ISOLATION AND PHENOTYPTIC CHARACTERISATION OF E. COLI O157 AND SALMONELLA SPP IN A SLAUGHTER HOUSE AND MEAT SALE TABLES AT NSUKKA MARKET, ENUGU STATE.

BY

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CERTIFICATION

This is to certify that Onwumere, Onyinye Stella, (PG/M.Sc/07/73955) has satisfactorily completed the requirements for the course and research work for the degree of Master of Science (M.Sc) in Veterinary Public Health and Preventive Medicine.

The work embodied in this dissertation is original and has not been submitted in part or full for any other Diploma or Degree of this or any other university

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DEDICATION

This work is dedicated to God Almighty, the Alpha and Omega, the Protector of my life, the reason for my existence. To my late Daddy, untied inspirational Mummy and comrd K.O Idolor for their love.

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Abstract:

Escherichia coli O157 and *Salmonella* spp. are major food borne pathogens and the emergence of these pathogens has been reported in many countries. The objectives of this study were to examine the meat contact surfaces in Nsukka slaughterhouse and meat sales environment for *E. coli* O157 and *Salmonella* species, determine the enterotoxigenic potential and antibacterial resistance profile of the isolated bacteria. For this study, a total of 448 swab samples randomly collected from slaughter slab (112), slaughter floor (112), meat holding drums (112) and meat sales tables (112) were pre-enriched in nutrient broth at 37^{o}C for 24hours. For *E. coli* O157 isolation, a loopful of each pre-enrichment broth was streaked on Cefixime Tellurite Sorbitol MaConkey (CT-SMAC) agar (Oxoid®). After 24 hours of incubation, colourless colonies were subcultured on plain MacConkey, incubated at 37^{o}C for 24 hours and thereafter a rose pink colony was subcultured on Eosin Methylene Blue (EMB) agar and observed for colonies with greenish metallic sheen appearance. *E.coli* O157 was further confirmed using Oxoid O157 latex agglutination kit. For *Salmonella* isolation, a loopful of the pre-enrichment broth was inoculated into Selenith F broth and incubated at 42^{o}C for 24hours after which loopful of the broth was streaked on Brilliance Salmonella agar (Oxoid®). After incubation at 37^{o}C for 18-24hrs, purple colonies (presumptive *Salmonella* organism) were further subjected to biochemical tests for confirmation. The saccharytic activity of the organisms were verified using 13 sugars – sorbitol, glucose, cellobiose, sucrose, saccharose, arabinose, maltose mannitol, mannose, dulcitol, xylose, Inositol and lactose. The ability to produce heat-labile and heat-stable enterotoxin was determined using infant mouse assay. Antibiogram of the test isolates was determined using disc diffusion technique with the following 11 antimicrobial agents - chloramphenicol, ciprofloxacin, ceftazidime, gentamicin, cefotaxime, ampicillin, aztreonam, ceftriaxone, sulphamethoxazole/trimethoprim, enrofloxacine and streptomycin. The results of this study have shown that *Escherichia coli* O157 was isolated from 3(0.68%) of the 448 samples analysed while *Salmonella* species were recovered from 10(8.93%) of the samples. The *E.coli* O157 strains were isolated from the slaughter floor 1(0.9), slaughter slabs 1(0.9), and sales tables 1(0.9). Four (3.57%) of the salmonellae were obtained from meat holding drums, and 4 (3.57%) from sales tables while 2 (1.79%) were isolated from slaughter floor. All the *E.coli* O157 isolated fermented glucose, sucrose, lactose, mannitol, dulcitol, saccharose, arabinose, maltose, cellobiose, xylose and mannose and none fermented inositol and sorbitol. All the *Salmonella* isolated fermented glucose, mannitol, dulcitol, saccharose, cellobiose and mannose, while 80% fermented xylose and none fermented sucrose, inositol, lactose, arabinose and maltose. The three *E.coli* O157 and

Salmonella isolates produced heat-labile enterotoxins as evidenced by death of all infected mice none of the *E.coli* O157 and *Salmonella* isolates produced heat-stable enterotoxins since the gut to carcass ratio produced ranged from 0.043-0.062. The three *E.coli* O157 isolates were sensitive to ceftriaxone, enrofloxacin, streptomycin, cefotaxime and gentamicin, but resistant to ampicillin and ceftazidime. All the *Salmonella* isolates were sensitive to enrofloxacin but resistant to ampicillin and ceftazidime. This study has shown that *E.coli* O157 and *Salmonella* are present on meat contact surfaces in Nsukka Slaughterhouse. This suggests that meat from these surfaces is likely to be contaminated by these organisms and may therefore predispose the consumer to bacterial food poisoning. The multiple antibiotic resistances displayed by zoonotic bacteria is of serious public health significance because infections in humans caused by these bacteria will be difficult to treat with the commonly available antibiotics.

CHAPTER ONE

INTRODUCTION

1.1 Background of Study

Abattoir is a special facility designed and licensed for receiving, holding, slaughtering and inspecting meat animals and meat products before release to the public (Alonge, 2005). Abattoir environment and slaughtering process play a very important role in the safety and wholesomeness of meat. Meat contamination by pathogens such as *Listeria monocytogenes, Salmonella spp*. and enterohaemorrhagic *E. coli* (Oboegbulem and Muogbo, 1981; Tabukun *et al.,* 1996, Tompkin, 2004; Abiade-Paul *et al.,* 2006) and chemicals in abattoir can persist in meat processing environment, contaminate meat as they are being processed and lead to food borne illnesses and intoxications (Okolocha *et al.,* 2002). Thus, meat borne diseases and intoxications are major public health concerns. The types and incidence of meat related illnesses are becoming increasingly widespread and consumers becoming more cognizant now than ever of the dangers associated with raw meat contamination.

*Escherichia coli O*157 and *Salmonella species* are two most important food borne bacterial pathogens, about 95% of the emerging diseases that have affected humans in the past years are caused by pathogens originating from these organisms (WHO, 2007). Diseases caused by these organisms range from bloody diarrhea, enteritis, hemorrhagic colitis (HC), hemolytic uremic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP) (Griffin and Tauxe, 1991; Nataro and Kaper, 1998). The growth of these organisms in the human intestine is known to produce large quantity of toxins, which can cause severe damage to the lining of the intestine and other organs of the body (Nataro and Kaper, 1998). The potentially high mortality associated with *E.coli* O157 and *Salmonella* infections, makes their presence in any food material worrisome and of serious public health concern as most of the outbreaks recorded has been traced to consumption of beef and food products contaminated with these organisms (Hussein, 2007., Mead *et al.,* 1999).

Domestic animals like cattle, sheep and goats are reported as sources and reservoirs of these organisms (Beutin *et a*l., 1993), these animals are being slaughtered and the meat sold in the slaughterhouse, probably contaminates the environment, and act as a vehicle for transmission of *E.coli O157 and salmonella species* to the public *(Doyle and Cliver 1990; Griffin and* Tauxe 1991*).* These pose serious public and environmental health risk.

Meat contact surfaces in the abattoir such as equipment (e.g. meat holding drums, knives, axes), walls and floors may serve as sources of infectious agents and are therefore considered important factors in the evaluation of the level of contamination in such establishment (Okolocha *et al.,* 2002., Bello and Son., 2009). Contamination occurs during slaughtering, handling, cutting, processing and storage (Madder, 1994). *Salmonella* species are primarily located in the gastro-intestinal tract of the sub-clinically infected animals and the epidemiology of *Salmonella* at the

slaughter house level is first of all a question of direct and indirect fecal contamination of carcasses (Oosteron, *et al.,* 1985). Carcasses may be contaminated or cross-contaminated by manual or mechanical handling. During slaughtering, transfer of microorganisms continues from carcasses to hands of workers and equipment surface and from them to other carcasses.

Although slaughter equipment is often the immediate source of contamination, the carrier animals play an important role in the dissemination of *Salmonella* and *E. coli O*157. As a result of the technical difficulties in detecting carrier animals before slaughtering or during meat inspection, carrier animals are continually serve as sources of contamination in slaughter houses and ultimately in food products (Haddock, 1970). Slaughter of cattle under poor hygienic conditions leads to the contamination of carcasses by *Salmonella* sp and *E. coli* O157 thus facilitating their transmission and increasing the risk of food borne diseases in humans (Mafu, *et al.,* 1989).

1.2 Statement of Problem

Escherichia coli O157 and *Salmonella* species are among the major food borne pathogens causing high mortality in animals and man (WHO, 2007; Bello and Son, 2009). Reports have shown that the intestinal tracts of humans and animals are major sources of these organisms in nature and approx 1 to 3% of all domestic animals are infected with Salmonella and these can be shed in their feaces (Litchfield, 1980).

Abattoir is a major link in the transmission of *E. coli* O157 and *Salmonella* spp to the food chain (Richard *et al.,* 1998); reports have shown that abattoir waste with all its contaminants are found littered on the slaughter floors and environment (Nwanta and Achi, 2002; Adeyemo 2002; Abiade-Paul, *et al.,* 2006). In many abattoirs, in Nigeria, meat is often

dipped into water in drums instead of a chilling room and this bad practice contaminates the meat (Ezenduka *et al*., 2010). Increasing numbers of human *E.coli* O157:H7 induced illnesses have been related to contact with animals or to water supply contaminated with runoff water from cattle farms or abattoir/slaughter houses (Adetosoye *et al.,* 1976., Adesiyun *et al.,* 1983., Oboegbulem and Muogbo 1981, Abiade-Paul *et al.,* 2006; Tabukun. 1996, Nwanta *et al*., 2010).

Studies have been carried out on the prevalence of *E.coli* O157 and S*almonella* species in Nsukka slaughter house (Abiade-Paul *et al.,* 2006; Oboegbulem *et al.,* 1981; Nwanta, *et al.,* 2010). Inspite of the wide knowledge of these organisms, data concerning isolation and characterization of these microorganisms from meat contact surfaces in Nsukka slaughter house and sales environment is lacking, it is therefore necessary that a study be conducted in Nsukka slaughter house to ascertain the presence of these organisms in Nsukka slaughter house meat contact surfaces and sales tables.

Antibiotic resistance is also a growing public health problem and has been attributed to overuse of antibiotics in humans, and its use as growth promoters in food animals (Johnson *et al*., 2006). Antibiotic resistant *E.coli* may also pass on the genes responsible for antibiotic resistance to other species of bacteria, such as *Staphylococcus aureus. E. coli* O157 often carries multidrug resistant plasmids and under stress readily transfers these plasmids to other species (Salyers *et al.,* 2004). Indeed, *E. coli* O157 is a frequent member of biofilms where many species of bacteria exist in close proximity to each other. This mixing of species allows *E. coli* strains that are piliated to accept and transfer plasmids from and to other bacteria. Thus *E. coli* and other enterobacteria are important reservoirs of transferable antibiotic resistant (Salyers *et al*., 2004; Enumeration of *Escherichia coli* and the Coliform Bacteria, 2002).

The wide spread occurrence of drug resistance *E.coli* and other pathogens in our environment has necessitate the need for regular monitoring of antibiotics susceptibility trends to provide the basis for developing rational prescription programs, making policy decisions and assessing the effectiveness of both (Omigie *et al*., 2006).

1.3 Objectives of the Study

The overall objective of the study is to examine the meat contact surfaces in Nsukka slaughterhouse and meat sales environment for *E. coli* O157 and Salmonell*a* species.

Specifically the study seeks to:

1. Determine the prevalence of *E. coli* O157 and *Salmonella* species on slaughter floor, slabs, sales tables and water in meat holding drums in Nsukka slaughter house.
2. Determine the saccharolytic activity of the *E.coli* O157 and *Salmonella* isolates.
3. Evaluate the E.coli O157 and *Salmonella* isolates for enterotoxin production.
4. Determine the antibiotic sensitivity profile of the E.coli O157 and *Salmonella* isolates.

1.4 SIGNIFICANCE OF THE STUDY

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The study will provide baseline data on the occurrence of *E. coli* O157 and *Salmonella* species on meat contact surfaces which are essential for the establishment of a Hazard Analysis Critical Control Point (HACCP) plan in any meat processing chain.

It will provide the information on the deleterious effect of location of abattoirs and discharge of its effluents near markets and residential areas.

Rational antimicrobial therapy requires the determination of the antimicrobial resistance or susceptibility pattern of any clinical isolates prior to drug administration. Unfortunately, in Nigeria, there are not enough veterinary diagnostic laboratories to achieve this purpose. The results of this study will also provide information on the antibiotic resistance profile and enterotoxin production profiles of the zoonotic bacterial isolates to the relevant public health authorities, abattior workers, livestock farmers, veterinary authorities and the Ministry of Health for the prevention, control and management of health problems.

CHAPTER TWO

LITERATURE REVIEW

2.1 Abattoir

2.1.1 Definition

An abattoir is defined as a premise approved and registered by the controlling authority for hygienic slaughtering and inspection of animals, processing and effective preservation and storage of meat products for human consumption (Alonge, 1991). Abattoirs act as the starting points of the meat industry, where stocks from farms and markets meant for slaughter enter the food chain (Louis Corinth, 1893).

2.1.2 Historical Background

Abattoirs have existed as long as settlements have been big enough for people to slaughter their own stock for personal consumption and sales. Public abattoir had been traced to Roman and in France by 15th and 16th centuries, public slaughter houses were among the public facilities (David-West, 2002).

In Italy, a law of 1890 required that public abattoir be provided in all communties of more than six thousand inhabitants. Similar enactments were reported in Norway, Sweden, Danmark, Netherlands and Rumania (Loverdo *et al.,* 1906). In Nigeria, nearly every town and neighborhood is provided with slaughter house or slaughter slab (David-West, 2002, Bello and Oyedemi, 2009). Edwards *et al*., (1979) suggested that abattoir should be situated in urban, rural, and nominated industrial sites well away from town boundries. He further recommended that abattoir should be built on firm gently sloping land away from other buildings, residential areas and factories.

Early maps of London show numerous stockyards in the periphery of the city, where slaughter occurred in the open air (Ebisike, 2009). In the 19^{th} and 20^{th} century, slaughterhouses were increasingly sited away from the public view in the developed countries. In Nigerian, the situation is far from this as abattoirs are sited right in the center of residential areas, market places and close to the high ways.

2.1.3 Abattoir Design/Facilities

The layout and design of most U.S slaughterhouses has been significantly influenced by the work of Grandin, (2000), whose objectives were to reduce stress, suffering of animals, contamination of environment, slaughter house, and meat. In his design each animal awaiting slaughter was prevented from seeing other animal being slaughtered. The dressing operation is recommended to flow from dirty end to a clean end, to reduce meat contamination (Grandin, 2000). Abattior facilities should be constructed so that the clean and unclean processes and products do not mix. The floor must be hard, smoot and impervious, sloping sufficiently towards a drain thus allowing cleaning with water. The walls should be made of materials that will allow

cleaning and strong. In Nigeria it is not so abattior are design and sited right inside market which is an unhealthy suitation, effluent channel towards market place, streets and farmland. There is also scarcity of water which has compelled butchers to use water from gutters and nearby streams contaminated by effluents for washing carcasses and slaughter floor. This development has always facilitated the contamination of meat, contact surfaces in the abattoir tables, utensils, equipment, walls and floors, being washed with this contaminated water hence posing a serious threat to human health.

2.1.4 Abattoir Operation and Management in Nigeria

Management and operation of abattoir, slaughter houses and waste in Nigeria have always been regarded as social services by the three-tiers of government – Local, State and Federal (Nwanta *et al.,* 2010). Each of these government authorities has for many years neglected its function and has been apathetic about taking over the responsibilities of abattoir. Some states have even leased out their abattoirs, slaughter houses and slaughtering of animal to individuals and private organizations with little or no knowledge of abattoir management. The 1999 constitution of the Federal Republic of Nigeria places the responsibility of establishment, maintainance and regulation of slaughter houses under local government councils. The local government on their own collects revenue without ploughing back the money to improve the environment. This development has resulted in deterioration of abattoirs and slaughter houses, improper meat inspection, poor environmental hygiene and waste management. The slaughtering facilities where available are dilapidated as a result of disuse and lack of maintainance. The slaughtering and handling of meat became generally poor as these abattoirs and slaughter houses operate in substandard and unsanitary conditions (Adeyemo, 2002; Cadmus *et al.,* 2008).

2.1.5 Source of Meat Contamination in abattoir

Abattoir environment and slaughtering process play very important roles in the safety and wholesomeness of meat (Bello and Son, 2009; Nwanta *et al.,* 2010). The environment in which meat is exposed determines to some extent safety of the meat and invariable, the health of the consumers (Okolocha, 2002; Bello and Son, 2009; Nwanta *et al.,* 2010). The inner tissue of healthy bovine carcasses is known to contain few or no micro organism (Nortj *et al*., 1990; McEvoy *et al.,* 2000). However, when animals are slaughtered, bacteria from their guts and processing environment may contaminate the meat (Shale, 2006). Schlegelov, *et al.,* (2004) reported that during meat processing, horizontal contamination may occur when the product is exposed to meat contact surfaces contaminated by bacteria. Schlegelov, *et al.,* (2004) also found that 92 – 100% of minced beef was cross-contaminated with Staphylococcus aureus in abattoir environment. When microbial contamination of meat from abattoir environment is high, the health of consumer is certainly threatened and such threats range from mild discomfort to serious and sometimes life threatening conditions (Doyle and Clive, 1990; Okolocha, *et al*., 2002). Pathogens such as *Salmonella* species, *Listeria monocytogenes* and *Enterohaemorrharic E. coli that* have been isolated in slaughter house equipment, environment, workers, solid waste and effluents are shown to persist in meat processing environment, contaminate meat as they are being processed and could lead to food borne illnesses and intoxication (Oboegbulem and Muogbo, 1981; Tabukun*.,* 1996; Okolocha *et al.,* 2002; Tompin, 2004; Abiade-Paul *et al.,* 2006, Bello and Son, 2009; Adetosoye *et al.,* 1976; Litchfield, 1980; Elder *et al.,* 2000; Callaway *et al.,* 2004; Akkaya *et al.,* 2008).

Meat contact surfaces in abattoir and slaughter houses such as table, utensils, instruments, equipment, walls and floors serve as sources of contamination of meat with infectious agents and

are therefore considered important factors in the evaluation of level of contamination in such establishment (Okolocha *et al.,* 2002, Bello and Son, 2009). Slaughtering and processing of animals in Nigerian abattoir and slaughter houses are done on the bare floor where evisceration and processing of guts and tripes are also carried out (Okoli *et al.,* 2006). Slaughter floors and environment are heavily contaminated by workers' dirty boots, animal feaces and urine (Gracey 1986). In Nsukka slaughter house, slaughtering, skinning, evisceration and cutting of carcasses into large chunks of meat are done on bare floors which are in most cases not washed or even disinfected (Nwanta, *et al.,* 2010). These chunks of meat are further dipped in meat holding drums that contain dirty water and micro-organisms (Ezenduka *et al.,* 2010). In some instances, the chunks of meat are loaded into open dirty vans or wheel barrows to market for sale. In the market, the meat is displayed on dirty tables these unhygienic practices expose the meat to contaminants. To compound this problem, is the practice where the sale of meat continues to evening and occasionally till the following day without chilling.

According to Bauman, (1990) hazard analysis critical control point (HACCP) is described as a practice of control particularly with regard to microbiological hazards. This involves careful evaluation or analysis of ingredients, products and processes so as to determine those areas or components that must be maintained under very strict control to ensure that the final product is safe to the consumer. It consists of the identification of hazards associated with any storage of food, processing or preparation, the assessing of related risk and the determination of the operation where control procedures will be effective. WHO (1982) and Bryan (1990) identified critical control point as a point by which prevention and control measure can be carried out to eliminate, prevent or minimize hazard. In Abattoir, there are critical control points which during meat slaughtering and processing if strictly control for microbial contamination

will help to reduce meat contamination. According to Umoh, 2002 there are different areas identified as points for prevention, reduction and control of meat contamination (Fig 1). These areas were identified by the asterisk (×)

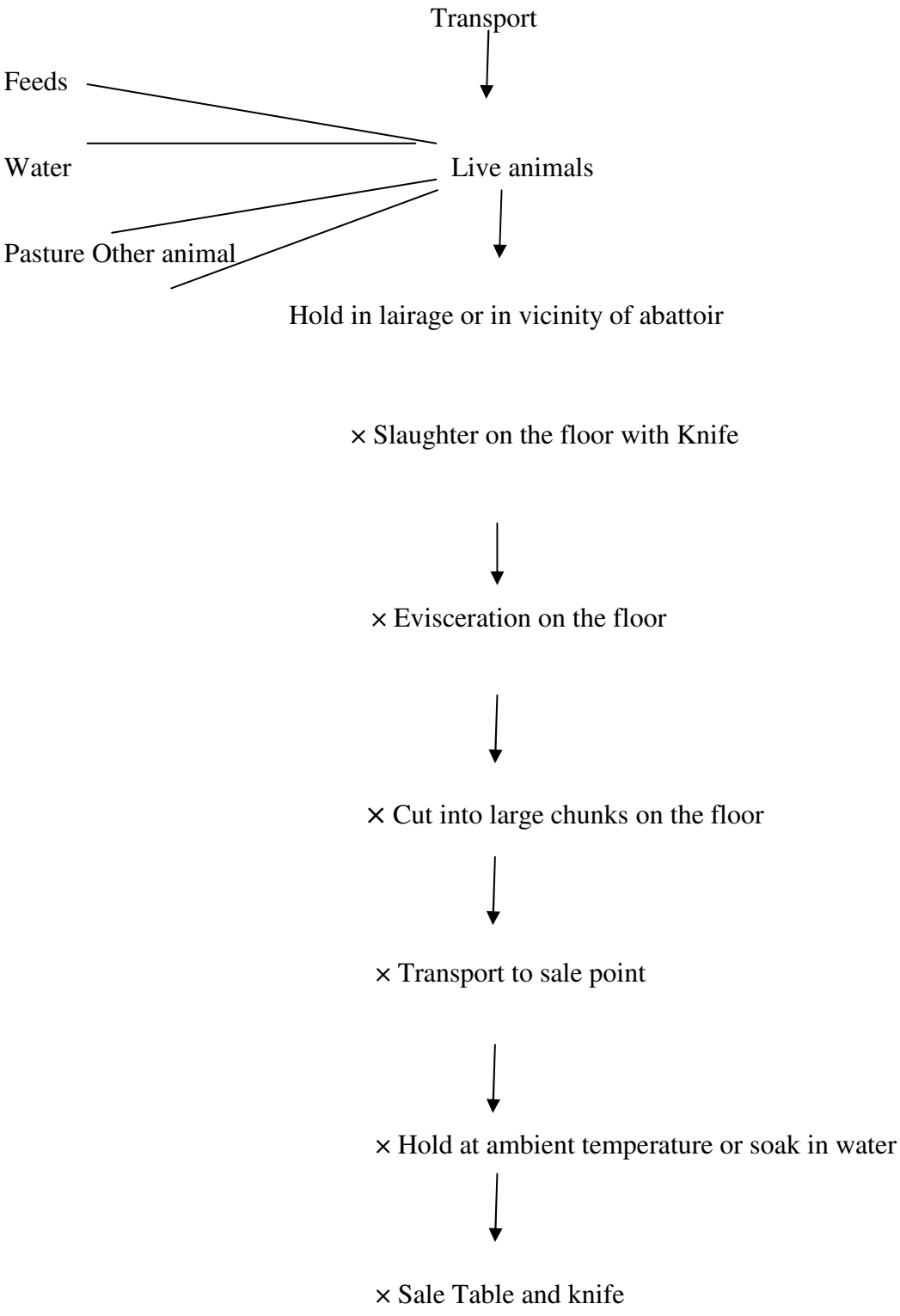

Fig. 1: Beef Processing and Possible Points of Contamination in Abattoir (Umoh, 2002)

2.2 Escherichia coli

2.2.1 Microbiology of Escherichia coli

E. coli is commonly found in the lower intestine of warm-blooded animals. It belongs to a family of bacteria known as enterobacteriacae or enteric bacteria or coliform bacteria that have as their natural habitat lower intestinal tract of animals and man (Nester *et al.,* 2004). *E. coli,* a normal flora of the intestinal tract has been associated with a variety of pathological condition in man, farm animals and poultry (Wray and Morries, 1985; Nataro and Kaper, 1998) *E. coli* that causes diarrhea are extremely common worldwide.

2.2.2 Classification of E.coli strains causing enteric infection

E. coli organisms are classified by the characteristics of their virulence (Todar, 2007) and each group causes disease by a different mechanism. Pathogenic E.coli strains can be classified into the following:

2.2.2.1 Enterotoxigenic *E. coli* (ETEC) – uses fimbriae as adhesin to bind to enterocyte cells in small intestine. It produces two proteinaceous enterotoxins: the larger of the two proteins, heat-labile (LT) enterotoxin, which is similar to cholera toxin in structure and function. The smaller protein, heat-stable (ST) enterotoxin, secretes fluid and electrolytes into the intestinal lumen.

2.2.2.2 Enteropathogenic *E. coli* (EPEC) – it lacks fimbriae, heat stable toxin (ST) and heat labile (LT) toxins, but they utilize an adhesion known as intimin to bind to host intestinal cells.

2.2.2.3 Enteroinvasive *E. coli* (EIEC) – causes a syndrome that is identical to shigellosis.

2.2.2.4 ,Enteroaggregative *E. coli* (EAEC) – have fimbriae which aggregate tissue culture cells. It binds to the intestinal mucosa and is non-invasive.

2.2.2.5 Enterohaemorrhagic *E. coli* (EHEC) - it is the most famous members of this serotype. It uses bacterial fimbriae for attachment (Rendon, 2007). It's name is based on it's capacity to cause haemorrhagic colitis and haemolytic ureamic syndrome and sudden kidney failure in human (Law, 2000; *Griffin and* Tauxe, 1991; Nataro and Kaper, 1998; Verweyen *et al.,* 2000; Karmali *et al.,* 1983), its ability to produce verocytotoxins (VT), to cause attaching and effecting lesions on epithelia cells, and in their possession of characteristics large plasmid (Nataro and Kaper, 1998).

Of these*, E.coli* O157, a strain of EHEC produces different type of toxins that cause illness. The toxin produced is similar to the toxin produced by *Shigella dysenrteriae* (Paton and Paton, 1998; Perna *et al.,* 1998; O'Brein and Laveck, 1983). EHEC strains however do not penetrate intestinal epithelial cells unlike shigellas; their toxin production depends on lysogenic conversion by a distinct bacteriophage (Nester *et al.,* 2004; Brussow, *et al.,* 2004). *Escherichia coli O*157, was first identified as a food-borne pathogen in 1982 during an outbreak that was traced to contaminated hamburgers (Riley and Remis, 1983). Since then, sporadic or epidemic cases have been reported in increasing numbers from North and South America, Europe, Asia and Africa. This has increased its important worldwide as a public health problem (OIE, 2008). The pathogen is associated with a range of symptoms, including watery or bloody diarrhea, vomiting, hemorrhagic colitis, thrombotic thrombocytopenic purpura (TTP) and Hemolytic Uremic Syndrome (HUS), which is characterized by acute renal failure affecting mainly children and the immunocompromised individuals (Griffin and Tauxe, 1991). While the majority of foods linked

to human outbreaks of *E. coli O*157 are not assessed quantitatively, some studies have indicated a low infective dose (Bell et al., 1994; Tilden *et al.,* 1996), highlighting the need for stringent control of contamination during food production. Cattle and other ruminants have been established as major natural reservoirs for *E.coli* O157 (Rassmussen, *et al.,* 1993) and play a significant role in the epidermiology of human infections (Griffin and Tauxe, 1991). It has been estimated that 1 to 4% of United Kingdom cattle are infected at slaughter (Chapman *et al.,* 1993; Richards *et al.,* 1998); however recently a prevalence rate of 8.6% has been reported from a farm in Scotland (Synge *et al*., 2000). In United States, breeding herd prevalences of 1% (Sargeant *et al.*, 2007) and 9.3% (Garber *et al*., 1999) have been recorded. In Africa three major outbreaks have been recoreded also (Isaacson *et al*., 1993; Germanii *et al.,* 1997; Cunin *et al.,* 1999; Effler *et al*., 2001). In Nsukka, E.coli O157 have been recorded in slaughter animals (Oguejiofor and Chah., 2010).

2.2.3 Structure of *Escherichia coli*

Escherichia coli cells propel themselves with flagella (long, thin structures) arranged as bundles that rotate counter–clockwise, generating torque to rotate the bacterium clockwise. It is a gram-negative, facultative anaerobic and non-sporulating. It is typically rod-shaped and is about 2 micrometer (μm) long and 0.5μm in diameter, with a cell volume of 0.6-0.7μm^3 (Kubitschek, 1990). The flagella have a peritrichous arrangement (Darton *et al.,* 2007). The strains that possess flagella can swim and are motile.

2.2.4 Biochemical Characteristics of *Escherichia coli*

Optimal growth of *E.coli* occurs at 37^{O}C but some laboratory strains can multiply at temperatures of up to 49^{O}C (Fotadar *et al.,* 2005). Growth can be drive by aerobic or anaerobic respiration, using a large variety of redox pairs, including the oxidation of pyruvic acid, formic acid, hydrogen and amino acid and the reduction of substrates such as oxygen, nitrate, dimethyl sulfoxide and trimethylamine N-oxide (Ingledew and Poole, 1984). *E.coli* is citrate negative, does not produce hydrogen sulfide, produce indole, methyl red positive, urease negative, and sorbitol fermenter and this makes the colony on cefixime tellurite sorbitol- MaConkey (CT-SMAC) to appear pink to red. However over 94% of *E.coli* O157 strains are sorbitol non fermenter but lactose fermenter, resulting in a colourless colony on CT-SMAC agar (Bopp *et al.,* 1999; Cary *et al.,* 1998; Chapman *et al.,* 1991). *E. coli* uses mixed acid fermentation in anaerobic condition, producing lactate, succinate, ethanol, acetate and carbon dioxide.

2.2.5 Transmission of E. coli

Transmission of pathogens *E. coli strains* often occurs via fecal-oral route (Evans *et al.,* 2007; Gehlbach *et al.,* 1973). Common routes of transmission include: direct or indirect contact with infected persons (Wilson *et al.,* 1997), unhygienic food preparation, from contamination due to manure fertilization (Sabin , 2006; Hilborn *et al.,* 1999), irrigation of crops with contaminated grey water or raw sewage (Heaton and Jones, 2008), feral pigs on cropland (Thomas, 2007) or direct consumption of sewage/feacal contaminated water (Adesiyun *et al.,* 1983; Alabi and Adesiyun, 1986; Chalmers *et al.,* 2003, Swersdlow *et al.,* 1992; Keene *et al.,* 1994; O'Connor, 2002; Feng *et al.,* 2002; Thompson, 2007). Dairy and beef cattle are primary reservoirs of *E.coli* O157: H7 (Bach *et al*., 2002; Chapman *et al*., 1993) and they can carry it

asymptomatically and shed the organism in their faeces (Bach *et al.,* 2002; Wallace and Jones, 1996). Food products associated with *E. coli* outbreaks include raw ground beef (Institute of medicine and National Academics, 2002; Riley *et al.,* 1983), raw seed sprouts or spinach (Sabin, 2006), raw milk, unpasteurized juice, and food contaminated by infected food workers via fecal-oral route (Boers and Heuvelink, 2001; Warburton *et al.,* 1998; Bolton *et al.* 1996, Okrend *et al.,* 1990; McDonough *et al.,* 2000; Igene, 1983; Shehu and Adesiyun, 1990). Shiga toxin-producing *E.coli* (STEC), specifically serotype O157; H7, have also been transmitted by flies (Szalenski *et al.,* 2004; Sela *et al,* 2005; Alam and Zurek, 2004), as well as direct contact with farm animals (Rahn *et al.,* 1998; Trevena *et al.,* 1999), petting zoo animals (Heuvelund *et al.,* 2002) and airborne particles found in animal-rearing environment (Varma *et al.,* 2003)

2.2.6 Clinical Manifestation

Enterohaemorrhagic *E. coli* can cause a broad spectrum of disease – watery diarrhea which is at first without blood but after 2 days becomes bloody, abdominal pain accompanied by colicky pain (Quinn, 2002). Vomiting is frequent but fever is rare at this stage. The abdomen is very tender to pressure. Symptoms appear after an incubation period of 3 to 4 days (range 1 to 8days). These symptoms last for 3 to 10 days. There is no immunity. It can cause serious food poisoning in humans and are occasionally responsible for costly product recalls (Vogt and Dippold, 2005). Haemorrhagic colitis, hemolytic uremic syndrome and thrombotic thrombocytopenic purpura (Bell *et al.,* 1994; Tilden *et al*., 1996; Anon, 1995; Nataro and Kaper 1998; Verweyen *et al*., 2000) develop approximately 1 week after the appearance of symptoms. These complications may not be separable, since in both microangiopatic hemolytic anemia with thrombocytopenia and involvement of the kidney, CNS and other organ system are present. This occurs mainly in

children and the immunocompromised individuals (Griffin *et al*., 1991) and this leads to mortality rate of 2-10% (Law, 2000).

Escherichia coli also cause gastroenteritis, urinary tract infection which is an ascending infection whereby fecal bacteria colonize the urethra and spread up to the urinary tract to the bladder as well as to the kidneys causing pyelonephritis (Nicolle, 2008) or prostate infection in male. Because women have shorter urethra than men, they are 14-times more likely to suffer from ascending urinary tract infection (Todar, 2007).

2.2.7 Laboratory Diagnosis of E. coli O157

Any person with bloody stools, Hemolytic-uremic syndrome (HUS), thrombotic thrombocytopenic purpura (TTP) or a history of bloody diarrhea should be evaluated for the presence of enteroheamorrhegic *Escherichia coli*. One should also be aware that the disease can cause non-bloody, watery diarrhea. In stool samples, microscopy will show gram negative rods, with no particular cell arrangement. Then, either MacConkey agar or Eosin Methylene Blue (EMB) agar are inoculated with the stool. On MacConkey agar, deep red colonies are produced as the organisms are lactose fermenters, and fermentation of this sugar will cause the medium's pH to drop, leading to darkening of the medium. Growth on EMB agar produces black colonies with greenish-black metallic sheen. This is diagnostic of *E. coli.* The organism is also lysine positive, and grows on Triple Sugar Iron (TSI) slant with a glucose positive and hydrogen sulfide negative (A/A/g+/H_2s-) profile. Also, *E. coli* is indole positive (red ring) and methyl red positive (bright red) but VP negative and citrate negative i.e. it does not utilize citrate as a sole source of carbon.

For *Escherichia coli O*157 there are several methods for its laboratory diagnosis (Bopp *et al.,* 1999; Cary *et al.,* 1998, Okrend *et al.,* 1990; Meng *et al.,* 1996; Wu and Pan*.,* 1999; Feng, 1997). Food or clinical faecal specimen can be directly placed on selective or differential agar (Nataro and Kaper, 1998). Samples can also be selectively enriched in broth media. Broth containing vancomycin cefixime and cefsinlodin, and modified trypticase soy broth containing novobiocin are two possible enrichment broths (Warbuton *et al.,* 1998; Okrend *et al.,*; 1990; Cizek *et al.,* 1999; Hardin *et al.,* 1995; Cary *et al.,* 1998, Wallace and Jones 1996). The selective agar, sorbitol MacConkey agar, is used since most *E. coli* O157: H7 and O157 non motile strains do not metabolize sorbitol. In formulation of sorbitol MacConkay agar, sorbitol is substituted for lactose in MacConkey agar. The medium also contain cefixime and potassium tellurite (CT-SMAC) and has been used as an important screening step (Chapman *et al.,* 1991; Zadik *et al.,* 1993; March and Ratnam, 1986; Nataro and Kaper, 1998; Fujisawa *et al.,* 2000; and Muller *et al.,* 2002). Unlike typical *E. coli, E coli O*157 do not ferment or produce acid from sorbitol within 24 hrs and lack glucoronidase activity (Manafi and Kremsmaier, 2001). *E. coli O*157 bacteria present as colourless colonies on sorbitol MacConkey (SMAC) medium (Manafi and Kremsmaier, 2001; Bopp *et al.,* 1999; Cary *et al.,* 1998; Chapman *et al.,* 1991; Zadik *et al.,* 1993). The addition of cefixime and potassium tellurite successfully increases the selectivity of sorbitol MacConkey agar.

Various researches have used polymerase chain reaction (PCR) techniques to confirm the genetic profiles of *E. coli O*157 (Paton and Paton, 1998). Other method for detecting *E. coli O*157 in stool include Enzyme Linked Immunosorbent Assay (ELISA) test, colony immunoblots, direct immunoflurescence, and microscopy of filters as well as immunocapture techniques using magnetic beeds (De Borer and Hewelink, 2000).

2.2.8 Treatment of E.coli O157 infections in humans

Fluid and electrolyte replacement is of prime importance. It contributes to kidney perfusion and prophylaxis of HUS. Antimicrobial treatment can be achieved but the organism are resistant to many antibiotics. Antibiotics which may be used to treat E.coli infection include amoxicillin as well as other semi-synthetic penicillins, many cephalosporins, carbapenems, aztreonam, trimetoprim-sulfamethoxazole, ciprofloxacin, nitrofurantoin and the aminoglycosides (Johnson *et al.,* 2006). Antibiotics treatment contributes to verotoxin release, and increases the risk of HUS motility (Walderhauy, M. 2001). Inhibiting drugs are also contraindicated since they lead to retention of enteroheamorrhagic *E. coli* and increase the possibility of verotoxin absorption. Clinical management of early dialysis, control of electrolytes, treatment of hypertension and plasmapheresis is necessary since it will help to reduce mortality (Walderhauy, M. 2001).

Phage therapy using viruses that specifically target pathogenic bacteria has been developed which is mainly used to prevent diarrhea caused by *E. coli* (Anonymous, 2007)

2.2.9 Prevention and Control of E.coli O157 in humans

Escherichia coli infection is prevented by general hygienic measures. Washing of hands, after contact with infected animals, particularly ruminants, and proper hygiene in the processing of food and preparation of meats .Also applying hazard analysis critical control point measures. Uncooked food should be kept in a refrigerator and heated to $70^{O}C$ for at least 10mins before consumption. Unpasteurized milk should be boiled prior to consumption. Meat should be well cooked before consumption (CDC.E.coli online 2008). Discharge of abattoir effluent into water

bodies and streets should be highly prohibited. Management practices to decrease EHEC in the environment include the storage of effluents on a cement floor for 3 months or longer before discharge, and the collection of all liquids in a trap to minimize leaching of liquid manure into groundwater. Some EHEC may remain after long-term storage, and untreated manure and effluents should not be used on fruit and vegetable crops that will be eaten raw. (Karch *et al.,* 2005).

Vaccines have been developed to lower the incidence of *E. coli* infection (Giraid *et al.,* 2006). A vaccine eliciting an immune response against *E. coli* O157 O-specific polysaccharide conjugated to recombinant exotoxin A of *Pseudomonas aeruginosa* was reported to be safe in children and adult (Ahmed *et a.l.,* 2006).

2.2.10 Prevention and control in Animals

Prevention of shedding in domesticated animals, particularly ruminants, is expected to decrease the number of human infections. Identifying and targeting super-shedders should be particularly effective (Sargeant *et al.,* 2007). In one study, allowing a pasture to lie fallow for the winter prevented the transmission of EHEC O157:H7 to susceptible animals the following spring (Chase-Topping *et al.,* 2008). Other proposed interventions include vaccination; vaccines have been developed by many researchers – live attenuated vaccine to control airsacculitis and peritonitis in chicken, which are genetically modified avirulent vaccine for protection against E.coli O78 (Anonymous, 2007). Also, a cattle vaccine which reduces the numbers of O157 shed in manure by a factor of 1000, to about 1000 pathogenic bacteria per gram of manure has been developed (Pearson, 2007; Bioniche Life Sciences, 2007), the application of disinfectants (e.g.,

chlorhexidine), various antimicrobial chemicals or bacteriophages to the terminal rectum; and the use of probiotics that would preferentially colonize the gastrointestinal tract (Naylor *et al*., 2007). To prevent transmission to animals, they should not be allowed to graze pastures for a period after effluent has been applied. Composting or treatment of manure before use as a fertilizer may reduce the risks from this source. During composting, the survival of the organism varies with the size of the compost heap, the temperature attained, and the dose of the organism. Other biological processes (aerobic and anaerobic digestion), heat drying, and/or chemical treatments have been proposed to sanitize farm effluents before discharge into the environment.

Methods to eliminate EHEC from infected companion animals have not been established. However, oral autovaccination with a heat-inactivated EHEC strain (O145:H–) stopped shedding of the organism in a persistently infected cat (OIE, 2008).

2.3 Salmonella species

2.3.1 Microbiology of Salmonella

Salmonella is a member of the family Enterobacteriaceae. The enterobacteriaceae are a large heterogeneous group of gram-negative rods whose natural habitat is the intestinal tract of human and animals (Murray *et al.,* 1995). The family includes many genera among which are Escherichia, Salmonella, Shigella, Enterobacter, Klebsella, Serratia, Proteus and others. Some enteric organisms such as Escherichia coli are part of the normal flora and can incidentally cause disease, while others such as Salmonella and Shigella, are regularly pathogenic for humans (Gray, 1995). They are facultative anaerobes or aerobes; except *S. gallinarum and S. pullorum* which are non-motile. They produce acid and gas from glucose, maltose, mannitol and salicin, do

not form indole, and coagulate milk are incapable of fermenting lactose, catalyase positive, oxidase negative and reduce nitrate to nitrite and have a 39-59% Guanine+ Cytosine DNA content (Adelberg *et al.,* 1998). They possess a complex antigenic structure, and have the ability of invading the intestinal mucosa, produce toxins and other virulent factors (Ewing, 1986). They possess the lipopolysaccharide (LPS) endotoxin characteristics of Gram- negative bacteria (Timoney *et al.,* 1992)

2.3.2 Prevalence of Salmonella species

Salmonella species occur worldwide. The bacteria are present in the intestine of mammals, birds and reptiles (Olson, 2001). Salmonella was first isolated in 1885 by Smith and Salmon from pigs which had died of hog cholera, and secondly by Gaitner (1888) from fatal case of gastroenteritis in a young man who had eaten raw meat taken from a diseased cow. Since its first isolation its concern has increase because of its effects on health food and economy (Chen and Griffiths, 2001). More than 2,400 Salmonella serovars are known at present and each of these may give rise to acute gastroenteritis (Gast, 1997). It occurs worldwide in both man and animal. The intestinal tracts of humans and animals are the major sources of Salmonellae in nature, and approximately 1% to 3% of all domestic animals are infected with Salmonella (Litchfield, 1980)

2.3.3 Transmission

One of the most challenging aspects of the Salmonella infection in animals is that most of the infected animals do not show any clinical sign. Many animals will be infected at younger ages while the gut flora is not well established. Following the infection, the bacterium will be

present in faeces for few days but will survive within lymph nodes for many weeks or months (Marg *et al*., 2001). After a stress period, such as transport to slaughter house, the positive animal will shed again the bacterium in feaces and possibly contaminate other animals or meat products by direct or cross-contamination at slaughter. In poultry, once birds are infected, the bacterium will persist in caecum until birds are shipped to abattoir (Asakura *et al*., 2001).

Transmission of salmonella from animals to humans can be through oral route either directly by consumption of contaminated foodstuff (Haddock, 1970; Mead *et al.,* 1999) like poultry, beef (Tauxe, 1991; Benenson *et al.,* 1995) and egg or contact with petting zoo animals (Geue and Loschner, 2002; Nakadai *et al*., 2005), milk and milk product, cereal and vegetables (Atanda and Ikenebomeh, 1991; Sierra *et al.,* 1995; Boonmar *et al*., 1998; Giovannnnacci *et al.,* 2001). Transmission can also be indirectly by contact with contaminated exhibition furnishing materials (Bauwems *et al*., 2006). Manure contaminated with salmonella which is spread on farmlands has been known to cause human outbreaks of salmonellosis when associated with lettuce, mushroom and alfalfa sprouts (Olson, 2001).

2.3.4 Clinical manifestations of Salmonelosis in man and animal

The incubation period depends on the member of bacteria ingested and it varies from 5-72hrs (Cowan, 1981). Affected individuals experience sudden nausea, abdominal cramps, vomiting and watery foul-smelling diarrhea which in most cases lasts only a few hours. Fever up to 39^0C (102^0F) is common. Convalescence starts within 1-2days. Enteric or typhoid fever occurs when the bacterial leaves the intestine and multiply within cells of the reticuloendothelia system (Cowan, 1981). The bacteria then re-enter the intestine causing gastrointestinal symptoms. Within the second week, the liver and spleen can become enlarged, and a distinctive

'rose spotted' skin rash may appear. This can cause other health problem like meninigitis, septiceamia,(usually caused by *S.cholerae-suis*), arthritis, osteomylelitis, peritonitis, urinary tract infection and endocarditis particularly in immunosuppressed patient, cancer-fighting drug patient, people with sickle cell disease and people taking chronic stomach acid suppression medication.

In animals, salmonellosis is either an acute gastroenteritis or a silent infection that results in a carrier state, particularlt in reptiles.

2.3.5 Laboratory diagnosis

Laboratory diagnosis of salmonella infection is by isolation and identification of the Salmonella species in faeces, blood, liver, and kidney, and spleen, intestinal segments at post mortem, aborted fetus, fetal membrane or urine. The appropriate specimen can be selectively enriched into an enrichement broth like Rappaport-Vassiliadis, selenite F and tetrathionate broths which selectively inhibits Gram positive organisms and other normal intestinal microflora. After the selective enrichment the sample is subcultured on selective media like Salmonella- Shigella agar (SSA), MacConkay agar (MAC), Deoxycholate citrate agar (DCA). Salmonella produces colourless colonies on SSA, MAC and DCA while on Brilliant Green agar it produces translucent pale/pink colonies and on Xylose Lysine Deoxycholate agar it gives rise to red colonies with a black center 'fish eye' (Cowan, 1981).

Salmonella can also be identified by inoculating directly on the Brilliance Salmonella agar supplemented with Salmonella selective supplement which produces purple colonies after incubating overnight at 37^0C for 24hrs (Quinn et al, 2002) . Suspected colonies are further subjected to a series of biochemical tests such as hydrogen sulfide production and gas on triple

sugar (TSI), citrate test, urease test, indole test, methyl red test, Voges proskauer test and sugar fermentation tests. The biochemical properties of Salmonella strains were described by Krieg and Holt (1984), Ewing (1986). Typical salmonella strains ferment glucose, mannitol, maltose, but do not ferment lactose, sucrose, malonate or salicin. They can produce hydrogen sulfide on many types of media, utilize citrate as a sole source of carbon and reduce nirates to nitrites. They do not produce indole and do not hydrolyse urea or gelatin.

Several techniques, such as polymerase chain reaction (PCR) (Hong *et al.*, 2003), immunological techniques such as (Fluit *et al.,* 1993) and automated identification systems, (Odumeru *et al*., 1991) are available for the detection of *Salmonella* in a variety of samples. These techniques are not routinely used in all diagnostic laboratories because of high capital expenditure and the need for highly skilled personnel to perform these tests.

2.3.6 Serological Identification

Early investigations into the classification of strains on a serological basis were not very successful owing to the fact that many were inagglutinable in the living state. It was not until the discovery by Kauffmann (1943) of a previously unsuspected heat-labile capsular (K) antigen, which prevented somatic "O" antigen agglutination, that it was possible to use agglutination method for this purpose. Precipitation tests were found to be of little value in the differentiation of strains (Lehmann and Jusatz, 1932). Enterobacteriaceae have a complex antigenic structure. They are classified by more than 150 different heat stable somatic "O" antigens, 100 heat-labile "K" antigens and 50 flagella "H" antigens (Morse, 1998).

The traditional Kauffmann white scheme for antigenic classification of Salmonella is based on both somatic and flagella "H" antigen (Ewing, 1986). The somatic "O" antigens are

determined by polysaccharides associated with the body of the cell and identified by Arabic numerals. Serotyping of isolates is generally accomplished using agglutination tests with batteries of specific antisera. Slide agglutination tests are first used to establish the somatic antigen content and flagella antigens content is later determined using tube agglutination test. Salmonella species can also be identified by bacterial phase typing.

2.3.7 Human Salmonella Infections

Salmonella typhi*, S.choleraesuis* and *S. Paratyphi* "A" and "B" are primarily infective for humans (Brooks *et al*, 1998). Thus, infection with these organisms perhaps implies acquisation from a human source. The vast majority of salmonella however, are chiefly pathogenic to animals that constitute the reservoir for human infection viz. Poultry, pigs, rodents, cattle, pets (e.g. turtles and parrots etc) (Oboegbulem, 1990).

The organism almost always enters through the oral route, usually from contaminated food and water. The mean infective dose to produce clinical or sub-clinical infection in humans is 10^5 -10^8 Salmonella (Blaser and Newman, 1982). The three major type of disease produced in humans by Salmonella are: enteric fever, bacteriamia with focal lesions and enterocolitis.

2.3.7.1 The "Enteric Fevers" (Typhoid fever)

This syndrome is produced by only a few of the Salmonella species of which *S.typhi* is the most important (Edelman and Levine, 1986). When ingested, salmonellae reach the small intestine and enter the lymphatics and the blood stream whence it is carried by the blood to many organs. It multiplies in the intestinal lymphoid tissues and is excreted from the stool (Homick, 1970). An incubation period of 10-14days usually elapses before the clinical signs of fever, malaise, headache, constipation, bradycardia and myaglia follows (Butler *et al.,* 1991). The fever

rises to a high plateau and the spleen and liver become enlarged, rose spots in some cases are usually seen on the skin around the abdomen or chest, the white blood cell count is normal or low. Butler *et al.,* (1991) also reported intestinal haemorrhage and perforation as the major complications of enteric fever leading to mortality rate of 10-15% prior to antibiotic era. However, Adelberg *et al.,* (1998) reported that treatment with antibiotic has reduced mortality rate to less than 1%. Edelman and Levine (1986) demonstrated the principal lesions seen in typhoid fever as hyperplasia and necrosis of the liver and inflammation of the gallbladder, periosteum, lungs and other organs.

2.3.7.2 Bacteriamia with focal lesions

This was described by Lee, (1994) to be commonly associated with *S. cholerasuis*. These lesions may be caused by any Salmonella serotype. Following oral infection, there is early invasion of the blood stream, with focal lesions in the lungs, bones meninges etc, but intestinal manifestations are often absent though blood cultures may be positive (Lee, 1994).

2.3.7.3 Enterocolitis

This has been reported by many researchers as the most common manifestation of Salmonella infection in humans whereas S. typhimurium is most commonly encountered in USA (Bela and Peter, 1999). S.enteritidis takes the lead in Europe (England, Scotland and Wales) (Gracey and Collins, 1992; Oboegbulem *et al*., 1991). Enterocolitis could result from any of the serotypes of Salmonella (Oboegbulem, *et al.,* 1991). Eight to 48 hours after ingestion of Salmonella, there is nausea, headache, vomiting and profuse diarrhoea, with few leukocytes in the stools, low grade fever is common, but the episode usually resolves in 2-3 days (Jones and Falkow, 1996). Although salmonella enterocolitis is generally a self-limiting illness, which may require only fluid and electrolyte replacement, the disease, can spread systematically and

degenerate into a chronic condition such as reactive arthritis, osteomyelitis, cardiac inflammation or neural disorders (D'Aoust, 1994). According to Jones and Falkow (1996), inflammatory lesions of the small and large intestine are present, bacteremia is rare 2-4% except in immunodeficient person; blood cultures are usually negative but stool cultures are positive for salmonellae and may remain positive for several weeks after clinical recovery.

2.3.8 Treatment of Salmonella

Enteric fever and bacteraemia with focal lesions has been reported to require antimicrobial treatment, while vast majority of cases of enterocolitis do not (D'Aoust, 1994). However, antimicrobial treatment of Salmonella enteritis in neonates still remains very important (Adelberg *et al.,* 1998)

Antimicrobial therapy of invasive salmonella infections is with ampicillin, trimethoprim-sulphonamide, or third generation cephalosporin. Otaka (1998) reported that quinolones (ciprofloxacin, norfloxacin, perfloxacin) are now the drug of choice for the treatment of enteric fever in Nigeria. Penicillin (especially amoxycillin) has been indicated to be the best alternative (Alstead and Girulead, 1985). Others include cotrimaxazole, and sulphonamide-trimethoprim combinations. Gastroenteritis should only be treated by replacing lost fluids, since antibiotic therapy does not affect the course of the disease and may only increase the number of resistant species (Helmuth and Protz, 1997; Rao, 1998; Witte, 1997).

2.3.9 Prevention and control

Edelman and Levine, (1986) proposed that human Salmonellosis can be controlled through sanitary measures in slaughter houses to prevent contamination of meat contact surfaces,

water and environment by rodents or other animal reservoirs and hygienic measures such as thorough boiling of meats and strict personal hygiene measures by the abattoir workers and meat handlers.

Salmonella also can be spread through cross contamination: therefore, when meals are being prepared, uncooked meats should be kept away from cooked and ready to eat foods. In addition, thorough washing of hands, cutting boards, counter, and knives after handling uncooked foods is necessary (Todar, 2004). Fecal matter is often the common source of salmonella contamination; hence thorough washing of hands is extremely important, particularly after using toilet and handling animals (http://www.kidshealth.org).

An improved hygienic practice at slaughter house and environment has been reported to prevent cross-contamination of animal products.

2.4 Enterotoxygenicity of E.coli O157 and Salmonella species

Enterotoxigenic *Escherichia coli* (ETEC) and *Salmonella* adhere to the microvilli of small intestinal epithelial cells without inducing morphological lesions and producing enterotoxins that act locally on enterocytes (Bela and Peter, 1999). They possess microbial attributes such as the production of cell wall or capsular lipopolysaccharide (LPS) antigens which help them to survive and flourish in the intestine (Elsinghorst and Weitz, 1994). They are well-recognized cause of acute dehydrating diarrhea worldwide (Sack, 1975; Kaura *et al.,* 1982). It occurs both in animals and man.

2.4.1 Enterotoxins

Enterotoxins are extracellular proteins or peptides (exotoxins) which are able to exert their actions on the intestinal epithelium. They are characterized by production of one or more of the following enterotoxin categories (Sherman *et al.,* 1983), all of which are plasmid regulated. These enterotoxins include that of large molecular weight of (ca.72,000) heat-labile enterotoxins (LT) (Kunkel and Robertson, 1979) which has been reported to be immunologically and structurally similar to cholera enterotoxin (Smith and Sack, 1973) and small molecular weight ranging from (1,800 to 5,100) which are heat-stable peptide toxins (ST) (Alderete, and Robertson 1978; Giannela and Staples, 1979).

These enterotoxins cause fluid accumulation when introduced into ligated intestinal loops prepared in rabbits or pigs (Evans, et al 1973, Kohler, 1968, Chah and Oboegbulem 1999)
LT enterotoxins are produced predominantly by human and porcine enterotoxigenic E.coli, while ST enterotoxins are produced by human enterotoxigenic E.coli of human, porcine and bovine origin. LT toxins have good antigenicity while ST toxins do not.

2.4.2 Mechanism of action of LT and ST enterotoxins

The common feature in the mechanisms of action of LT and ST enterotoxins is that they do not produce pathological lesions or morphological alterations on the mucosa. They only produce functional changes such as an increased secretion of H_2O, Na^+ and Cl^- and a concomitant decrease of fluid and absorption (Bela and Peter, 1999). As a result, fluid and $NaHCO_3$ is lost from the body, leading to dehydration and acidosis. The mechanism of action and structure of LT enterotoxin are similar to the heat-labile toxin of Vibrio cholerae (CT). CT and LT consist of a single A domain and five B subunits containing 240 and 103 amino acids

respectively. The B subunits bind predominantly to the GMI ganglioside acting as receptors on the cell surface (O'Brien and Holmes, 1996) once this is fixed, the fragments will translocate into the cell where they activate the adenylate-cyclase system resulting in increased fluid and electrolyte secretion and decreased absorption. For ST, it's mechanism of action can be assayed in suckling mice or in ligated intestinal loops of pigs and calves (Frantz, et al 1984).

2.4.3 Labouratory detection of enterotoxin production

The diagnosis of enterotoxigenic *E.coli* 0157 and Salmonella spp infections requires the detection of virulence factors (adhesions, enterotoxin) using in vitro tests (slide or latex agglutination or ELISA) in most cases (Thorns *et al*., 1989). Adhesive fimbriae can, however, be most effectively detected in vivo, by an immunofluorescent method using absorbed polyclonal antifimbrial antibodies (Isaacson *et al.,* 1978). In contrast to fimbriae, enterotoxins produced in vivo are much more difficult to detect, therefore, at the early ages of these studies in vitro-produced toxins could only be tested by biological assays; weaned pig ligated loop assay (Smith and Halls, 1967), neonatal pig ligated loop assay (Smith and Halls, 1967; Sack, 1975), piglet oral dosing assay (Kohler, 1968), rabbit ligated loop assay (Evans *et al.,* 1973; Chah and Oboegbulem, 1999) and calf ligated loop assay (Smith and Halls, 1967) (for all enterotoxins) or suckling mouse assay (Dean *et al.,* 1972) for ST, followed by cell cultures for LT, and later on by ELISA assays (for LT and ST) (Czirok *et al.,* 1992; Yolken *et al.,* 1977). Now, with the advent of molecular methods in the diagnostic laboratories, the cumbersome biological assays can be replaced by so-called gene probes of different virulence characters (Franck *et al*., 1998; Mainil *et al*., 1990; Tsen and Jian, 1998) or by immunological assays such as passive immune hemolysis (Evans and Evans, 1977), staphylococcal coagglutination (Brill

et al., 1979), radioimmunoassay (Greenberg *et al.,* 1979), or by tissue culture (Guerrant, 1974; Sack and Sack, 1975)

2.5 Antibacterial agents

Antibiotics are substances produced by microorganisms that kill or inhibit other microorganisms (Rang *et al*., 1995). They are products of the earth more specifically of soil (Trease and Evanse, 1972). They are also chemicals that interfere directly with the proliferation of microorganisms at concentrations that are tolerated by the host (Davies *et al.,* 1973).

Many of the antibiotics are now produced semi-synthetically by chemical modifications of naturally occurring compounds (Craig and Stitzel, 1994). Antibiotics used in the treatment of disease must be effective against the pathogenic microorganism and not the host (patient taking the antibiotics) essentially must be resistant to the action of the drug (Todar, 2002).

2.5.1 Mechanism of action of antibiotics

Most antimicrobial agents used for the treatment of bacterial infections may be categorized according to their principal mechanism of action. There are four major modes of action viz:

A. interference with cell wall synthesis

B. inhibition of protein synthesis

C. interference with nucleic acid synthesis

D. inhibition of a metabolic pathway

Antibacterial drugs that work by inhibiting bacterial cell wall synthesis include the β-lactams, such as the penicillins, cephalosporins, carbapenems and monobactams, and glycopeptides, including vancomycin and teicoplanin (Neu, 1992; McManus, 1997). Beta-lactams, agents inhibit synthesis of the bacterial cell wall by interfering with the enzymes required for the synthesis of the peptidoglycan layer (McManus, 1997).

Macrolides, aminoglycosides, tetracyclines, chloramphenicol, streptogramins and oxazolidinones produce their antibacterial effects by inhibiting protein synthesis (Neu, 1992; MaManus, 1997). Bacterial ribosomes differ in structure from their counterparts in eukaryotic cells. Antibacterial agents take advantage of these differences to selectively inhibit bacterial growth. Macrolides, aminoglycosides, and tetracyclins bind to the 30S subunit of the ribosome, whereas chloramphenicol binds to the 50S subunit (Tenover, 2006).

Fluoroquinones exert their antibacterial effects by disrupting DNA synthesis and causing lethal double stranded DNA breaks during DNA replication (Drlica and Zhao, 1997), whereas sulfonamides and trimethoprim block the pathway for folic acid synthesis which ultimately inhibits DNA synthesis (Yao and Moellering, 2003; Petri, 2006).

2.5.2 Antibiotic resistance

Antimicrobial resistance represents an important current and future problem in infectious disease. Antimicrobial resistant to bacterial pathogens are continually emerging, and these present specific challenges to disease treatment and control. It is generally accepted that antimicrobial resistant bacteria are produced, maintained and disseminated as a result of selection pressure induced by the use of antimicrobial drugs (Van den Bogaard and Stobberingh, 2000). Antibiotic resistant strains of bacterial known to be food borne pathogens including

Salmonella spps, *E. coli* and *Campylobacter* spp have been isolated from farm animals (Fairchild *et al.,* 2000; Beach *et al*., 2002; Altekruse *et al.,* 2002; Hayes *et al*., 2004; Aarestrup, 2005; Alcaine *et al.,* 2005; Belloc *et al.,* 2005; Bywater *et al.,* 2005; Chapin *et al*., 2005; Englen *et al.,* 2005; Farnell *et al.,* 2005). These resistant bacteria could cause human and animal diseases that are difficult to treat. Antibiotic resistant bacteria have frequently been isolated from livestock and farms, and these constitute a serious public health risk. It can be transmitted to humans, through direct contact with livestock and production environments, but particularly in the form of antimicrobial resistant food-borne pathogens (Van den Bogaard and Stobberingh, 2000; White *et al*., 2001). Multi-state outbreaks of enteritis caused by multi-drug resistant strains of Salmonella enterica serotype Typhimurium DT104 (Dechet *et al.*, 2006) and *E. coli* (Salyers *et al.,* 2004; Fluit *et al*., 2001) were reported to be associated with ground beef. An epidemic clonal ecology in which a single clone can disseminate broadly, even globally, to and between livestock farms has been observed (Hancock *et al*., 2000; Threifall, 2002). Even if the antibiotic-resistant bacteria in animal meat are not human pathogens they may pass their resistance genes to other human pathogenic bacteria (Alcaine *et al*., 2005; Kim *et al*., 2005; Lester *et al*., 2006; Gould, 2008).

2.5.3 Mechanisms of antibiotic resistance

Bacteria may manifest resistance to antibacterial agents through a variety of mechanisms. These mechanisms often interplay synergistically to raise resistance level significantly (Li and Nikaido, 2004).

The basic mechanisms by which a bacterium can resist antibacterial agent include the following.

A. target alteration e.g. fluroquinolone resistance (Hooper, 2000)

B. exclusion from the cell e.g. by outer membrane of Gram negative bacteria (Hancock, 1998)

C. enzymatic inactivation e.g. beta-lactamase (Thomson and Smith, 2000)

D. Active efflux from the cell e.g. for tetracycline (Chopra and Robert, 2001).

Gram-negative bacteria express various types of resistance. Intrinsic resistance is as a result of cooperation of outer membrane barrier and multidrug efflux pump (Beovic, 2006). Some Gran-negative bacterial possess drug specific efflux pump, which mediate resistance to certain classes of antibacteria (Beovic, 2006). In Gran-negative bacteria, outer membrane permeability barrier and multidrug efflux pumps play synergistically an important role in intrinsic resistance of these bacteria (Li et al., 1994; Li and Nikaido, 2004).

2.5.3.1 Target alteration

Changes in drug targets that interfere with or limit antibiotic interaction also prevent the bacteriastatic and bactericidal effects of these agents and, thus promote resistance (Poole, 2002). The most common mechanism of resistance to macrolides, for example, involves modification of their target site on the ribosome, specifically methylation of an adenine residue in domain V of the 23SrRNA (Schmitz *et al*., 2000; Leclercq and Courvalin, 1991). Beta-lactams resistance due to target site (i.e. penicillin-binding protein) alteration is also common (Poole, 2004; Livermore, 1995).

Similarly, resistance to fluoroquinolones has traditionally been attributed to mutations affecting the fluoroquinolone targets, DNA gyrase and topoisomerase (Hooper, 2000; Li, 2005; paddock, 2002). The tetL and tetM determinants of tetra- cycline resistance encode a poorly

understood ribosomal protection mechanism that ultimately compromises tetracycline association with its ribosomal target (Chopra and Roberts, 2001). Resistance to aminogylcoside can also arise from target site (i.e. ribosomal) mutations (Wright *et al.,* 1998).

2.5.3.2 Cell wall impermeability

In order for antibiotics to exert their bacteriostatic or bactericidal actions on bacteria they must access intracellular targets (Poole, 2002). In gram negative bacteria, they cross the outer membrane, a substantial permeability barrier and a major determinant of antimicrobial resistance in these bacteria (Nikaido, 1994; Hancock, 1997a). Acquired resistance to β-lactams in a number of Gram negative bacteria has been attributed to outer membrane changes that correlate with reduced permeability (Chen and Livermore, 1993; Charrel *et al.,* 1996; Martinez-Martinez *et al.,* 1996).

The importance of the outer membrane barrier as a determinant of antibiotic resistance is highlighted by observations that perturbation of this membrane by chemical means enhances antimicrobial susceptibility (Vaara, 1992). The outer membrane barrier as a resistance mechanism is only significant in the context of additional resistance mechanisms (efflux and β-lactamases) that work synergistically with it to promote resistance (Nikaido, 1994).

2.5.3.3 Enzymatic modification or destruction

The predominant mechanism of resistance to β-lactams remains β-lactamase enzymes that inactivate the antibiotic by hydrolyzing the β-lactam ring of the molecule (Thomson and Smith, 2000; Li *et al.,* 2007). Over 400 β-lactamases have been reported and new beta-lactamases continue to emerge rapidly worldwide (Jacoby and Munoz-Price, 2005; Miriagou *et al.,* 2004).

Beta-lactamases are classified into four molecular classes (the Anbler classes A to D, where the classes A, C and D enzymes are serine enzymes and the class B includes metallo beta-lactamases (Li *et al.,* 2007; Thomson and Smith, 2000).

Resistance owing to enzymes-medicated inactivation is also known in tetracycline (Chopra and Roberts, 2001). Chloramphenicol resistance by bacteria is typically afforded by acetyltransferases (Murray and Shaw, 1997).

2.5.3.4 Active efflux from the cell

Antibiotic efflux as a mechanism of resistance was first recognized in the late 1970s (Levy and McMurry, 1978). Drug efflux system pumps out a broad range of chemically and structurally unrelated compounds from bacteria in an energy-dependent manner without drug alteration or degradation (Kumar and Schweizer, 2005). R-glycoproteins were the first example of an efflux pump implicated in the resistance of mammalian cancer cells (Juliano and Ling, 1976).

Efflux as a means of antibiotic resistance is most commonly associated with tetracycline (Tet A, Tet B and Tet K pumps) and the fluoroquinolones in both Gram positive and Gram negative bacteria (Chopra and Roberts, 2001; Poole 2000a, 2000b; Paddock, 2002; Li, 2005). These pumps contribute to both intrinsic and acquired resistance, the latter arising from mutational hyper expression of these chromosomally encoded efflux systems (Poole, 2000a).

Elevated expressions of multidrug efflux pump have been observed in *E.coli* and S*almonella spp* from food animals (Baucheron *et al.,* 2002; Oliver *et al.,* 2005). Although exclusion from the cell due to reduced outer membrane impermeability was thought to play a key role in the intrinsic resistance of P*seudomonas aeroginosa* and related bacteria to many

antimicrobial compounds, this is now attributed to synergy between low permeability outer membrane and active efflux from the cell (Poole, 2000a, Poole and Srikumar, 2001).

2.5.4 Genetic Basis and Spread of Antimicrobial Resistance

Acquired resistance to an antimicrobial drug is the result of an alteration of the genome of a microorganism. The primary pathway through which the alteration of the genome can occur is the mutation of a gene (*de novo* resistance). The secondary pathway is the incorporation of such resistance genes (determinants) by donor bacteria into acceptor bacteria via conjugation, transformation or transduction (Quinttiliani *et al.,* 1999; Schwarz and Chaslus-Dancla, 2001).

Genetic mutation is a normal process that occurs during bacterial replication and occurs spontaneously in DNA replication at a rate of 1 mutation per million bases per cell division (Prescott and Baggot, 1985). Mutation or changes in the genome, mostly in the chromosome, occur spontaneously during the replication of bacteria genome. Although this is a low probability event, it may be relatively common place in the ubiquitous huge bacterial population (Magee, 2001). Conjugation, transformation and transduction imply the horizontal transfer of resistance genes from a donor bacterium into an acceptor bacterium via a protein tunnel, naked DNA and viral phages, respectively. Conjugation is the most important transfer mechanism (Hawkey, 1998; Rowe-Magnus and Mazel, 1999). It is estimated that 85% of therapy failure in staphylococci (Beta-lactams), *enterobacteriaceae* (ampicillin, sulpha/trimethoprim, gentamicin, and chloramphenicol) and enterococci (vancomycin) can be attributed to conjugation-induced antimicrobial resistance (Berends *et al.,* 2001). The rapid horizontal gene transfer which occurs in conjugation consists of the spread of genetic elements such as plasmid (able to replicate automatically), transponons (not able to replicate automatically, movement by transposases) or

integrons/ gene cassettes (not able to replicate automatically, movement by site-specific recombination) (Bennet, 1999; Schwarz and Chaslus –Dancla, 2001).

CHAPTER THREE

MATERIALS AND METHOD

3.1 Sample collection

A total of 448 samples were collected from meat contact surfaces such as floor (112), slabs (112), meat holding drums (112) in Nsukka slaughter house and meat sales tables (112) at the meat sale section of Nsukka market. Surfaces of selected sites were randomly swabed using sterile swab sticks (evepon®). Samples were cultured once a week between September and December 2009. The samples were immediately transported to the Veterinary Microbiology laboratory, University of Nigeria, Nsukka for analysis.

3.2 Culture media and their preparation

All bacteriological media were prepared according to the directions of the manufacturers and they include: Sorbitol MacConkey agar (SMAC) Oxoid®, Brilliance Salmonella agar (BSA) Oxoid, MacConkey agar, Eosin Methylene Blue agar, and Mueller Hinton agar.

3.2.1 Preparation of Sorbitol MacConkey Agar

A total of 28.25gm SMAC powder were weighed and suspended in 500ml of distilled water. It was then boiled for 10minutes to dissolve. After boiling it was sterilized using autoclave at 121^0C for 15minutes. After sterilization, the media was placed in a water bath at 42^0C allowed to cool and Cefixime supplement was mixed with 2ml of distilled water and added to the medium and the medium rotated gently for thorough mixing of supplement with medium and it

was then poured into sterile Petri-dishes (20ml per plate) and allowed to solidify on a level laboratory bench.

3.2.2 Preparation of Brilliance Salmonella Agar

A total of 27gm BSA powder was weighed and suspended in 500ml distilled water, mixed and boiled for 10minutes to dissolve and thereafter sterilized at 121^{o}C for 15minutes. Salmonella selective supplement (Oxoid, SRO 194E) was mixed with 2ml distilled water and was added to the homogenized BSA, this was allowed to continue boiling by sterilization. After it has all homogenized, it was allowed to cool and poured into sterile Petri-dishes (20ml per plate) and allowed to solidify on a level laboratory bench.

3.2.3 Preparation of MacConkey Agar

A total of 10.4gm MacConkey agar (MAC) powder was weighed and suspended in 200ml of distilled water and boiled for 10minutes to dissolve and thereafter the agar was sterilized by autoclaving at 121^{o}C. The sterilized medium was allowed to cool in a water bath and thereafter poured into plates and allowed to solidify on a level laboratory bench. The prepared media were then incubated at 37^{0}C to check for their sterility before use.

3.2.4 Preparation of Mueller-Hinton Agar

A total of 7.6gm Mueller-Hinton (MH) agar powder was weighed and suspended in 200ml of distilled water and boiled for 10 minutes to dissolve. The agar was sterilized by autoclaving at 121^{0}C for 15 minutes. The agar was allowed to cool in a water bath and thereafter

poured into sterile Petri dishes to solidify. The prepared media plates were incubated overnight at 37^{o}C to check for their sterility before use.

3.2.5 Preparation of Eosin Methylene Blue

A total of 6gm Eosin Methylene Blue powder was suspended in 200ml of distilled water. The agar was sterilized by autoclaving at 121^{0}C for 15 minutes. The agar was allowed to cool in a water bath and thereafter poured into sterile Petri-dishes to solidify.

3.2.6 Preparation of Nutrient Agar slant

A total of 1.3gm nutrient agar powder was dissolved in 100ml distilled water. This was boiled to dissolved and sterilized by autoclaving at 121^{o}C for 15minutes after dispensing in bijou bottles. The medium was allowed to solidify in a slanting position and later packed and stored at 4^{o}C in the refrigerator.

3. 2.7 Preparation of Urea Agar

This was prepared by suspending 2.4gm of urea base in 95ml of distilled water. It was dissolved by shaking and the suspension was sterilized by autoclaving at 121^{o}C for 10minutes. Then 2gm of urea was dissolved in 5ml of distilled water and boiled for about 30minutes. The two solutions were allowed to cool in a water bath set at 45^{o}C. The urea was added to the urea base and the mixture was then dispensed, in 5ml amounts into half ounce bottles. The media bottles were then kept in a slant position to solidify.

3.2.8 Preparation of Simmons citrate Agar (SCA)

A total of 4.6gm SCA powder was suspended in 200ml of distilled water. This was boiled to dissolve completely. It was then dispensed into half ounce bottles (5ml per bottle) and sterilized by autoclaving at 121^{o}C for 10 minutes. They were then placed in a slanting position to solidify.

3.2.9 Preparation of triple sugar iron agar (TSI)

A total of 6.5gm TSI powder was suspended in 100ml deionised water, soaked for 10minutes, swirled and mixed then boiled. It was then dispensed into tubes and sterilized by autoclaving for 15minutes at 121^{o}C. They were later paced in a slanting position to solidify, ensuring that the slant is over a butt at about 3cm deep.

3.2.10 Preparation of Brain-Heart Infusion Broth (BHI)

A total of 3.7gm BHI powder was suspended in 100mls of distilled water, soaked for 10minutes, swirled and mixed, then boiled gently to dissolve. It was then dispensed in 5ml amounts into half ounce bottles and sterilized by autoclaving for 15minutes at 121^{o}C.

3.2.11 Preparation of Selenith F. Broth

A total of 3gm Selenith F. Powder was suspended in 100mls of distilled water, this was boiled to dissove completely. The medium was sterilizedby authoclaving for 15minutes at 121^{o}C , cooled and stored at room tempreature.

3.3 Isolation of Escherichia coli and Salmonella species

Isolation of E.coli O157 was done according to the procedures described by Nataro and Kaper, (1998) while salmonella isolates was carried out as described by Leifson, (1936). Each swab sample was inoculated into buffered peptone water and incubated at 37^{o}C overnight for pre-enrichment.

For *E. coli* O157 isolation, a loopful of pre-enrichment broth was streaked on sorbitol MacConkey (SMAC) agar. Inoculated plates were incubated at 37^{o}C for 24hrs. After incubation 2 to 4 colourless colonies (non sorbitol fermenters) were subcultured on plain MacConkey agar plates. Inoculated MacConkey plates were incubated at 37^{o}C for 24hrs and thereafter a rose pink colony (lactose fermenter) from each plate was subcultured on Eosin Methylene Blue (EMB) agar. Inoculated EMB agar plates were incubated at 37^{o}C for 24hrs and observed for colonies with greenish metallic sheen appearance. Such colonies were stocked on nutrient agar slants for further confirmation using Oxoid O157 latex agglutination kit. The latex agglutination test was conducted according to manufacturer's guidelines.

For Salmonella isolation, a loopful of the pre-enrichment broth was inoculated into Selenith F. broth for selective enrichment. Inoculated broths were incubated at 42^{o}C for 18-24hrs. After selectve enrichment a loopful of the broth culture was streaked on Brilliance Salmonella agar (BSA) and inoculated plates incubated at 37^{o}C for 18-24hrs. After incubation about 2 to 4 purple colonies (presumptive Salmonella organism) were picked and each colony subcultured on plain MacConkey and inoculated plates incubated at 37^{o}C for 24hrs. Medium size colourless colonies (non-lactose fermenters) were further subjected to biochemical tests such as

urease test, methyl red test, citrate utilization, gas production, glucose fermentertion as described below.

3.3.1 Urease Test

A portion of each test colony was streaked on urea agar slant and incubated for 24hrs at 37^{o}C. Urease positive organisms turned the medium red. Salmonella is urease negative. Urease positive isolates were discarded.

3.3.2 Citrate utilization Test

A colony of the test organisms was inoculated on the surface of simmon's citrate agar slant and incubated overnight at 37^{o}C for 24hrs. The colour changed to blue indicating that the organism utilized the citrate as a sole source of carbon. Most Salmonellae grow on citrate agar.

3.3.3 Indole Test

Tryptone water was inoculated with a colony of the test organisms and incubated at 37^{o}C for 48hrs. 1ml of Kovac's reagent was run down the side of the tube into the medium. This test was for the ability of the organism to convert tryptophan (amino acid) to indole. Indole production was indicated by a deep-red colouration at the top of the broth, production of a yellow ring indicated indole negative, which is expected of Salmomellae.

3.3.4 Methyl Red Test

This test determines the ability of the test organism to ferment glucose and produce a pH of 4.5. Peptone water culture of suspected Salmonella organism was inoculated into glucose phosphate peptone water and incubated at 37^{o}C for 48hrs. Thereafter 5 drops of methyl red indicator were

run down the side of the tube. A pink ring on the surface of the medium indicated a methyl red positive reaction, salmonella is methyl red positive.

3.3.5 Reaction on triple sugar iron (TSI)

This test determines the ability of the test organism to produce hydrogen sulfide, gas and ferment glucose. A colony of the test organism was stabbed and the slant surface streaked and incubated overnight at 37^{o}C for 24hrs. Hydrogen production, gas production and glucose fermentation indicated TSI positive, which is expected of salmonella.

3.4 Sacchalytic activity of the E.coli O157 and Salmonella isolates

The ability of the isolates to ferment sugars was carried out as described by Crichton *et al.,* (1979). The following thirteen (13) sugars were used sorbitol, sucrose, dulcitol, mannitol, mannose, arabinose, glucose, lactose, maltose cellobiose, saccharose, inositol, and xylose. 0.5gm of each sugar was weighed and added to 50mls of nutrient broth, plus 0.5ml bromothymol blue indicator, dispensed into half ounce bottles and was sterilized at 121^{o}C for 10minutes. After which it was allowed to cool and a colony of each suspected organism was inoculated into the sugar broths and incubated at 37^{o}C for 24hrs.The results were recorded as either fermented which was indicated by change in colour from blue to yellow or non-fermented indicated by no change in colour (colour remains blue).

3.5 Enterotoxigenic potentials of the isolates

Overnight broth cultures were centrifuged at 1500rpm for 20 minutes and supernatant collected and transferred into new vials: Gentamycin was added at 8µg/ml and kept at room temperature

overnight, following the method described by Gianella (1976). The supernatant was used for detection of heat-labile and heat-stable (ST) toxin by suckling mice and Infant Mouse Assay (IMA) respectively.

For detection of heat-labile enterotoxins forty five Swiss Albino suckling mice of 1-4 days old were used. The mice were separated from their mother immediately before used and randomly assigned into two groups; A and B, consisting of three mice to represent each isolate. A 25µl crude culture supernatant of each test isolate was administered to the mice of group A through oral route with the help of micropipette. Mice of group B were kept as control which was dosed orally with brain-heart infusion broth. Inoculated mice were incubated at 37^{o}C for 24hrs and observed for mortality.

IMA was used for the detection of heat-stable toxin. Day old Swiss Albino mice were used for the test. The mice were inoculated orally with 0.1ml of crude culture supernatant and kept at room temperature for 4 hours, then sacrificed using chloroform anesthesia. The abdomen was opened and the entire intestine was removed. For each mouse, the weight of the gut and the remaining carcass was taken and the ratio of gut weight to carcass weight calculated. The average ratio of less than 0.070 was considered negative while 0.070-0.085 was considered positive for heat-stable toxin (Gianella, 1976).

3.6 Antibiotic sensitivity profile

Antibiogram of the test isolates was determined using disc diffusion technique (Bauer *et al.,* 1966; CLSI 2008). Antimicrobial disc were obtained from Oxoid Limited, Basingstoke Hampshire, England. Each colony of the test isolate was picked with a wire loop and inoculated into nutrient broth and incubated for 3hours. The turbidity of each broth culture was adjusted to

correspond to 0.5 McFarland turbidity standards (corresponding to approximately 10^8cfc/ml). Each standardized broth culture was used to inoculate the surface of the Mueller-Hinton (MH) agar plates and the excess fluid drained into disinfectant jar. The surface of each inoculated plate was allowed to dry. Using a disc dispenser, the antibiotic discs were aseptically placed on the surface of the inoculated agar plates and then incubated for 24hrs at 37^0C. After incubation, the plates were examined for zones of inhibition round each disc. The diameters of the zones were measured with a meter rule and recorded. Each test was conducted three times and the mean inhibition zone diameter (IZD) recorded to the nearest millimeter. Each test isolates was classified as either resistance, intermediate or sensitive to the test antibiotics in accordance with the criteria given by NCCLS (2008). However, for the purpose of this study, all bacteria showing intermediate susceptibility were recorded as being susceptible.

CHAPTER FOUR

RESULTS

4.1 Prevalence of *E.coli* O157 in Nsukka slaughterhouse meat contact surfaces and sales tables

The prevalence of *E.coli* O157 on meat contact surfaces and sales tables is shown in Table 1. Out of the 448 samples collected from the slaughter house floor, slabs, water in meat holding drums and sales tables, 3(0.68%) were positive for *E.coli* O157.

4.2 Prevalence *Salmonella* species in Nsukka slaughter house meat contact surfaces and sales tables

The prevalence of Salmonella on meat contact surfaces and sales tables is shown in Table 2. Out of the 448 samples collected from the slaughter house floor, slabs, water holding drums and sales tables, 10 (8.93%) were positive for Salmonella species.

4.3 Sugar fermentation activity of *E.coli* O157

The results of sugar fermentation activities of *E.coli* O157 showed that all (100%) of the isolates fermented -glucose, sucrose, lactose, mannitol, dulcitol, saccharose, arabinose, maltose, cellobiose, xylose and mannose, while non of the three isolates fermented inositol and sorbitol (Table 3).

4.4 Sugar fermentation activity of *Salmonella* species

The results of the sugar fermentation activites of *salmonella* isolates are presented in Table 5. Glucose, mannitol, dulcitol, saccharose, cellobiose and mannose were fermented by all

the 10(100%) *Salmonella* isolates while 8(80%) of the isolates fermented xylose. None of the Salmonella isolates fermented sucrose, inositol, lactose, arabinose and maltose.

4.5 Enterotoxigenic potential of *E.coli* O157

4.5.1 Heat-labile enterotoxin production

The effect of oral adiministration of *E.coli* O157 supernatants is shown in Table 5. All the the mice inoculated with each of the test *E.coli* O157 supernatant died within 24 hours following inoculation, indicating that the strains produce heat-labile enterotoxin. None of the mice inoculated with BHI broth died or none 0157 *E.coli* (normal flora) died.

4.5.2 Heat-stable enterotoxin production by *E.coli* O157

The results of infant mice assay are shown in Table 6. As shown in the table the average gut weight to carcass ratio produced by the three *E.coli* strains ranged from 0.044-0.062. None of the mean gut to carcass ratio fell within the cut-off value (0.070) for positive heat-stable enterotoxin production indicating that none of the test isolates produced heat-stable enterotoxin.

4.6 Enterotoxigenic potential of *Salmonella* species

4.6.1 Heat-labile enterotoxin production

The effect of oral administration *salmonella* species supernatant is shown in Table 7. As shown in the table all isolates were found to produce enterotoxins since mice inoculated with supernatants of the test isolates died within 24 hours of incubation while mice inoculated with Brain Heart infusion broth did not die.

4.6.2 Heat-stable enterotoxin production by *Salmonella* species

The results of infant mice assay are shown in Table 8. As shown in the table the average gut weight to carcass ratio produced by each of the ten *Salmonella* strains ranged from 0.047-0.056. None of the mean gut to carcass ratio fell within the cut-off value (0.070) for positive heat-stable enterotoxin.

4.7 Antibacterial resistance profile of the *Escherichia coli* O157 isolates

Results of the antibacterial resistance profile of the *E.coli* O157 strains are presented in Table 9. The results showed that among the 3 *E.coli* O157 isolated, none was resistant to gentamicin, ceftriaxone, enrofloxacin, streptomycin, and cefotaxime, and 3(100%) resistant to ampicillin and ceftazidime.

4.8 Antibacterial resistance profile of the *Salmonella* isolates

The antibacterial resistance profile of the *Salmonella* isolates is presented on Table 10 As shown on the table, none of the isolates was resistance to enrofloxacin and 2(20%) were resistant to gentamicin and ceftriaxone while 10(100%) were resistant to ampicillin and ceftazidime and 8(80%) resistant to ciprofloxacin, cefotaxime and aztreonam.

4.9 Resistance patterns of *E.coli* O157 isolated from Nsukka slaughter house meat contact surfaces and environment

The three *E.coli* O157 strains exhibited two resistance patterns as shown in Table 11.

4.10 Resistance patterns of *Salmonella* isolated from Nsukka slaughter house meat contact surfaces and environment

The ten *Salmonella* strains exhibited eight resistance patterns with sulphamethoxazole-ceftazidime-ampicillin-cefotaxime-aztreonam-chloramphenicol-ciprofloxacin being the most prevalent (Table 12).

4.11 Number of antibacterial agents *E. coli* O157 and S*almonella* strains were resistant to

Of the three *E.coli* O157 strains, two were resistant to two antibacterial while one was resistant to more than five antibacterial agents. Of the ten *Salmonella* strains three were resistant to five antibacterial while seven were resistant to more than five antibacterial agents (Table 13).

Table 1 Prevalence rates of E.coli O157 in Nsukka slaughter house meat contact surfaces and sales tables

Sample Sites	No of Sample Tested	No of Samples Positive (%)
Slaughter Slabs	**112**	**1(0.9)**
Slaughter Floor	**112**	**1(0.9)**
Drums	**112**	**0(0)**
Sales Tables	**112**	**1(0.9)**
Total	**448**	**3(0.68)**

Table 2 Prevalence rates of *Salmonella* species in Nsukka slaughter house meat contact surfaces and sales tables

Sample Sites	No of Samples Tested	No of Samples Positive (%)
Slaughter Slabs	**112**	**0(0)**
Slaughtre Floor	**112**	**2(1.79)**
Drums	**112**	**4(3.57)**
Sales Tables	**112**	**4(3.57)**
Total	**448**	**10(8.93)**

Table 3 Sugar fermentation activity of *E.coli* O157 isolates

Isolate	Glu	Suc	Ino	Lac	Manl	Sor	Dul	Sacc	Arab	Mal	Cello	Xyl	Mann
E.c f 20	+	+	-	+	+	-	+	+	+	+	+	+	+
E.c s 44	+	+	-	+	+	-	+	+	+	+	+	+	+
E.c s 44	+	+	-	+	+	-	+	+	+	+	+	+	+
% Positive	100	100	0	100	100	0	100	100	100	100	100	100	100

Glu = Glucose, Suc =Sucrose, Ino = Inositol, Lac = Lactose, Manl = Mannitol, Sor = Sorbitol, Dul = Dulcitol, Sacc = Saccharose, Arab = Arabinose, Mal = Maltose, Cello = Cellobiose, Xyl = Xylose, Mane = Mannose.

+ = fermenter, - = non-fermenter

Table 4 Result of sugar fermentation activity of *Salmonella* isolates

Isolates	Glu	Suc	Ino	Lac	Manl	Sor	Dul	Sacc	Arab	Mal	Cello	Xyl	Mane
D 24	+	-	-	-	+	-	+	+	-	-	+	+	+
F 22	+	-	-	-	+	-	+	+	-	-	+	-	+
F 20	+	-	-	-	+	-	+	+	-	-	+	+	+
D 36	+	-	-	-	+	-	+	+	-	-	+	+	+
SS 23	+	-	-	-	+	-	+	+	-	-	+	+	+
D 40	+	-	-	-	+	-	+	+	-	-	+	-	+
ST 13	+	-	-	-	+	-	+	+	-	-	+	+	+
D 19	+	-	-	-	+	-	+	+	-	-	+	+	+
ST 31	+	-	-	-	+	-	+	+	-	-	+	+	+
SS 20	+	-	-	-	+	-	+	+	-	-	+	+	+
%	100	0	0	0	100	0	100	100	0	0	100	80	100
Positive													

+ = fermenter

- = non-fermenter

Table 5 Effect of oral administration of E.coli 0157 supernatant on mortality of suckling mice

Isolates	No injected/No dead	% Mortality	Inference
E.coli F 7	3/3	100	LT-Positive
E.coli SS 44	3/3	100	LT-Posiive
E.coli ST 44	3/3	100	LT-Positive
Control (BHI Broth)	3/3	0	LT-Negative
Control (none O157)	3/3	0	LT-Negative

LT = Heat-labile enterotoxin

BHI = Brain Heart Infusion broth

Table 6 Effect of oral adminisration of *E.coli* O157 using Infant Mouse Assay (IMA)

Isolates	Gut wt.: Carcass wt. ratio				Inference
	Mouse A	MouseB	MouseC	Mean Ratio	
E.coli F. 7	0.045	0.052	0.055	0.051	ST- Negative
E.coli SS. 44	0.082	0.049	0.056	0.062	ST- Negative
E.coli ST.44	0.080	0.052	0.051	0.060	ST-Negative
Control(BHI)	0.044	0.055	0.044	0.048	ST-Negative
Control(non O157)	0.045	0.052	0.055	0.051	ST-Negative

ST=Heat-stable enterotoxin

Table 7 Effect of oral administration of Salmonella species supernatant on mortality of suckling mice

Isolates	No injected/No dead	% Mortality	Inference
D 24	3/3	100	LT-positive
F 22	3/3	100	LT-positive
F 20	3/3	100	LT-positive
D 36	3/3	100	LT-positive
SS 23	3/3	100	LT-positive
D 40	3/3	100	LT-positive
ST 13	3/3	100	LT-positive
D 19	3/3	100	LT-positive
ST 31	3/3	100	LT-positive
SS 20	3/3	100	LT-positive
Control (BHI)	3/0	0	LT -negative

LT= heat labile enterotoxin

BHI= Brain Heart infusion broth

Table 8 Determination of heat-stable (ST) Toxin of Salmonella sp. by Infant Mouse Assay (IMA)

	Gut weight: Carcass weight ratio				
Isolates	**Mouse A**	**Mouse B**	**Mouse C**	**Mean Ratio**	**Inference**
D 24	0.049	0.056	0.045	0.050	ST-Negative
F 22	0.051	0.049	0.046	0.049	ST-Negative
F 20	0.047	0.046	0.056	0.050	ST-Negative
D 36	0.051	0.041	0.051	0.048	ST-Negative
SS 23	0.044	0.056	0.056	0.052	ST-Negative
D 40	0.055	0.046	0.060	0.054	ST-Negative
ST 13	0.051	0.046	0.056	0.051	ST-Negative

ST=Stable-heat enterotoxin

BHI= Brain Heart Infusion

Table 9 Antibacterial resistance profile of the 3 *E.coli* O157 isolates

Antibacterial Agents (Disc potency)		**No (%) Resistant isolates**
Chloramphenicol	(30µg)	1(33%)
Ciprofloxacin	(5µg)	1(33%)
Gentamicin	(10µg)	0
Ampicilin	(10µg)	3(100%)
Aztreonam	(30µg)	1(33%)
Ceftriaxone	(30µg)	0
Sulphamet/Trimet	(25µg)	1(33%)
Ceftazidime	(5µg)	3(100%)
Enrofloxacin	(5µg)	0
Streptomycin	(10µg)	0
Cefotaxime	(30µg)	0

Table 10 Antibacterial resistance profile of 10 Salmonella isolates

Antibacterial Agents (Disc potency)	No (%) resistance isolates
Chloramphenicol (30µg)	7(70)
Ciprofloxacin (5µg)	8(80)
Gentamicin (10µg)	2(20)
Ampicilin (10µg)	10(100)
Aztreonam (30µg)	8(80)
Ceftriaxone (30µg)	2(20)
Sulphamet/Trimet (25µg)	6(60)
Ceftazidime (5µg)	10(100)
Enrofloxacin (5µg)	0
Streptomycin (10µg)	4(40)
Cefotaxime (30µg)	8(80)

Table 11 Resistance pattern of *E.coli* O157 isolated from Nsukka slaughter house meat contact surfaces and environment

Resistance pattern	No(%) of isolates
SXT + CAZ + AMP + ATM + C +CIP	1(33.3%)
CAZ + AMP	2(67%)

STX-Sulfamethoxazole/Trimethoprim, CAZ-Ceftazidime, AMP-Ampicillin

C-Chloramphenicol, CIP-Ciprofloxacin

Table 12 Resistance patterns of Salmonella isolated from Nsukka slaughter house meat contact surfaces and environment

Resistance Patterns	No(%) of isolates
SXT + CAZ + AMP + CRO + CTX + S + CN + ATM	1(10%)
SXT + CAZ + AMP + CRO + CTX + ATM + CIP	1(10%)
SXT + CAZ + AMP + CTX + C + CIP	1(10%)
SXT + CAZ + AMP + CTX + ATM + C + CIP	3(30%)
CAZ +AMP + S + CN + ATM + C + CIP	1(10%)
CAZ + AMP + S + C + CIP	1(10%)
CAZ +AMP + CTX + S + ATM	1(10%)
CAZ + AMP + CTX + ATM + C + CIP	1(10%)

SXT-Sulphamethoxazole CAZ-Ceftazidime AMP-Ampicillin CTX-Cefotaxime

S-Streptomycin CN-Gentamicin ATM-Aztreonam C-Chloramphenicol

CIP-Ciprofloxacin CRO-Ceftriaxone

Table 13 Number of anibacterial agents E. coli O157 and salmonella strains was resistant to

No of Drugs resistant to strains	E.coli O157	Salmonella
1	-	-
2	2	-
3	-	-
4	-	-
5	-	3
>5	1	7

CHAPTER FIVE

DISCUSSION AND CONCLUSION

5.1 DISCUSSION

Escherichia coli O157 isolation rate of 0.68% recorded in this study is lower than the 1.7% recorded by Oguejiofor and Chah (2010) in cattle slaughtered at Nsukka slaughter house. The higher isolation recorded by Oguejiofor and Chah (2010) in the same study area may be due to the fact that they used faecal samples collected from animals prior to slaughter while in the present study swabs of meat contact surfaces were used as culture materials. Akkaya *et al.,* (2008) reported a prevalence of 15, 10, 10 and 0% of E.coli O157 in abattior environment, equipment, abattior workers and water respectively in Turkey. In United States (Hancock *et al.,* 1997), Britain (Paiba *et al.*, 2002) and Italy (Bonarrdi *et al.,* 1999) reported a prevalence range of 1.8 - 7.5% in cattle brought for slaughter. However, other studies in UK (Low *et al.*, 2005), Canada (Van Donkersgoed *et al.*, (1999), US (Elder *et al,* 2000) reported a higher prevalence between 12 and 28%. The low prevalence in this study may also be attributed to time of sample collection (Sept to Dec) since this period is defined as period of low prevalence by Hancock *et al*., (2001). Gansheroff and O' Brien, (2000); Heuvelink *et al*., (1998) and Chapman *et al.,* (1997) in their studies demonstrated seasonal influence in occurance of *E.coli* O157 with prevalence of 38% in cattle faece in the spring and 4.8% during the winter. Seasonal variations

of *E. coli* O157 in food poisoning were reported by CDC (2000); Infectious Diseases and Immunization Committee, Canadian Pediatric Society, 1995. The differences in the prevalence of *E.coli* O157 from different studies is complicated by the fact that shedding of the organism may be transient and is almost certainly influenced by a range of factors e.g. diet, stress, population density, geographical region, and season (Clarke *et al.,* 1994, Kudva *et al.,* 1996). Furthermore, prevalence estimates are influenced by the differences in sensitivites of numerous methods used for detecting *E.coli* O157 (Rice *et al.*, 2003).

The 8.92% *Salmonella* isolation rate recorded in this study is similar to the 10% recorded in the same abattior by Abiade-Paul *et al.,* (2006) from samples collected at different points along the collecting channels of the effluent. The prevalence of *Salmonella* recorded in this study was lower than the 25% prevalence reported by Amaechi and Ezeronye (2006), from swab samples collected from slaugtering tables, wash hand basins and floor in abattior environment at Umuahia, Abia State, Nigeria. The detection of *Salmonella* on the slaughter floor and meat holding drums agrees with Mckeen *et al.,* (2001), who pointed out that cattle and swine are the principal sources of *Salmonella* contamination in abattior and that slaugtering process promotes dissemination of the contamination. The isolation of the bacteria from the meat holding drums further proved the cross-contamination of carcass by abattior workers.

The level of contamination in Nsukka slaughterhouse meat contact surfaces and sales environment with *E.coli* O157 and *Salmonella* spp. could be as a result of such factors as high number of cattle from different ranches slaughtered per day and poor sanitation practices associated with the environment. Contamination of these contact surfaces by zoonotic bacteria constitutes serious environmental and public health hazards since these contact surfaces may be washed and the effluent channelled to drainage system or washed by flood into market places,

major roads and streets, streams, and farmlands. In the market and streets, food items on sale are observed displayed in close proximity to this effluent, and this food may be contaminated by these pathogenic zoonotic bacteria. Consumption of such contaminated food may result in food poisoning (Okolocha *et al*., 2002; Nwanta *et al.*, 2010; Abiade-Paul *et al*., 2006; Oguejiofor and Chah, 2010). In the study area there seems to be no available reports on human *E.coli* O157 and *Salmonella* infection established to be associated with consumption of food item sold around the abattoir. However, this does not suggest that such situations may not exist but could rather be attributed to the fact that such cases are never reported and epidemiological associations are hardly followed up. Contamination level of *Escherichia coli* O157 and *Salmonella* species at Nsukka slaughterhouse meat contact surfaces may be reduced by applying harzard analysis critical control points (HACCP) procedures. Since *E.coli* O157 and *Salmonella* species can cause food poisoning, it is an obligation to eliminate or minimize the presence of these bacteria (Lowe *et al*., 2001). Attention needs to be given to training and supervision to ensure appropriate cleaning and sanitation procedures to reduce or eliminate cross-contamination (Kusumaningrum *et al*., 2003).

In this present study the three *E.coli* O157 isolates that did not ferment sorbitol and inositol were confirmed as *E.coli* O157. Thus, none fermentation of these two sugars can be used as phenotypic marker for detection of O157 (Bopp et al, 1987, Farmer, et al 1985, Haldane et al 1986, March and Ratnam, 1986). Fermentation of other substrate within 24hours of incubation was indicated by all the 3 *E.coli* O157 isolates. For *Salmonella* isolates the 80% xylose fermentation can be used as phenotypic marker to detect *Salmonella* since *Salmonella typhi and Salmonella paratyphi* are xylose non fermenter (Brenner and MeWhorter-Murlin., 1998). Betty *et al.,* (1980) explained that dulcitol fermentation is one of the characteristics of distinguishing

*Salmonell*a from other groups of the family Enterobacteriaceae. The findings of this study showed that 100% of Salmonella strains were fermented by dulcitol. However, the 100% Salmonella fermentation of glucose, mannose, mannitol, cellobiose and saccharose is in line with the findings of Charles, (1945), Yoshikazu, *et al., (*1960).

Enterotoxigenic *E.coli* O157 and *Salmonella* isolates were detected based on mortality and survivability of 3-day old mice for each isolate within 24 hours of post-inoculation. All the the mice inoculated with each of the test *E.coli* O157 supernatant died within 24 hours following inoculation, and none of the control mice inoculated with BHI broth died. For Salmonella species, all isolates were found to produce enterotoxins since the inoculated mice died within 24 hours of incubation and the 3 for control injected with BHI survived. This result is in agreement with Hossain *et al.,* (2008), Yamamoto and Yokota, (1983) who stipulated that the toxic effects could be due to heat-labile (LT) toxin produced which lead to the death of the mice used. The result of Infant Mouse Assay (IMA) showed that all the *E.coli* O157 and *Salmonella* species isolates, tested using IMA were found negative for heat stable (ST) toxin.

Haemorrhagic isolates of *E.coli* have relatively high poterntials for developing resistance (Karlowsky *et al.,* 2004). A high resistance of ampicilline was observed for *E.coli* isolates. This is in agreement with the report of Schroecler *et al* (2002) who recorded 100% resistance in their study. Aibinu *et al*., (2004) and Kwaga *et al*, (1984) also recorded multidrug resistant to *E.coli* isolates from abattior effluent. This drug resistance might be due to frequent use of drugs in Veterinary practice at sub-optimal concentration. The *E.coli* O157 strain in this study were senstivity to gentamycin, enrofloxacin, streptomycin, ceftriaxone and cefotaxime and this finding is in agreement with the work of Osek, (2004). *Salmonella* isolates were resistant to ceftazidime, ampicillin, cefotaxime, aztreonam, chloramphenicol and ciprofloxacin. This is of serious public

health concern since these drugs are still considered the most efficacious and recommended for treatment of complicated Salmonella infection in man and animal (Greenwood, *et al*, 1992, Shehu, *et al.,* 1990). The 80% sensitivity of Salmonella to ceftriaxone recorded in this study is similar to the work of Karachi (1992) and Syed, (2010) who reported 87.2% sensitivity. This means that ceftriaxone can still be used effectively for the treatment of *Salmonella* infections.

A report of transmission of resistant strains to people, and transmission of multi-resistance encoding determinants from these bacteria of animal origin to human strains has been established (Oppegaard *et al.,* 2001). The large number of resistance patterns recorded in this study as well as previous works (Chah *et al.,* 2003) suggests that several selective pressures may be involved in the induction of drug resistance among the strains. The presence of these strains with high rates of resistance to commonly avaliable antimicrobial agents, pose a threat to veterinarians, abattior workers, consumers and people living close to the abattior because they represent a 'gene pool' from which drug resistant infections might arise.

All the isolates in this study exhibited multiple resistances to antibacterial agents used. Similar findings have been reported from this abattior (Abiade-paul *et al*., 2006). Due to indiscriminate exploitation of antimicrobial agents, such high incidence of multi drug resistant organisms may apparently occured and ultimately replaces the drug sensitive microorganisms from antibiotic saturated environment (Van den Boogard and Stobberingh, 2000). Conjugal transfer of drug resistant determinants in bacterial has been reported to occur over species and genus border (Teuber *et al.,* 1996). Transfer of drug resistance factors to human pathogens such as Salmonella and E.coli strains will render human infections caused by these pathogens difficult to treat with the commonly available antimicrobial agents. The strains may colonise the human

intestines where it transfers its resistance factor (R-factor) to human indigenious flora (Chah *et al*, 2003).

5.2 CONCLUSION

This study has shown that *E.coli* O157 and *Salmonellae* are present on meat contact surfaces in Nsukka Slaughterhouse. This suggests that meat from these contact surfaces are likely to be contaminated by these organisms and may predispose the consumers to food poisoning.

The findings from this study have also shown that the zoonotic pathogens from the meat contact surfaces are multiple antibiotic resistance to antibacterial agents used. This development is of serious public health significance because the resistant isolates may be transferred to the consumers of such meat who will subsequently develop resistance to the therapeutic agents. The bacterial isolates are also enterotoxigenic elaborating heat-labile enterotoxins.

RECOMMENDATIONS

1. Local government authorities should enforce proper hygiene maintainance within the slaughterhouse environment, using clean water and disinfectants in washing meat contact surfaces and sales tables thoroughly before and after use.
2. Provision of appropriate facilities such as chilling room and overhead rail that helps to minimize contamination is imperative.

Although sterile production of meat is not possible, the processes involving slaughtering, dressing, and handling must be geared towards minimizing the number of potential

pathogenic organisms this can be achieved by separation between ‘dirty’ and ‘clear’ operation.

3. Implementation of Hazard Analysis Critical Control Point (HACCP) principles in the slaughterhouse meat processing chain is highly recommended to reduce gros contamination of meat.
4. Livestock farmers, animal health workers and veterinarians should avoid indiscriminate use and abuse of antibiotics in the management of livestock diseases and health problems
5. Consumers of meat should cook their meat properly before consumption.

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