria of Kaduna State, Nigeria

3.4 Sample Size

Sample size was calculated using the formula by Thrusfield, (2005) at the Confidence Interval of 95% and 5% precision.

N = Z2Pq

d2

Where N =sample size

Z = Standard deviation at 95% confidence interval

P = prevalence of 8.4% obtained from raw meat (Kwaga *et al*., 1984)

Q = 1- P

D = Level of significance

$$N = \frac{1.96^2 \times (0.084 \times 0.916)}{(0.05)^2}$$

$$= \frac{3.8416 \times (0.084 \times 0.916)}{0.0025}$$

$$= \frac{3.8416 \times 0.076944}{0.0025}$$

N = 118.23. The actual sample size was 118 however, 200 samples were taken to increase precision

3.5 Inclusion Criteria

All the cattle slaughtered in the abattoir with no distinction to breed and age were included in this study

3.6 Exclusion Criteria

Any other livestock slaughtered in the abattoir that was not cattle was excluded from this study.

3.7 Sample Collection and Transportation

Samples were obtained as described by Teagasc (2008). The neck, brisket, flank and rump were swabbed vertically, horizontally and diagonally using commercially prepared swab sticks covering 10 x 10 cm^2. A total of 400cm^2 were swabbed for each carcass as pooled sample. After swabbing, the pooled samples were immediately placed into a 5ml of maximum recovery diluents which was used as the transport and pre - enrichment media after swabbing. The swab sticks were cut-off and immersed into the diluents. Each sample container was identified with carcass number, time and date of sampling and the samples were stored between 0-4°C in ice park cooler and transported immediately to the Bacterial Zoonoses Laboratory of the Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine Ahmadu Bello University Zaria.

3.8 Sample Processing

One (1) mililitre of the overnight culture was then added into selenite "F" broth as an enrichment media in a ratio of 1: 10 and incubated at 37°C for 24 hours. A loopful of the overnight culture was then streaked on selective media (*Salmonella* - *Shigella* Agar) and incubated at 37°C for 24 hours. The colonies suspected to be *Salmonella* based on colonial

morphology that is those that appared colorless with dark centres were inoculated onto nutrient agar slant and incubated at 37°C for 24hours and thereafter stored at 4°C in the refrigerator for further analysis.

3.9 Conventional Biochemical Characterisation

This was carried out based on standard techniques (Barrow and Felthan, 1995). All the isolates that tested positive as*Salmonella* in all or most of the test substrates were considered to belong to the genus *Salmonella*. Typical *Salmonella* reactions were indole negative , methyl red positive Voges Proskauer negative, citrate positive, motile in motility medium, produces H_2S niitrate positive, lysine decarboxylase positive, oxidase negative, ferments glucose, mannitol, dulcitol, maltose but fail to ferment lactose, sucrose, adonitol and raffinose.

3.9.1 Triple Sugar Iron Agar test (TSI)

This was prepared according to manufacturer's instruction. The medium was inoculated with the isolate using inoculation needle by first stabbing through the centre of the medium through the bottom of the tube and then the surface of the slant was streaked and incubated at37°C for 24 hours. Typical *Salmonella* reaction on TSI was acid/ alkaline + H_2S gas

3.9.2 Methyl Red- Voges Proskauer test

Isolates were inoculated onto 5ml of MR-VP broth and incubated for 48 hoursat 37°C. After incubation, 2.5ml of the broth was transferred to a second sterile tube and 3 drops of methyl red was added. The appearance of a red coloration was indicative of positive methyl red test. Five drops of 40% potassium hydroxide (KOH) was added to the remaining broth in the original tube followed by another 5 drops of 5% of alcoholic (ethanol) Alpha-

Naphthol and then shaken. The development of a red color starting from the liquid-air interface within 1hwas indicative of a positive test.

3.9.3 Citrate utilization test

Isolates were picked using sterile wire loop and then lightly streaked on the surface of the Simon Citrate Agar slant. This medium contains bromothymol blue as a PH indicator. Following 24 hours incubation at 37°C, citrate utilization was indicated by a change of color to blue while a negative test retains the original color of the Citrate Agar Slant

3.9.4 Urea test

A colony was picked with a sterile wire loop and streaked lightly on the surface of the sterile Urea Agar Slant in the test tube. This was then incubated at 37°C for 24 hours. Retention of color was an indication of a negative reaction while a change in color to bright pink was an indication of a positive reaction which indicated that the organism was capable of hydrolyzing urea to produce carbon dioxide.

3.9.5 Sulphur – Indole Mortility tests (SIM)

Suspected isolates were inoculated onto the medium by stabbing. The tube was then incubated at 37°C for 24 hours. Production of H_2S was indicated by a black discoloration while motility was indicated by migratory movement away from the line of stabbing giving a blue like appearance. Indole positive reaction was indicated by the appearance of a red colored ring on the surface of the medium within a few minutes following the addition of Kovac's Indole Reagent.

3.9.6 Oxidase test

Fresh colony was picked and transferred onto4.5ml nutrient broth. 0.2 ml of 1% naphthol was added followed by 0.3ml of 1% P- amodymethylandine oxalate (Gabby and Hadley Reagents). It was shaken vigorously to ensure mixing and thorough oxygenation of the culture. Observation for color change was made immediately. Color changes to blue within 15 to 30 seconds indicated positive test, color changes to purple in 2 to 3 minutes in weak organisms and organisms are negative when there is no color change.

3.10 Microbact 12E Gram-Negative Bacillus (GNB) Identification System

A 24 hours culture of presumptive *Salmonella* on selective media was obtained. One to three (1-3) colonies of each isolate were then selected and emulsified in 3 ml sterile saline. The micro plates were placed in a holding tray and the seal peeled back. Four (4) drops of bacterial suspension was added to each well, covered back with its seal and incubated at 37°C. After 24 hrs of incubation, appropriate reagents were added to well 8, 10 and 12. Two drops of the Kovac's reagent was added to well 8 and observed for 2mins and I drop each of VPI (Voges Proskauer) and VP2 to well 10 and observed for 15-30 mins and one drop of EDTA in well 12 which was interpreted immediately.

Results were recorded in report forms containing the substrate that were tested. Twelve substrates were tested, lysine, ornithine, hydrogen sulfide, glucose, mannitol, xylose, ONPG, Indole, urease, VP, Citrate, TDA. Three substrates formed 1 group with each substrate assigned a number, when a substrate gave a positive results, the corresponding number for the group was summed up and recorded. The results of the consecutive 3 wells were added and a four digit code (Octal Code) was then obtained which was fed into computer identification software .This immediately gave the probable identity of the

organism tested in percentage. The microbact software permits a 75% cut-off point for a probable identification. All tested organisms that gave less than 75% probability were not accepted as *Salmonella*.

3.11 Rapid Latex Agglutination Test for *Salmonella*

Agglutination tests for the presumptive identification of *Salmonella* species were carried out. A wide range of *Salmonella* flagella antigens have been used to produce polyvalent antisera in rabbits. The presumptive overnight growths on *Salmonella Shigella* agar plate were mixed with the Latex Test Reagent and the presence of agglutination indicated that *Salmonella* was present as described here.

For rapid control, one drop of *Salmonella* Latex Reagent (Oxoid)® was added to one drop of saline in the circle provided on the reaction card. This was mixed for two minutes and observed for agglutination. No agglutination occurred indicating no contamination.

For the positive control, a smooth suspension of a known *Salmonella* species was prepared on a reaction card and observed for 2 minutes. One drop of Latex reagent was then added and mixed with a mixing applicator stick. Agglutination occurred indicating a positive reaction.

A reaction card was placed on a work bench and one drop of sterile saline was added within one circle in the reaction card. The suspect colony was emulsified in the drop of sterile saline to produce a smooth suspension. The reagent was then added to the saline suspension. This is further mixed with clean mixing stick for 30 seconds and gently rocked for 2-3minutes. Agglutination was observed within a maximum of 2minutes indicating *Salmonella* was present in the test culture.

3.12 Antibacterial Susceptibility

All the isolates identified as *Salmonella* were tested for their susceptibility to 12 antibacterial agents using disk diffusion method. Each isolate was diluted in sterile saline to a 0.5 McFarland standard. The diluted bacterial suspension was transferred onto nutrient Agar Plates using sterile swabs by swabbing the entire surfaces of the agar plates and allowed to dry for 10minutes (Bauer *et al*., 1966). The discs of the antibacterial agents were placed onto the inoculated surfaces of the agar using a multiple disc dispenser. The discs were further pressed with a sterile forcep to ensure complete contact with the surface of the agar plates. The petri dishes were then incubated at 37°C for 18-20 hours after which the zone of inhibition were measured and interpreted using an interpreting chart for zone sizes (CLSI, 2011). The following were the antibacterial agents with their concentrations used; Nalidixic acid (30µg), Amikacin (30µg), Kenamycin (30µg), Ciprofloxacin (5µg), Chloramphenicol (30µg), Nitrofurantoin (300µg), Imipenem (10µg), Streptomycin (10µg), Gentimicin (30µg), Tetracycline (30µg), Amoxicillin + Clavulanic acid (30µg).

Interpretation of the growth inhibition zones and classification of isolates as susceptible, intermediate and resistant was done following the guidelines of the Clinical and Laboratory Standards Institute (CLS, 2011).

3.13 Data Analysis

Data were subjected to descriptive statistics and presented in tables, pie– chart and bar–chart.

CHAPTER FOUR

RESULTS

4.1 Occurrence of *Salmonella* Species in Cattle Carcasses from Zango Abattoir, Zaria Nigeria

Ten out of the 200 samples tested yielded *Salmonella* species giving a prevalence of 5% in this study. *Salmonella arizonae* recorded the highest prevalence of 80%and *Salmonella* cholerasuis and *Salmonella* species (unclassified) had 10% prevalence respectively.

4.2Morphological and Biochemical Results of *Salmonella* Species from Zango Abattoir, Zaria Nigeria

Ninety eight (98) out of the two hundred (200) samples showed the biochemical characteristics typical of *Salmonella* on *Salmonella Shigella* Agar and fortyeight (48) of the ninety eight (98) *Salmonella* suspected isolates showed characteristics typical of *Salmonella* species following biochemical tests

4.3 Microbact 12E Identification System Results of *Salmonella* Isolates from Zango Abattoir, Zaria Nigeria

Nineteen (39.9%) out of the ninety eight (98) isolates suspected were *Salmonella arizonae*, 3 (6.25%) were *Salmonella* cholerasuis, 2(4.16%) were(unclassified) *Salmonella* species, 5 (10.4%) were *Serrattia liguefaciens,* 3 (6.25%) were *E.coli,* 4 (8.33%) were *K. pneumoniae,* 2 (4.16%) were *E. tarda,* 2 (4.16%) were *C. diversus* and 1 (2.08%) for each of the following: *H. alvei, E. vulneris, E. gergoviae, A. baumanni, A. iwoffii, E. agglomerans, K. rhinoscleromatis* and *K. azaenae*. However, 25 out of the 48 isolates did not meet the75% cut-off for the species identification by microbact 12E test, 2 out of the 25

isolates were *Salmonella Cholerasuis* with 42.85% and 56.56%, 5 were *Serrattia liguefaciens* with 54.60%, 44.75%, 54.60%, 44.75% and 44.75%, 4 were *Salmonella arizonae* with 31.16%, 54.19%, 31.16%, and 52.53%, 4 were *K. pneumoniae* with 46.73%, 46.73% 65.38% and 65.38% and *E. tarda*, *E. vulneris, E. gergoviae, A. baumanni, A. iwoffii*, *E. agglomerans* and *K. azaenae* with 42.23% , 40.47% 39.04% 73.62% 49.62% 65.38% and 38.75% respectively (Table 4.1)

4.4 Rapid Latex Agglutination Test of *Salmonella* Isolates from Zango Abattoir, Zaria Nigeria

Ten(10) out of the eighteen(18) *Salmonella* isolates that met the cutoff point of 75% probability of microbact agglutinated while 8 did not.. Of the 10 that agglutinated, 8 (80%) were *Salmonella arizonae*, while *Salmonella* specie (unclassified) and *Salmonella* Cholerasuis were 1 (10%) each. (Fig. 4.2)

4.5 Determination of Susceptibility of *Salmonella* Isolates to 12 Antibacterial Agents from Zango Abattoir, Zaria Nigeria.

All the 10 *Salmonella* isolates showed 100% susceptibility to Gentamicin and varying percentages to the rest of the antibacterial agents used. These were Ciprofloxacin (90%), Amikacin (70%), Chloramphenicol (60%), Nitro-furantoin (40%), Trimethoprim (40%), Nalidixic acid (40%), Kenamycin (40%), Tetracycline (30%), Streptomycin (20%), Imipenem (20%) and Amoxicillin + Clavulanic acid (20%) (Table 4.2)

Table 4.1. Distribution of bacterial isolates from swab samples based on Microbact Identification system.

Bacterial isolates	Frequency of isolation	% Frequency
Salmonella enterica **subspp** ***arizonae***	**19**	**39.9**
Salmonella **specie (unclassified)**	**2**	**4.16**
Salmonella enterica **subspp Cholerasuis**	**3**	**6.25**
Serrattia liguefaciens	**5**	**10.4**
E.coli	**3**	**6.25**
K. pneumoniae	**4**	**8.33**
E. tarda	**2**	**4.16**
C. diversus	**2**	**4.16**
H. alvei	**1**	**2.08**
E.vulneris	**1**	**2.08**
E. gergoviae	**1**	**2.08**
A.baumanni	**1**	**2.08**
A.iwoffi	**1**	**2.08**
E.agglomerans	**1**	**2.08**
K. rhinoscleromatis	**1**	**2.08**
K. Azaenae	**1**	**2.08**
Total	**48**	**100**

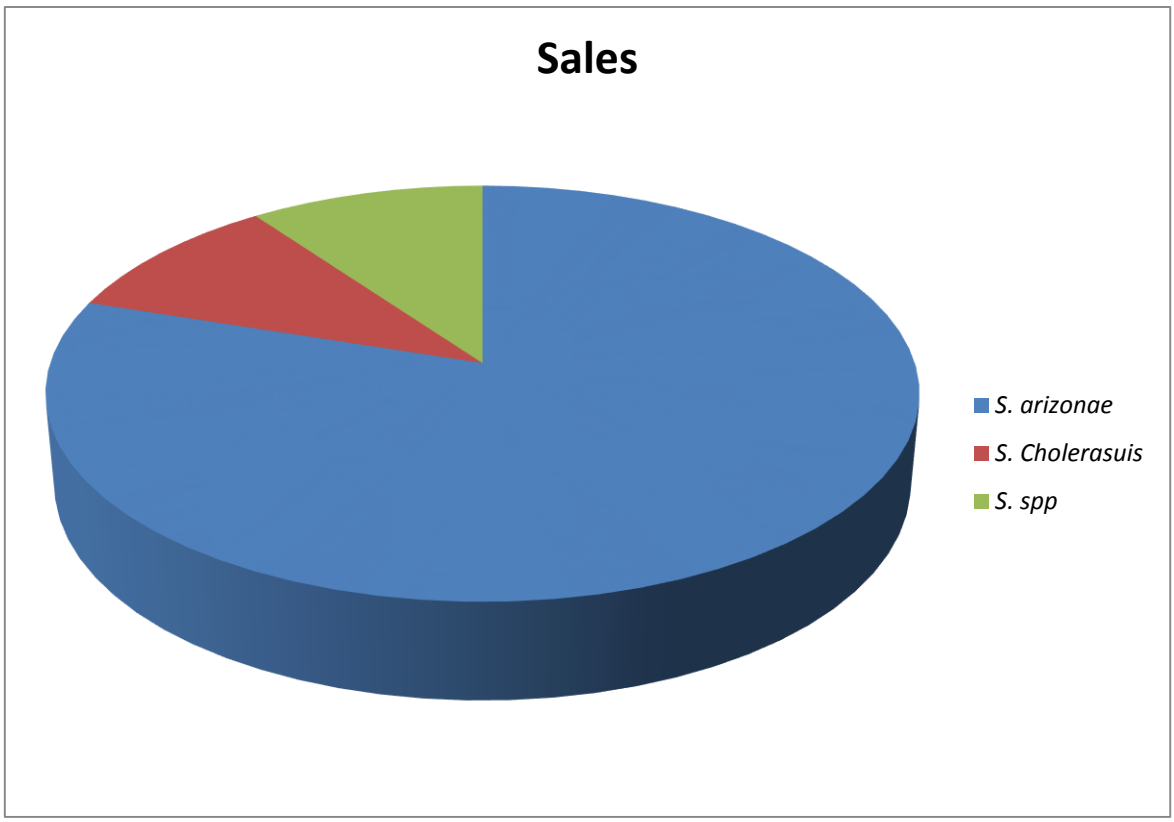

Fig 4.1: **The percentage of *Salmonella* species following Agglutination test.**

4.6 Multiple Drug Resistance Patterns of *Salmonella* Isolates from Zango Abattoir, Zaria Nigeria

The result gave patterns of drug resistance with AMC appearing in 8 patterns, TE in 7, S in 7, NA and W appearing 5 times each, F and IPM in 4 patterns each, K and C appearing 3 times each and AK in 3 patterns .(Table 4.3)

4.7 MAR Index of *Salmonella* Isolates from Zango Abattoir, Zaria Nigeria

None of the isolates showed a multiple drug resistance index that is less than two (2). All the isolates showed multiple drugs resistance with the least showing resistance to two (2) antibiotics and the highest showed resistance to seven (7) antibiotics (Fig. 4.2)

Table 4.2 Antibacterial Susceptibility test of Salmonella isolates from Zango abattoir Zaria, Nigeria

S/NO	Antibacterial Agents	Disk Content(µg)	Percentage (%)
1	Gentamicin	10	100
2	Ciprofloxacin	5	90
3	Amikacin	30	70
4	Chloramphenicol	30	60
5	Nalidixic acid	30	40
6	Kenamycin	30	40
7	Nitro-furantoin	300	40
8	Trimethoprim	5u	40
9	Tetracycline	30	30
10	Streptomycin	10	20
11	Imipenem	10	20
12	Amoxicillin+Clavulanic acid	30	20

Table 4.3: Multiple Atimicrobial Resistance Patterns of *Salmonella* Isolates from Zango abattoir, Zaria, Nigeria

S/No	Resistance Patterns	Frequency
1	**NA, K, F, C, W, TE, AMC**	1
2	**S, W, TE, AMC**	1
3	**S, TE, AMC**	1
4	**S, AMC**	1
5	**S, IPM**	1
6	**NA, IPM, TE, AMC**	1
7	**NA, AK, K, F, S, IPM, TE**	1
8	**NA, S, IPM, W, TE, AMC**	1
9	**F, S, C, W, TE, AMC**	1
10	**NA, K, F, C, W, AMC**	1

KEY

NA	Nalidixic acid
AK	Amikacin
K	Kenamicin
C	Chloramphenicol
W	Trimethoprim
TE	Tetracycline
AMC	amoxicillin+ Clavulanic acid
S	Streptomicin
IPM	Imipenem
F	Nitrofurantoin
CIP	Ciprofloxacin
CN	Gentamicin

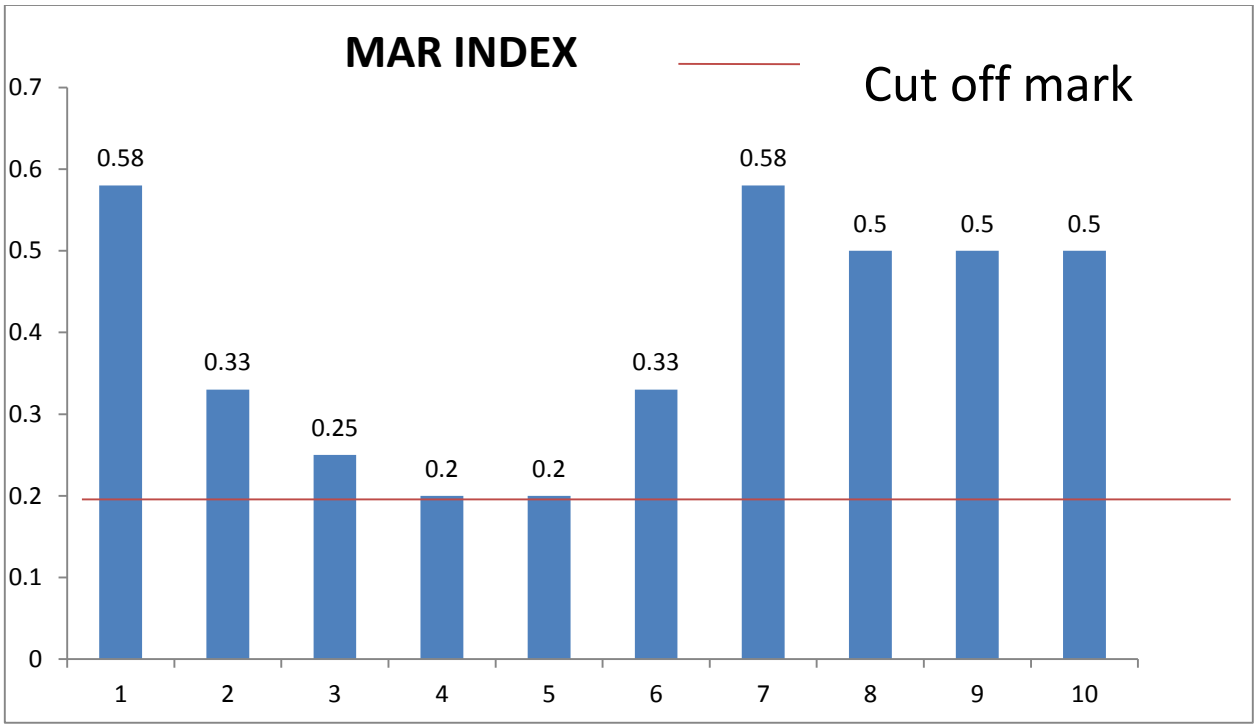

Figure 4.2 MAR Index of *Salmonella* isolates from Zango abattoir Zaria, Nigeria

CHAPTER FIVE

DISCUSSION

A prevalence of 5% of *Salmonella* species from cattle carcases swab was recorded from this study. This recorded prevalence indicates a significant level of contamination of beef in the abattoir. The contamination may occur at the abattoir from the excretion of assymptomatic animals, abattoir floor and poor personnel hygiene (Foley and Lynne, 2008). This high level of contamination could also be associated to the fact that most of the animals slaughtered were brought directly from the market driven on foot and without allowing them to rest in the lairage. This stressful condition might have contributed to the excretion of large numberss of *Salmonella* into the faeces of these animals (Venter *et al.*, 1994).

The finding in this study is similar to the reports of Adesiyun and Oni. (1989), Okoli *et al.* (2006) and Esona *et al*. (2004) who all reported 6% prevalence of *Salmonella* species from Zaria, Abakaliki and Zaria respectively. This may indicate that the ddeterminants of *Salmonella* species in these areas were similar. Lower prevalences of 2.43% and 2.08% were reported by Tafida *et al.* (2013) and Abakpa *et al.* (2011). Higher prevalences of 14.4%, 25.3% and 97.6% were reported by Ejeta *et al.* (2004), Samaxa *et al.* (2012) and Kessel *et al.* (2013) from Ethiopia, Bostwana and United States respectively. The differences in the prevalences could be due to differences in geographical location and determinants.

A combination of different *Salmonella* serovars were identified by the Microbac kit in this study namely; *Salmonella* Arizonae, *Salmonella* Cholerasuis and *Salmonella* species

unclassified. This finding is comparable to that of Tafida *et al.* (2013) and the similarity could be due to the fact that the isolates from this present study and that of Tafida *et al.* (2013) were both from meat samples and meat/ food processing plants could share common contaminants (Declan *et al.*, 2004). The kit was observed to be less laborious, rapid and convenient mehod for the identification of *Salmonella* over the conventional biochemical methods as found by Mugg and Hills. (1989).

Salmonella Arizonae was found to be the most prevalent serovar in this study. The presence of this serovar on cattle carcasses may be due to contamination either from the environment or from cold blooded animals in the premises of the slaughter house (Silham and Taha, 2009). The significance of *Salmonella* Arizonae has not been clarified. However, every serovar must be regarded as potential pathogen for the consumer (Bouvet and Grimont, 1997). Not all the isolates identified by the biochemicak kit as *Salmonella* agglutinated following serological test because the kit was not 100% sensitive.

There are reports of resistance of *Salmonella* isolates to different antimicrobial agents from different countries like Taiwan (Lauderdale, 2006), Netherlands (Duijkeren *et al.*, 2013), France (Weill *et al.*, 2006), Ethiopia (Molla *et al.*, 2003) and in Nigeria (Tafida *et al.*, 2013). The *Salmonella* serovars isolated from this study showed significant susceptibilities to Gentamin (100%), Ciprofloxacine (90%), Amikacin (70%) and Chloramphenicol (60%). They are moderately sensitive to Kenamicin (40%), Nalidixic acid (40%), Nitrofurantoin (40%) and Trimethoprim (40%) and were less sensitive to Strptomycin (20%), Imipenem (20%), Amoxicillin+Clavulanic acid (20%) and Tetracycline (30%). These findings of higher susceptibilities to Gentamicim, Ciprofloxacin, Amikacin and Chloramphenicol is similar to the reports of Tafida *et al.* (2013), Gorbindha *et al.* (2013), Garedew *et at.* (2015)

and Bouchrif *et al.* (2007) in Nigeria, India, Ethiopia and Morocco respectively. The resistance to Imipenen, Tetracycline, Streptomicin and Amoxicillin+ Clavulanic acid were also in line with Sisak *et al.* (2006) and Skov *et al.* (2007) in Czech Republic and Denmark respectively.

Several lines of evidences indicate that antibiotic resistance among humans results from the use of antimicrobial agents in food animals (Vydrin, 2003). The agents are used for 3 main reasons; to treat infections in humans, animals and plants, it is also used prophylactically inhumans, animals and plants and also sub-therapeutically in food animals as growth promoters and feed conversion (Vydrin, 2003). When antibiotic use become the norm of both human and animal medicine, selection pressure increases the bacterial advantage of maintaining and developing new resistant genes that could be shared among the bacterial population (Vydrin, 2003). The bacteria can acquire the resistance either by the production of enzymes that either destroy the antibiotic before it reaches the target site or modify the drug so that it no longer recognize the target. The cell wall of the bacteria may become impermeable to the antimicrobial agents, target site may be altered by mutation so that it no longer binds the antimicrobial agents, bacteria may poses an efflux pump that expels the antimicrobial agents from the cell before it reaches the target or the specific metabolic pathways in the bacteria may be genetically altered so that the antimicrobial agent cannot exert its effect.

This study reports that among the *Salmonella* strains, the most common antibiotic resistance was towards Amoxicilin- Clavulanic acid, Imipenem, Streptomycin and Tetracycline. Based on previous literatures, resistance to more than one class of antibiotic is defined as multidrug resistance (Butaye *et al.*, 2006). Steveson *et al.* (1996-2003)

reported the common resistance pattern towards Nalidixic acid, Amoxicillin, Streptomycin and Tetracycline. These reports are similar to our findings and the findings of Sultan *et al.* (2009). The reasons for the increasing reports on antimicrobial resistance are not readily available but may not be unconnected with the consumption of contaminated food by humans and animals (Benacer *et al.*, 2010). Limiting the use of antibiotics in both humans and animal, carrying out sensitivity tests and considering the dosage and doses of treatment may decrease the spread of resistant strains.

Based on the findings of this study, there is the risk of dessimination of resistant strains of *Salmonella* in the environment in the study area in which infections with such strains may pose a serious threat to publichealth.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusion

This study established a prevalence of 5% of *Salmonella* from slaughtered cattle in Zango abattoirof Zaria, Kaduna State. The contamination could either be as a result of infection from slaughtererd animals or faecal contamination of the carcasses during dressing, theabattoir floor or the materialsused in dressing the carcasses. It is also reported from this study that *Salmonella* arizonae, *Salmonella* specie (unclassified), and *Salmonella* Cholerasuisexhibited multiple drug resistance with 10 different patterns. This also showed that Gentamicin and Ciprofloxacin were the best drugsto be used for the treatment of infection with *Salmonella* since the isolates showed 100 and 90% susceptibilities respectively.

6.2 Recommendations

1. Abattoirs should be properly equipped and the buildings maintained so as to observe better hygienic conditionss.
2. Butchers should be sensitized on the importance of maintainanceof hygiene and of use of protective clothing during handling and processing of meat at the abattoir.
3. Based on the findings of this study, Gentamicin and Ciprofloxacin should be the drugs of choice to be used in the treatment of *Salmonella* infections in animals
4. There is need to develop, validate and verify critical control points and critical limits for food borne pathogens. Control should be through the introduction of

Hazard Analysis and Critical Control Points (HACCP) as policy for food safety in Nigerian abattoirs.

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APPENDICES

Appendix I: Maximum Recovery Diluent Preparation (Peptone Saline Diluent)

Composition in gram/litre

Peptone	1.0
Sodium chloride	8.5

PH 7.0± 0.2 @ 25°C.

Directions

Dissolve 9.5g in a litre of distilled water. Dispense into final containers and sterilize by autoclaving at 121°C for 15minutes.

Appendix II: selenite “f” brothpreparation

Composition in gram / litre

Peptone	5.0
Lactose	4.0
Sodium phosphate	10.0

Directions

Disslve 4g of Sodium biselenite in 1litre of distilled water and then add 19g of selenite broth base. Warm to dissolve, mix well and fill out into containers. Sterilise in a boiling water. DO NOT AUTOCLAVE.

Appendix III: *Salmonella Shigella* Agar Preparation

Composition in gram/litre

Lab Lemco' powder	5.0
Peptone	5.0
Lactose	10.0
Bile salts	5.5
Sodium citrate	10.0
Sodium thiosulphate	8.5
Ferric citrate	1.0
Brilliant green	0.00033
Neutral red	0.025
Agar	12.0

PH 3 ± 0.2 @ 25°c

Directions

Suspend 57g in a litre of distilled water. Bring to boil with frequent agitation and allow to simmer gently to dissolve the agar. DO NOT AUTOCLAVE. Cool to about 50°C, mix well and pour into sterile petri dishes.

Appendix IV: SIM Agar Preparation

Composition in gram/litre

Tryptone	20.0
Peptone	6.1
Ferrous ammonium sulphate	0.2
Sodium thiosulphate	0.2
Agar	3.5

P^H 7.3 ± 0.2 @ 25°C

Directions

Suspend 3.6g in 120ml of distilled water and boil to dissolve the medium completely. Dispense 5ml each into final containers and sterilize by autoclaving for 15minutes at 121°C.

Appendix V: Nutrient Agar Preparation

Composition in gram/litre

Lab Lemco' powder	1.0
Yeast extract	2.0
Peptone	5.0
Sodium chloride	5.0
Agar	15.0

Directions

Suspend 28g in a litre of distilled water. Bring to boil to dissolve completely. Cool to 50°C.

Mix well and pour into sterile petri dishes.

Appendix VI: TSI Agar Preparation

Composition in gram/litre

Lab Lemco' powder	3.0
Yeast extract	3.0
Peptone	20.0
Sodium chloride	5.0
Lactose	10.0
Sucrose	10.0
Glucose	1.0
Ferric citrate	0.3
Sodium thiosulphae	0.3
Phenol red	0.024
Agar	12.0

P^H 7.4 ± 0.2 @ 25°C.

Directions

Suspend 7.8g in 120ml of distilled water. Bring to boil to dissolve completely. Mix well and distribute. Sterilise by autoclaving at 120°C for 15minutes. Distribute 7ml into each test tube and allow to set in sloped form.

Appendix VII: Methyl Red – Voges Proskauer Preparation

Composition in gram/litre

Suspend 2.4g in 120ml of distilled water. Mix well and distribute 10mls into final containers, then sterilize by autoclaving at 121°C for 15minutes. Inoculate and incubate at 35°C for 18-24h. Divide it into two portions.

To the first portion, add 5drops of 0.4%w/v methyl red solution and the color on the surface of the medium is read immediately.

To the second portion, 3ml of 5% w/v a-naphthol solution and 3ml of 40% w/v KOH solution and observe after shaking,

Appendix VIII: Urea Preparation

Composition in gram/litre

Peptone	1.0
Glucose	1.0
Sodium chloride	5.0
Disodium phosphate	1.2
Potassium dihydrogen phosphate	0.8
Phenol red	0.012
Agar	15.0

P^H 6.8 ± 0.2 @ 25°C

Directions

Suspend 3.02g in 120 ml of distilled water. Bring to boil to dissolve completely. Sterilize by autoclaving at 115°C for 20minutes. Cool to 50°C and aseptically introduce 5ml of sterile 40% urea solution. Mix well, distribute 10ml amount into sterile containers and allow to settle in a slope position.

Appendix IX: Citrate AgarPreparation

Composition in gram/litre

Magnesium sulphate	0.2
Ammonium dihydrogen phosphate	0.2
Sodium ammonium phosphate	0.8
Sodium citrate, tribasic	2.0
Sodium chloride	5.0
Bromothymol blue	0.08
Agar	15.0

P^H 7.0 ± 0.2 @ 25°C

Directions

Suspend 3.02g in 120ml of distilled water, bring bto boil to dissolve completely. Sterilise by autoclaving at 121°C for 15minutes.

Appendix X: Microbact Identification System Results

S/No	Sample No	Octal code	Percentage	Org. I dentified
1	Z6	3300	94.46	S CHOLERAESUIS
2	Z14	4662	67.35	E COLI
3	Z16	3742	94.82	S CHOLERAESUIS
4	Z19	7732	99.79	S SPP
5	Z30	3342	94.82	S ARIZONAE
6	Z23	3142	92.68	S ARIZONAE
7	Z32	6752	44.75	S LIQUEFACIENS
8	Z35	2002	49.62	A IWOFFII
9	Z36	7552	83.77	S ARIZONAE
10	Z39	3142	92.68	S ARIZONAE
11	Z43	6752	44.75	S LIQUEFACIENS
12	Z44	7463	46.27	E TARDA
13	Z48	3102	42.85	S CHOLERAESUIS
14	Z52	7752	54.19	S ARIZONAE
15	Z54	700	88.67	K RHINOSCLEROMATIS
16	Z55	3340	31.16	S ARIZONAE
17	Z61	6752	47.75	S LIQUEFACIENS
18	Z62	7752	84.99	S ARIZONAE
19	Z63	6572	81.82	C DIVERSUS
20	Z65	7742	98.29	S ARIZONAE
21	Z66	5552	46.73	K PNEUMONIAE
22	Z72	2772	99.96	C DIVERSUS
23	Z76	3102	42.85	S CHOLERAESUIS
24	Z82	2342	38.75	K OZAENAE
24	Z91	7572	34.26	E COLI
26	Z102	7753	84.99	S ARIZONAE
27	Z115	6742	54.6	S LIQUEFACIENS
28	Z118	4510	65.38	E AGGLOMERANS
29	Z126	3340	31.16	S CHOLERAESUIS
30	Z131	6100	56.56	S CHOLERAESUIS
31	Z132	102	73.62	A BAUMANNI
32	Z139	4352	65.38	K PNEUMONIAE
33	Z141	7741	87.15	S ARIZONAE
34	Z146	6460	52.53	E COLI
35	Z147	6552	39.04	E GERGOVIAE
36	Z148	7762	97.78	S ARIZONAE
37	Z149	3542	92.68	S ARIZONAE
38	Z153	3342	94.82	S ARIZONAE

39	Z164	6340	80.01	H ALVEI
40	Z168	3742	94.82	S ARIZONAE
41	Z169	5340	40.47	E VULNERIS
42	Z171	4752	65.38	K PNEUMONIAE
43	Z173	6742	54.6	S LIQUEFACIENS
44	Z174	3302	73.05	S SPP
45	Z175	3742	94.82	S ARIZONAE
46	Z177	7002	41.23	E TARDA
47	Z179	7743	98.29	S ARIZONAE
48	Z181	5152	46.73	K PNEUMONIAE

Appendix XI: Susceptibility of Isolates to Commonly Used Antimicrobial Agents

S/No	Sample No.	NA	AK	K	CN	F	S	CIP	IPM	C	W	TE	AMC
1	Z6	0	21	10	27	0	17	29	21	0	0	11	0
2	Z16	17	22	20	17	15	10	29	12	22	0	10	0
3	Z19	0	20	19	22	20	10	29	12	22	0	10	0
4	Z23	28	28	28	27	27	0	25	30	29	11	0	0
5	Z39	20	22	15	21	22	10	22	30	17	8	17	0
6	Z65	0	15	15	20	15	13	20	17	20	18	0	0
7	Z102	8	21	0	23	0	15	23	26	0	16	0	0
8	Z126	25	20	18	20	20	0	36	29	32	25	29	37
9	Z175	7	14	7	17	10	10	30	7	20	15	0	20
10	Z179	22	15	18	20	15	10	35	22	22	20	15	0

Appendix XII: Interpretation of Susceptibility Test Results

S/No	Sample No.	NA	AK	K	CN	F	S	CIP	IPM	C	W	TE	AMC
1	Z6	R	S	R	S	R	S	S	I	R	R	R	R
2	Z16	S	S	S	S	S	R	S	S	S	R	R	R
3	Z19	S	S	I	S	S	R	S	I	I	S	R	R
4	Z23	S	I	S	S	I	R	S	I	S	S	S	R
5	Z39	S	S	S	S	S	R	S	R	S	S	S	S
6	Z65	R	I	I	S	I	I	I	R	S	S	R	R
7	Z102	R	R	R	S	R	R	S	R	S	I	R	S
8	Z126	I	S	S	S	R	R	S	I	R	R	R	R
9	Z175	R	S	S	S	S	R	S	R	S	R	R	R
10	Z179	R	S	R	S	R	S	S	S	R	R	S	R

Appendix XIII: Performance Standards for Antimicrobial Susceptibility Testing for *Salmonella* species

	Antimicrobial Agent	Disc Code	Potency	Resistant	Intermediate	Susceptible
1	Amikacin	A	30ug	≤14	15-16	≥17
2	Amoxicillin-clavulanic acid	AMC	30 µg	≤13	14-17	≥18
3	Chloranphenicol	C	30 µg	≤12	13-17	≥18
4	Ciprofloxacin	CIP	5 µg	≤15	16-20	≥21
5	Gentamicin	CN	10 µg	≤12	13-14	≥15
6	Imipenem		10ug	≤19	20-22	≥23
7	Kanamycin	K	30 µg	≤13	14-17	≥18
8	Nalodixic Acid	NA	30µg	≤13	14-18	≥19
9	Streptomycine	S	10µg	≤11	12-14	≥15
10	Tetracycline	TE	30 µg	≤11	12-14	≥15
11	Trimethoprim	W	5µg	≤10	11-15	≥16
12	Nitrofurantoin	F	300µg	≤14	15-16	≥17

Appendix XIV: MAR index result

S/No	No Of Antibiotics	No Resistant	MAR Index
1	12	**7**	**0.58**
2	12	**4**	**0.33**
3	12	**3**	**0.25**
4	12	**2**	**0.2**
5	12	**2**	**0.2**
6	12	**4**	**0.33**
7	12	**7**	**0.58**
8	12	**6**	**0.5**
9	12	**6**	**0.5**
10	12	**6**	**0.5**

Appendix XV: **Percentages of *Salmonella* species following Agglutination test**

Isolates	**No of isolates**	**Percentages (%)**
S. Cholerasuis	8	80
S. arizonae	1	10
Unclassified *Salmonella* species	1	10

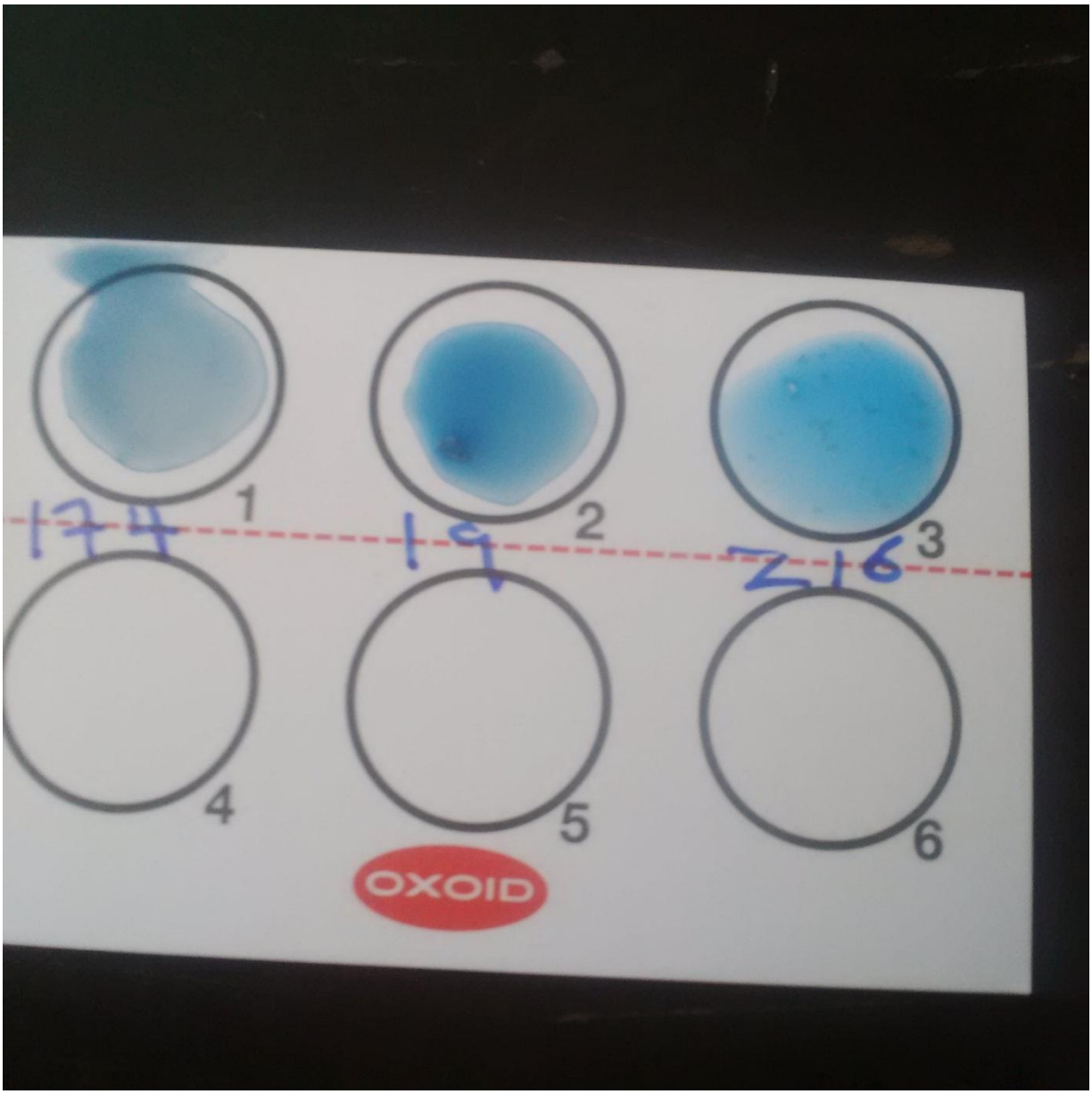

Plate I: Rapid Latex Agglutination test result of the *Salmonella* isolates

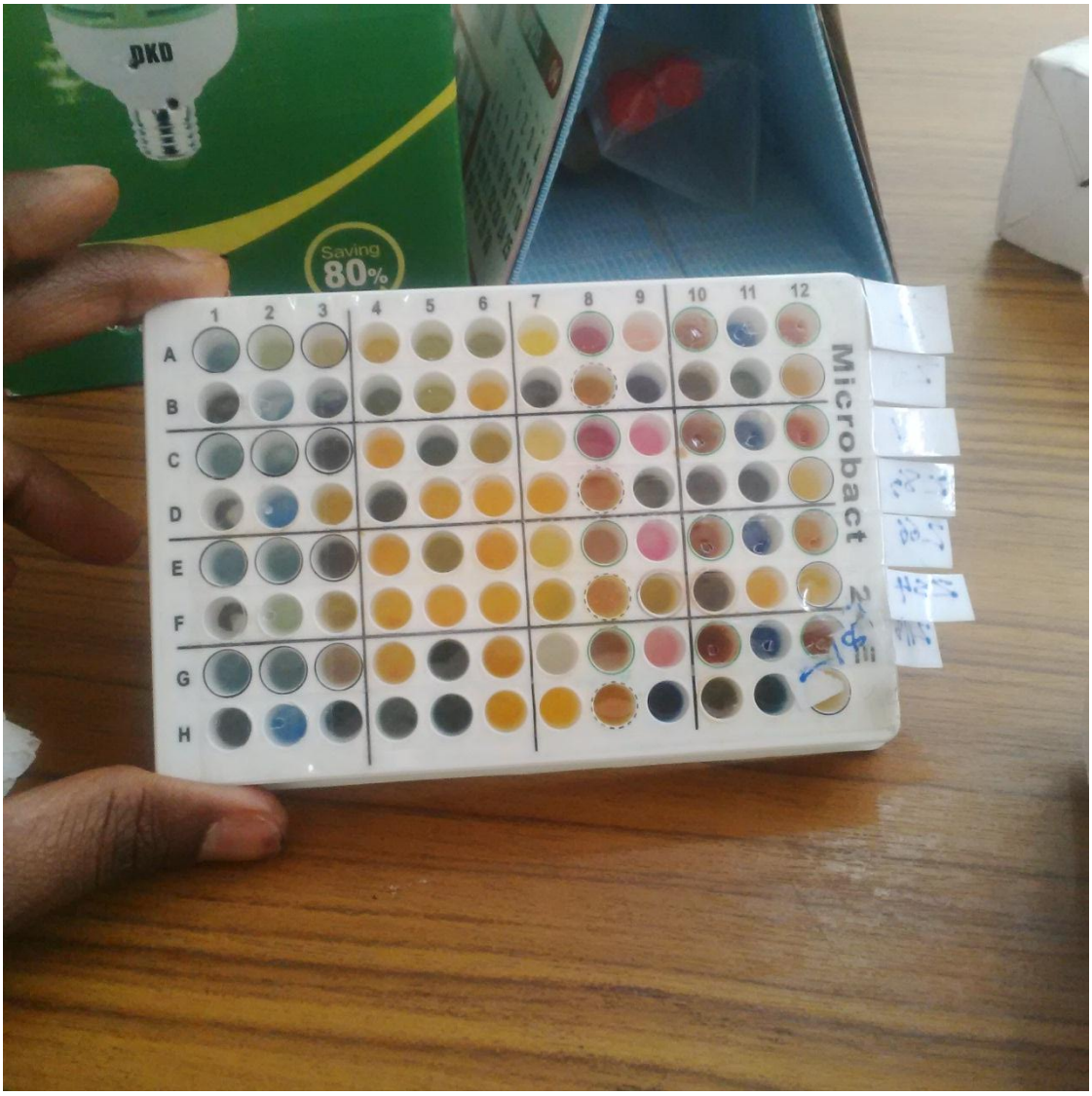

Plate II: Microbact test plate for the presumptive *Salmonella* isolates.

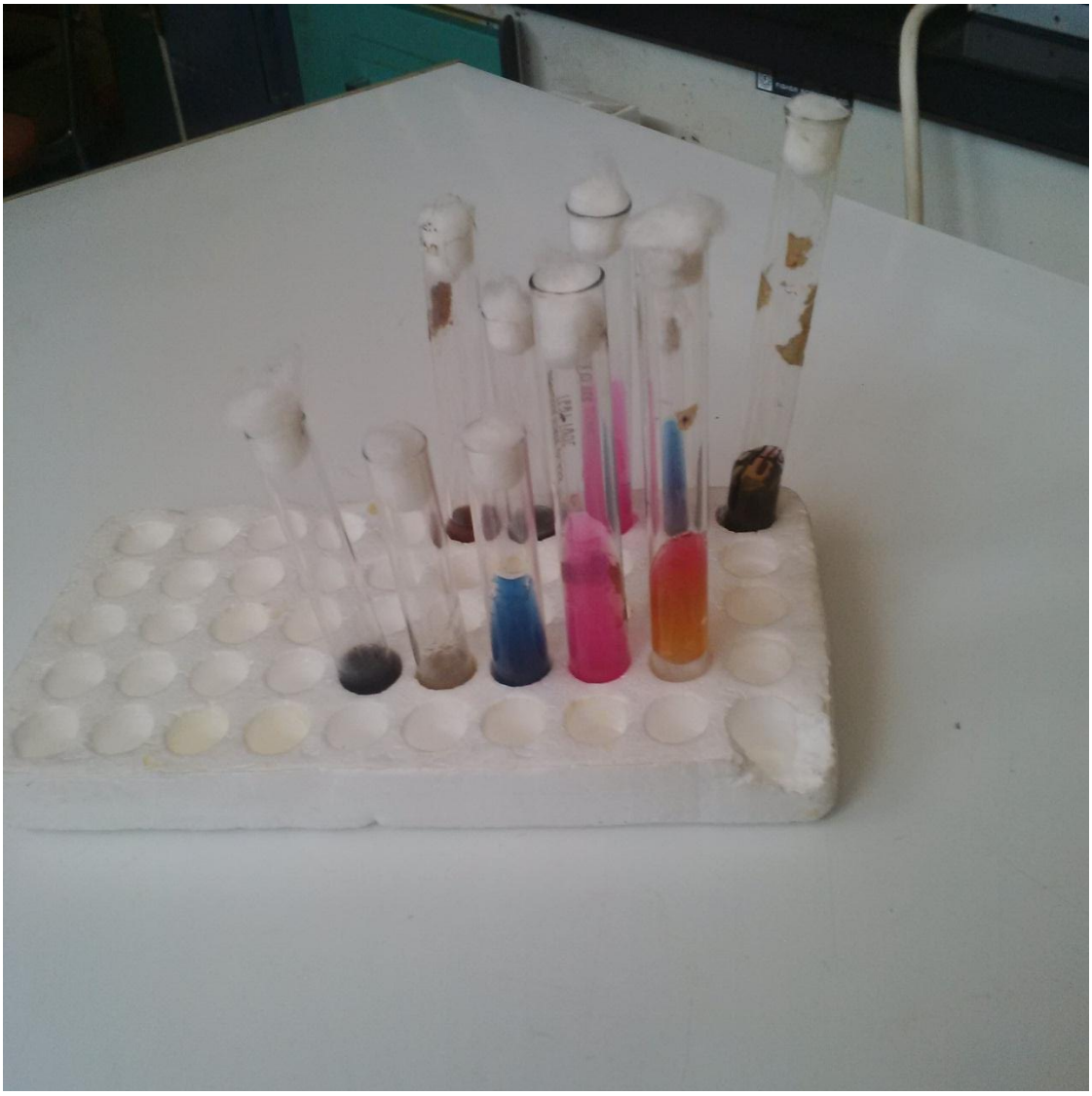

Plate III: Conventional Biochemical tests

Plate IV: Recommended sampling sites for cattle carcasses.

Source: Teagasc, 2008