THE PREVALENCE AND ANTIBIOGRAMS OF SALMONELLA AND E. COLI ISOLATED FROM MEAT, MILK, BOVINE FAECES AND HUMAN STOOL IN ZARIA

A Thesis Presented to the Postgraduate School,
Ahmadu Bello University, Zaria

BY

MATHEW DIOH ESONA
B.Sc (HONS), (1994), Usman Danfodio University, Sokoto

In Partial Fulfillment of Requirements
for the
Degree of Master of Science
(MICROBIOLOGY)

Department of Microbiology
Ahmadu Bello University, Zaria, Nigeria

CERTIFICATION

This thesis titled "THE PREVALENCE AND ANTIBIOGRAMS OF SALMONELLA AND ESCHERICHA COLI FROM MEAT, MILK, BOVINE FAECES AND HUMAN STOOL IN ZARIA" by MATHEW DIOH ESONA meets the regulations governing the award of the degree of Master of Science of Ahmadu Bello University, Zaria and is approved for its Scientific contribution to knowledge and literary presentation.

Dr. (Mrs) V.J. Umoh
Chairperson, Supervisory Committee — 16-9-97 Date

Dr. J.K.P. Kwaga
Member, Supervisory Committee — 16-9-97 Date

Dr. J.B. Ameh
Head of Department of Microbiology — 26/1/98 Date

Dean, Post-Graduate School
Ahmadu Bello University, Zaria. — 29/5/98 Date

DECLARATION

I hereby declare that the work presented in this thesis is original. It was carried out by me under the supervision of Dr. (Mrs.) V.J. Umoh, and Dr. J.K.P. Kwaga in the laboratory of Microbiology, Ahmadu Bello University, Zaria.

Works of other investigators have been cited and are duly acknowledged. No part of this thesis has previously been submitted for a degree or any other qualification.



M.D. ESONA
Ahmadu Bello University,
April 1997.

DEDICATION

This work is dedicated to My Dad, Mom, Step Mom and to the entire Ituka's family.

ACKNOWLEDGEMENT

In the course of undertaking this study, I have depended on the assistance of numerous individuals. The successful completion of this research would not have become a reality but for the contributions of these people. While it is impossible to mention the names of all those who helped me, there are some to whom I am so indebted to, that I must thank them individually.

First and foremost, my deep appreciation go to my Supervisor, Dr. (Mrs) V.J. Umoh for her guidance, cooperation, patience and expertise.

My thanks is ineffable to my second Supervisor, Dr. J.K.P. Kwaga, Head of Department of Veterinary Public Health and Preventive Medicine, Ahmadu Bello University, Zaria. He did not only allow me the use of all his laboratory materials, but also gave me some academic materials to read. Infact, his constructive suggestions, contributions and penetrating questions kept the study on an even keel.

I am grateful to the entire staff members of the Department of Microbiology, A.B.U., Zaria, especially Mr. E.D. Jatau and Mr. Machido D.A. who provided the sensitivity discs used in this research. They also made valuable suggestions which led to the completion of this research.

I particularly wish to acknowledge the interest and concern of the Director of Sick Bay, A.B.U., Zaria, Dr. A. Usman and the Zongon abattoir Manager, Mr. Abdumalik Sambo who contributed in no small measure to the provision of samples. Also to the staff of the Department of Medical Microbiology, A.B.U.T.H, Tudun-wada, who were more of friends and members of family to me than staff, I say "thank you".

My sincere gratitude goes to my friends, especially, Teba, T.; Fonge, F.; Yenkong, R.; Awasum, A.; Nfor, N.; Egbom, C.E. and Edith, T.; whose constant

encouragement, unequivocal faith, and sincere belief in me, guided this research to a successful completion.

My indebtedness, grateful and sincere gratitude to Ethel, T., for understanding that knows no bounds.

My thanks also goes to my brothers and sisters particularly to my Senior brother, Mr. Maurice Ituka for untold hours of assistance.

I also extend my appreciation to Miss Patience Attai for typing this work.

I would like to acknowledge the gratitude which I owe to my dad, Mr. M.E. Ituka, Mom. Mrs. Sussana Ituka and Step-Mom, Mrs. Sophie Ituka for their parental care, untiring supports, concern and understanding.

Finally, to God be the glory.

A B S T R A C T

A total of 700 specimens comprising of 50 specimens each of raw meat, "suya", "Kilishi", Lymph nodes, bile, kidney and 100 specimens each of fresh milk, fermented milk ("nono"), bovine rectal swab and human stool were examined for Salmonella and Escherichia coli. Salmonella - Shigella agar and Eosin methylene blue agar were used as isolation media for Salmonella and E. coli respectively.

The range of aerobic plate and coliform counts in some specimens analysed were 5.0 x 10^1 - 4.95 x 10^J CFU/g and 1.0 x 10^1 - 5.05 x 10^1 CFU/g of unprocessed meat products (raw meat and kidney) respectively. For the processed meat products ("Suya" and Kilishi) the range of aerobic plate and coliform counts were 2.0 x 10^l - 6.3 x 10^J CFU/g and 1.0 x 10^1 - 5.0 x 10^T CFU/g of specimens respectively. The range of aerobic plate and coliform counts of fermented milk ("nono") were 5.0 x 10^s - 4.95 x 10^1 CFU/ml and 1.05 x 10^1 - 4.85 x 10^5 CFU/ml of specimen respectively, with a pH range of 4-5.2. For fresh milk, the range of aerobic plate and coliform counts were 5.0 x 10 - 6.0 x 10 CFU/ml and 1.0 x 10 - 4.5 x 10 CFU/ml of specimen respectively.

Ten Salmonella isolates and fifty nine E. coli isolates were obtained. The isolation frequency of Salmonella were human stool (3%), raw meat (630 and lymph nodes (8%). Salmonella was not isolated from bovine rectal swabs, from processed meat, bile, kidney, fermented milk (nono) and fresh milk. All the isolates biochemically identified as Salmonella agglutinated in Salmonella polyvalent "O" group A-S antiserum.

The Salmonella isolates were tested for susceptibility to nine antimicrobial agents and nine of the isolates were sensitive to Tetracycline and Nalidixic acid. All the isolates were resistant to Ampicillin, Cotrimoxazole and Chloramphenicol. The percentage of isolates found

to be resistant to four or more, five or more, six or more and seven or more drugs were 100. 70 and 50 respectively. Of the six patterns of multiple resistance seen, the most common was cotrimoxazole - streptomycin -Nitrofurantoin-Augmentin-Gentamicin-Ampioillin- chloramphenicol occurring in five isolates. None of the isolates was resistant to all the drugs tested.

The minimum inhibitory concentration (MIC) for all the nine Salmonella isolates were 3ug/ml for Ampicillin, 0.8ug/ml for Gentamicin, 2ug/ml for Streptomycin and 1.5ug/ml for Chloramphenicol.

Out of the fifty nine E. coli isolates, 19(32.2%) were classified enteropathoccenic E. cpli. The percentage isolation of E. coli from the following specimens were as follows: human stool (16%), raw meat (18%), "suya" (4%), Lymph node (20%). bile (30%), kidney (4%), fermented milk (nono) (1%) and fresh milk (4%). E. coli was not isolated from bovine rectal swab and "Kilishi".

Thirty five E. coli isolates were tested for susceptibility to eight antimicrobial agents and one isolate from raw meat was resistant to all the drugs. All thirty five E. coli isolates were resistant to Cotrimoxazole and Nitrofurantoin. Out of the thirty five E. coli isolates tested for susceptibility, only the patterns for the 19 enteropathogenio E. coli were studied. Ten patterns of resistance were obtained and the most common pattern was Ampicillin - Augmentin - Nalidixic acid - Nitrofurantoin -Tetracycline - Cotrimoxazole occurring 4 times. The MIC of Tetracycline, Ampicillin, Gentamicin and Streptomycin for all studied enteropathogenio E. cpli were 6ug/ml, 6ug/ml,1.6iig/ml, and 3ug/ml, respectively.

The presence of Salmonellae and enteropathogenic E. coli in any of the specimens could be a health risk as Salmonellosis and E. coli associated gastro-intestinal disorder may result. Furthermore, it was observed that multiple resistance to antibiotics in current application is widespread amongst the local isolates of Salmonellae and E. cpli.

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CHAPTER ONE

INTRODUCTION

The infectious and contagious nature of Salmonella and the diseases it cause was demonstrated by Budd in 1856 (Burrows, 1973). Salmonellae are a major cause of food-borne infections worldwide (Greenwood et al., 1992) and concern about the organism has increased since its first isolation because of its effects on health, food and economy of man (Al-Hindawi and Rished, 1979). Apart from causing heavy economic losses to the animal industry, enteric infections caused by salmonellae have assumed great zoonotic importance chiefly due to their ubiquitous distribution, the growing number of serotypes, wide host spectrum, complex pathogenesis and the complicated epidemiological chain involving animals, man and the environment (Tiwari and Prasad, 1972). Also the ubiquity of this group of bacteria and with few exceptions, the lack of host specificity displayed by these pathogenic organisms emphasize both their economic importance and danger to public health (Assoku and Corkish, 1975).

The intestinal tracts of animals are the major source of salmonellae in nature and approximately 1% to 3% of all domestic animals are infected with these organisms (Litchfield, 1980). Salmonellae have been found in all domestic mammals and birds and have been isolated from a wide range of wild and zoo animals including bats, birds, rats, mice, squirrels, elephants, lions, snakes, lizards, fishes and the environment (Bryan, 1980; D'Aoust et al., 1980; Kwaga et al., 1984; Tabukum, 1990).

Animal products are the most common source of salmonellosis for man (Anderson, 1972). The vehicles of transmission include, meat and meat products, poultry, egg and egg products, milk and milk products, fish and marine products, cereals and vegetables, water and drugs and therapeutic agents of animal origin (Morgan, 1965; Silliker, 1980, Bryan, 1981; Atanda and Ikenebomeh, 1991; Morgan et al., 1994). Contaminated products cause disease as a result of inadequate cooking or cross-contamination in the kitchen environment (Bryan, 1981). Other modes of transmission which have accounted for a significant number of outbreaks include person-to-person spread and pet-to-person exposures (WHO, 1983).

Members of the genus have been recognised for many years and are responsible for the disease of man. The degree of host adaptation varies and affects the pathogenicity for man in three ways:

(1) Serovars adapted to man such as Salmonella typhi, S. paratyphi and S. sendai; usually cause grave diseases with septicaemic-typhoidic syndrome (enteric).

(2) Ubiquitous serovars such as S. typhimurium which affect both man and a range of animals cause gastro-intestinal infections of varying severity (but usually less severe than enteric fever). In addition to classical food poisoning, these serovars are involved in infantile and travellers' diarrhoea.

(3) Serovars which are highly adapted to an animal host such as S. abortus-ovis (sheep) and S. gallinarum (poultry) usually produce none or very mild symptoms in man. Also S.

choleraesuis which has the pig as the primary host, also causes a severe systemic illness in man (NRC, 1985).

Despite the fact that in many countries methods of control have been well established, Salmonella remains the most important reported cause of food infection and recent years have seen both massive outbreaks and major new vehicles of infection (Okolocha, 1994).

In Nigeria extensive literature on Salmonella exist especially when it comes to food animals, but accurate epidemiological data are not available. The surveillance and reporting are extremely poor, making it difficult to estimate the true situation (Okolocha, 1994). However, more work needs to be done (Olayemi, 1978; Kwaga et al., 1989).

Although E. coli has long been recognised as a normal inhabitant of the gastro-intestinal tract of man and animals, under certain conditions it may assume the role of a pathogen, especially in the invasion of organs anatomically related to the intestinal tract such as appendix, gall bladder, peritoneal cavity, kidney and bladder (Goullet et al., 1994). On several occasions it has been responsible for acute gastro-enteritis, diarrhoea and extra-intestinal infections (Goullet et al., 1994).

Enterotoxigenic strains of E. coli (ETEC) have been associated with epidemic and sporadic outbreaks of infantile diarrhoea in humans and some diarrhoeal diseases in domestic animals (Adetosoye, 1980). Recent food-borne outbreaks of haemorrhagic colitis caused by enterohaemrrhagic E. coli (EHEC) have heightened the demand for rapid detection of this organism in

food. Strains classified as EHEC posses the virulence characteristics of both enteropathogenic E. coli and Verotoxigenic E. coli (VTEC), a larger group of E. coli producing exotoxins known as vertoxins (also termed Shiga-like toxins) that are linked causally to haemolytic uraemic syndrome (Karmali, 1989). Since the recognition of E. coli 0157:H7 as a food-borne pathogen in 1982 (Padhye and Doyle, 1992) about 16 documented outbreaks of food borne infection have been recorded (Mermelstein, 1993). So far the most prevalent of the numerous serotypes of VTEC implicated in human disease is E. coli 0157:H7 (Karmali, 1989; Griffin and Tauxe, 1991; Thomas et al., 1993).

E. coli has been isolated from water as a faecal contaminant and from various foods and food products of animal origin including market raw milk (Sack et al., 1977; Bonita and Steven, 1980; Adesiyun et al., 1983; Alabi and Adesiyun, 1986). The organism has also been implicated in food-borne and water-borne outbreak world wide (Schroeder et al., 1968). Of the various locally produced food products in Nigeria, a report exists on a study of E. coli contaminants on "suya", a roasted beef product (Igene, 1983).

Although some reports exist on the enterotoxigenicity of E. coli strains isolated from animals in Nigeria (Adetosoye et al., 1976), information is not available on food isolates (Shehu and Adesiyun, 1990). This work was carried out with the following specific objectives:

1. To determine the aerobic plate and coliform counts in fresh milk, fermented milk ("nono") "suya", "kilishi", kidney and raw meat.

2. To determine the recovery frequency of Salmonella and E. coli from raw meat, fresh milk, fermented milk ("nono"), "suya", "kilishi", kidney, bile, lymphnodes, rectal swab and human stool.
3. To determine the antibiogram of the Salmonella and E. coli isolates.

CHAPTER TWO

LITERATURE REVIEW

A. SALMONELLA:

2.1.0 HISTORICAL BACKGROUND

Before the development of modern bacteriology and the isolation of the Typhoid bacillus, William Budd, in 1856, made a strikingly accurate study of typhoid fever out breaks which led him to believe that the disease was contagious and that the infectious agents was excreted in the faeces of patients. Salmonella typhi was the first member of the genus to be recognized as a pathogen. It was first seen in 1880 by Eberth and isolated by Gaffky in 1884 (Morgan, 1965; Burrows, 1973; Difco 1977; Hickman and Farmer, 1978; Ewing, 1986; Edelman and Levine, 1986). Since 1880, the following names have been used; Bacillus typhosus, Bacillus typhi, Vibrio typhosus, Bacterium typhi, Bacterium typhosum, Eberthella typhosus, Salmonella typhosa and Salmonella typhi. The name Salmonella typhi is currently used (Hickman and Farmer, 1978). Later, other salmonellae associated with disease processes were isolated. Salmon and Smith isolated S choleraesuis in 1885 (Difco, 1977; Edelman and Levine, 1986). Gaertner in 1888 isolated S. enteritidis from a patient who died following the consumption of meat contaminated with this organism and shortly afterward Durham and DeNoeble described another organism, S.typhimurium, isolated from patients suffering with gastroenteritis following ingestion of infected meat (Morgan,

1965). Loeffler also isolated S typhimurium in 1892 (Morgan, 1965; Burrows, 1973; Difco, 1977).

The generic name Salmonella was given to this bacterium in 1900 by Lignières in honour of the Late Veterinarian, Dr. Daniel Elmer Salmon, then Chief of Bureau of Animal Industry of the United States Department of Agriculture. He co-discovered S choleraesuis which was first isolated from swine suffering from hog cholera. The name has been in universal use since 1933 (Edwards and Ewing, 1972).

2.1.1 Definition

The genus Salmonella is a member of the family Enterobacteriaceae. The genus consists of a group of related species in the tribe Salmonellae. Other members of the tribe are Arizona and Citrobacter. The family consists of eight tribes namely, Escherichiae, Salmonellae, Klebsiellae, Proteae, Edwardsiellae, Erwinieae and Yersinae (Ewing, 1986; Jawetz et al., 1989; Greenwood et al., 1992). Members of the family are closely related (Bryan et al., 1979; Ewing, 1986). Salmonellae are primarily intestinal parasites, widely distributed in man, mammals and birds. They are pathogenic for man and animals including birds, reptiles and fishes (Morgan, 1965; Edwards and Gatlon, 1967; Jawetz et al., 1989).

Salmonellae are grouped on the basis of their biochemical and antigenic characteristics and because they are pathogenic for man and other animals (Smith, 1967; GreenWood

et al., 1992), also their ability to grow on a wide range of relatively simple media (GreenWood et al., 1992).

The genus Salmonella is composed of motile bacteria that conform to the definition of the family Enterobacteriaceae and the tribe Salmonellae (Ewing, 1986). Salmonella is a gram negative rod, with varying length (Jawetz et al., 1989). Most species except S pullorum and S gallinarum are motile with peritrichous flagella (Ewing, 1986; Jawetz et al., 1989). They produce colonies 2-4 millimetres in diameter. They are non-capsular and non-sporing. Salmonellae are mesophilic and are aerobic to facultative anaerobes. Most strains are aerogenic but anaerogenic variants occur. They are catalase positive, oxidase negative, Methyl red positive, Voges Proskauer test negative. Indophenol test negative and rarely form indole. They normally do not liquefy gelatin, pectate and alginate and do not produce urease or deaminate phenylalanine. Salmonellae produce hydrogen sulphide (H_2S) and decarboxylate lysine, arginine and ornithine. They reduce nitrates, reduce trimethylamine, produce acid and gas from glucose and mannitol, usually also from dulcitol and sorbitol. Lactose, sucrose, Salicin and raffinose are not fermented. Inositol is utilized by many strains, sodium malonate is not utilized and acid is produced in Jordan's tartrate medium. They form arginine dehydrolase, esculin is not hydrolysed, growth occurs on sodium acetate medium but few grow in potassium cyanide medium (Ewing, 1986; Hickman-Brenner and McWhorter-Murlin, 1994). However, Hickmen-Brenner and McWhorter-Murlin (1994)

reported that most strains belonging to subspecies IIIa and IIIb ferment lactose and only strains of subspecies I, II and V ferment dulcitol. The type species is S. enterica subspecies enterica, as exemplified by serotype Typhimurium (Ewing, 1986). Also strains of subspecies VI as described by Le Minor et al., (1986) are inositol and sorbitol negative; 22% ferment lactose, and 67% ferment dulcitol. Le Minor et al., (1982) reported that subspecies V was distinct from the other subspecies of Salmonella and Reeves et al., (1989) proposed that subspecies V strains be placed in a second species which they designated S. bongori. Thus the genus Salmonella currently has two species: S. enterica and S. bongori (Hickman-Brenner and McWhorter-Murlin, 1994), with S. enterica divided into six subspecies namely: S. enterica subspecies enterica (I or 1), S. enterica subspecies salamae (II or 2), S. enterica subspecies arizonae (IIIa or 3a), S. enterica subspecies diarizonae (IIIb or 3b), S. enterica subspecies houtenae (IV or 4) and S. enterica subspecies indica (IV or 6). These species and subspecies can be differentiated biochemically (Hickman-Brenner and McWorter-Murlin, 1994).

2.1.2 Classification:

There are several methods of classification that have been proposed, but the two most commonly used are those of Kauffmann and White, and of Ewing (Edwards and Ewing, 1972; Kauffmann, 1975). Knowledge of this subject is largely based on the fundamental work of White who investigated the

serotyping of the Salmonella organisms. Later Kauffmann greatly extended the work, publishing the Kauffmann-White scheme (Taylor, 1967). The Kauffmann-White scheme which was accepted by the International society for Microbiology accords a specific status to each antigenically distinguishable Salmonella type. Kauffmann divided the genus into 4 biochemically defined subgenera (I-IV) (Kauffmann, 1975; Ewing, 1986).

Serotypes belonging to Salmonella subspecies I are designated by a name usually related to the geographic location where this serotype was first isolated. This serotype name is written in Roman letters and the first letter is Capital letter. For example, Salmonella serotype Typhimurium and S. ser. Dublin (Hickman-Brenner and McWhorter-Murlin, 1994). However, World Health Organization collaborating Centre has dropped the term "Serovar" for serotypes. Also the centre has dropped all names for serotypes in species other than subspecies I (Le Minor and Popoff, 1987). Also the Centre has combined O-groups E_1, E_2 and E_3 (Le Minor and Popoff, 1987). But the Centres for Disease Control (CDC) is retaining this old names for serotypes in these O groups for surveillance purposes, that is names are easier to recognize than formulas and they make reporting less error-prone (Hickman-Brenner and McWhorter Murlin, 1994).

2.1.3 Antigenic Variation and Formulae:

Antigenic structure is important, but in day-to-day practice the demonstration of a small number of biochemical characters together with the demonstration of some Salmonella antigens is sufficient to decide that the organism is a Salmonella specie. To give the Salmonella a name demands a more complete antigenic analysis (Lewis, 1992). The typical Salmonella possesses two sets of antigens which are readily demonstrable by serological reactions in the laboratory and these include the somatic or O and flagella or H antigens. Some other salmonellae possess the Vi antigen (Lewis, 1992). Other antigens that have been described are "M" and "S" fimbrial (or pili), "X" and "T" antigens (Stewart, 1968; Edwards and Ewing, 1972; Kauffmann, 1975; Ewing, 1986).

The Vi and M antigens are grouped under K antigens, they are either capsular or envelope antigens. The mucoid (M) antigen appears to be identical in all types of Salmonella. The Vi antigen is presumed to be present in a layer covering or masking the "O" antigens and prevents the agglutination of the organism by O antiserum. The Vi antigen is non-toxic on ingestion and organisms with this antigen but no "O" antigen have little or no pathogenicity for mice. The Vi antigen appears to protect the organism against phagocytosis and bactericidal effect of serum (Wilson and Miles, 1955). The "X" antigen is a thermostable antigen of certain salmonellae that cross-agglutinates with unrelated serotypes. The fimbrial antigen prevents agglutination with "O" antiserum (Kauffman,

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1975). According to Kauffman, the fimbrial antigen has the same practical importance in Salmonella diagnosis as Vi antigen because it can produce "O" inagglutinability.

The Somatic (O) or body antigens are said to be carbohydrate in nature, but are probably phospholipid polysaccharide conjugated protein complexes with the antigenic specificity residing in the polysaccharide component (Kauffmann, 1975).

The flagella or H antigens are heat-labile and are formed from the structural proteins which make up the flagella that endow the organism with mobility. In most salmonellae strains the flagella exhibit the property of diphasic variation. That is, the flagella antigens exist in two alternative forms defined by two different sets of genes in the bacterial genome. When one of these sets is expressed, the flagella consists of one or more phase 1 antigens, while when the other set is operative two or more phase 2 antigens are synthesized (Lewis, 1992).

The Kauffman - White method for labelling and identifying salmonellae consists of 3 parts, describing the somatic, phase 1 and 2 flagella antigens respectively. The three parts are separated by colons and the components of each part by commas. If the designation of an antigen is enclosed in parenthesis it means the complete antigen is not present and if it is enclosed in brackets it means the antigen or complex may be present or absent. In the notation, a dash is inserted where a flagella antigen is missing.

The "O" antigens are designated by Arabic numerals and are numbered consecutively from 1 to 67 though some are missing and others are subdivided. The O antigen 67 was described by (Rowe et al., 1976) in S. crossness. The phase 1,H antigens are accorded lower case Roman letters from a to z with the exception of j and then z1, z2, z3 etc. Phase 2,H antigens are designated by Arabic numerals 1 to 12 with some missing in between. Some phase 2H antigens are also contained in the e, n --- complex series. The terms phase 1 and 2 were chosen merely to provide a neutral designation and to simplify the antigenic scheme (Kauffman, 1975).

In the Salmonella diagnostic scheme, the groups provided share a common antigen. The first 26 groups are designated by capital letters and subsequent one by the group determining antigens. The group letters form no constituent part of the name of a serotype or of its antigenic formula (Edwards and Ewing, 1972).

Knowledge of antigenic variation is indispensable for understanding of the antigenic structure of the genus Salmonella and in practical work if uniform results with proper interpretation are to be obtained (Stewart, 1968; Kauffmann, 1975). The following types of variation and their significance have been described by various authors (Wilson and Miles, 1955; Stewart, 1968; Burrows, 1973; Kauffmann, 1975).

(i) Phase variation is a fluctuating completely reversible immunological variation, a phenomenon in which the H antigens

of certain salmonellae can be separated into relatively stable components. Phase variation is a definite qualitative change of the flagella antigen between two serologically contrasting states, which consists of the alternate expression of one or the other of these two genes on the loci of phase 1 and 2. The three types of phase variation that have been described are specific-non-specific variation of Andrews and B phase variation of Kauffmann and Mitsui and the phase variation of Edwards and Brunner.

(ii) Form, or "O" antigen variation caused by absence or poor development of "O" antigen components.

(iii) VW or Vi antigen variation involving loss of Vi antigen.

(iv) S-R variation result from loss of normal smooth antigen with appearance of rough antigen, which is loss of "O" antigens and manifested by alternation in colonial morphology from smooth to rough. The rough strain is characterized by loss of virulence and autoagglutination of rough culture in saline.

(v) OH-O variation due to irreversible loss of flagella causing complete or partial loss of motility.

(vi) M-N variation involves variation from mucoid to non-mucoid or normal form. The M-form is characterized by special M-antigen which causes "O" and "H" inagglutinability.

Other variations that might affect Salmonella are transduction, phage or lysogenic variation and conjugation. Variation involving "T" and "X" antigens have also been described (Ewing, 1986).

2.1.4 Salmonella Antibodies:

The "O" antibodies are the first to appear on infection with Salmonella and may persist for about one year. The "H" - antibodies appear towards the end of the infection and may persist for many years after recovering. In infection with strains that possess the Vi antigens, Vi-antibodies are produced but disappear soon after recovery from infection. The level of antibodies may depend on the frequency and duration of the infection. Also treatment with Chloramphenicol may prevent the rise of antibody titre (Farez et al., 1957).

Some investigators have obtained results suggesting that the H-agglutinins may be stimulated by non-specific stimulus (Parry, 1965). This makes H-agglutinins very unreliable in diagnostic procedures. It has also been observed that carriers usually show a high titre of Vi-agglutinins but low "H" and "O"- agglutinins (Schubert et al., 1959).

2.1.5 Host-Adapted Serotypes

Members of the genus Salmonella have been recognised for many years and are responsible for Salmonellosis of man. The degree of host adaptation varies and affects the pathogenicity for man in three ways:-

(i) Serotypes adapted to man such as S. typhi, S. sendai and S. paratyphi A, B and C usually cause grave diseases with septicaemic - typhoidal syndrome (enteric). S. paratyphi B, while essentially a human pathogen, is occasionally isolated from cattle, pigs, poultry and other animals, although cycles

of transmission in these host have not been demonstrable (Lewis, 1992).

(ii) Salmonellae adapted to particular animal hosts include S. choleraesuis (pigs), S. dublin (cattle), S. gallinarum and S. pullorum (poultry), S. abortus-equi (horses) and S. abortus-ovis (sheep). These are all responsible for considerable morbidity, mortality and economic losses among domestic animals. Although all are capable of causing illness in man, only S. choleraesuis and S. dublin do so regularly (Lewis, 1992).

(iii) Non-host adapted serotypes such as S. typhimurium which affect both man and a range of animals, cause gastro-intestinal infections of varying severity and usually less severe than enteric fever.

Out of the 2339 serotypes of Salmonella so far identified (Hickman-Brenner and Mcwhorter-Murlin, 1994) about 2000 or more serotypes show no apparent host preference. All can potentially cause human infection, but the extent to which they do so is determined by their prevalence in domestic food animals at any particular time and the possibility for cross-contamination of foods and the ability to multiply in such foods. In developed countries most human infections are caused by a relatively small number of locally prevalent serotypes (Lewis, 1992).

2.1.6 Serological Identification of Salmonellae:

The principle of the serological identification of Salmonella involves the ultimate mixing of the organism (the antigen) with the immune system (the antibody). If the serum contains agglutinins for the antigen present on the organism, a rapid clumping (agglutination) of the organism will occur. This is known as a homologous reaction. In some cases a reaction in the immune serum with another species of Salmonella might occur. This reaction is due to the presence of one or more commonly shared antigens between various species and is called heterologous reaction (Difco, 1977). According to Burrows (1973), the serological identification of Salmonella strains by the use of antisera containing appropriate antibodies is known as Salmonella typing. In determining the Salmonella serotype, the somatic antigens are first identified using slide agglutination test with polyvalent Salmonella "O" antiserum and with antisera corresponding to the main "O" groups. The next stage is to confirm by the identification of its flagella (H) antigen by the use of tube agglutination tests (Ewing, 1986).

There are three stages in the identification of Salmonella (Ewing, 1972, Ewing and Martin, 1974; Hickman-Brenner and McWhorter-Murlin, 1994). The first stage involves complete identification of important species and serotypes including S. typhi, S. paratyphi B, S. choleraesuis and S. typhimurium. A few antisera are required for this stage. The second stage is intermediate and involves identification of 50

to 60 commonly occurring serotypes. This stage may be modified by the addition of antisera for complete serotyping of particular strains that may become epidemic or endemic in an area or of serotypes that are of special interest. The third stage involves the complete serological identification of all serotypes of Salmonella and characterization of new serotypes. This stage requires numerous antisera and this is restricted to few reference laboratories. Emphasis is given to complete serological identification of Salmonella regardless of the stage that may be adopted. If serological identification of strain is not completed, they should be forwarded to a Central or reference laboratory, together with the minimal information required regarding each isolate. The biochemical reactions of cultures should be determined before forwarding to a reference laboratory (Edwards and Ewing, 1972).

2.1.7 Typing Methods:

Numerous methods are now available for classifying, differentiating and identifying bacteria. These include many new biochemical tests, drug resistance typing, species or group specific bacteriophages, plasmid profiles analysis, biotyping, lipopolysaccharide (LPS) typing, plasmid finger printing, virulence properties, plasmid typing, IS 200 typing, phage typing (Kwaga, 1984; Threlfall and Chart, 1993).

Phage typing is used to subdivide strains within a particular sertotype. Different phage typing schemes have been developed for important serotypes and those occurring with

greater frequency in certain countries include: S. enteritidis, S. typhimurium, S. virchow and S. typhi in the UK (Threlfall et al., 1990), S. enteritidis in USA and Europe (Hickman-Brenner et al., 1991). Phage typing is a very important epidemiological tool for tracing outbreaks of infections due to Salmonella (Ward et al., 1987).

Plasmid typing is a method of strain discrimination based on the numbers and molecular weights of plasmids carried and the resultant plasmid profile of a strain can be used to supplement the more traditional methods of strain identification. The technique has proved useful for the discrimination of serotypes such as S. gold-coast (Threlfall et al., 1986) and S. berta (Threlfall et al., 1992).

Plasmid finger printing, using a variety of restriction endonucleases is a technique which may extend the results of plasmid profile typing (Platt et al., 1986). However, finger printing of plasmids in the type strains of the S. enteritidis phage types using restriction endonucleases Hind III and Pst I has demonstrated a remarkable homogeneity amongst the plasmids of 38 MDa, identified in 18 of the 27 phage type strains studied (Brown et al., 1993).

Restriction fragment length polymorphism (RFLP) was only observed in the 38 MDa plasmid in the type strain of S. enteritidis phage type 15 and it was concluded that for S. enteritidis, plasmid finger printing was unlikely to significally extend the degree of discrimination achieved by plasmid profile typing (Threlfall and Chart, 1993).

Antimicrobial drug resistance is uncommon in S. enteritidis, in contrast to S. typhimurium and S. virchow, the second and third most prevalent serotypes from humans in England and Wales between 1981 and 1990 (Threlfall et al., 1992). Subdivision by antibiogram has proved useful in some instances of an outbreak of S. enteritidis phage type 4 (Threlfall et al., 1992).

IS 200 is a Salmonella - specific insertion element first described by Lam and Roth (1983) and distributed on conserved loci on the chromosome of many Salmonella serotypes (Gibert et al., 1990), which has not been specifically identified in other members of the Enterobacteriaceae (Threlfall et al., 1994). Certain characteristics of IS 200 favour its use as a probe for strain discrimination. By using IS200, three "clonal lineages" designated SEC 1I, SEC 1II and SEC 1III have been identified within the chromosomes of 27 of S. enteritidis phage type strains examined (Stanley et al., 1991). Unlike other enterobacterial insertion elements such as IS1 and IS5, IS200 transposes only rarely under laboratory conditions (Casedesus and Roth, 1989), and this stability has favoured its use as a probe for discrimination within serotype and for phytogenetic purposes.

2.1.8 Transmission:

Salmonellosis is a bacterial disease commonly manifested by an acute enterocolitis, with sudden onset of headache, abdominal pain, diarrhoea, nausea and sometimes vomiting. It

is caused by a genus of bacteria called Salmonella (Benenson, 1990). Animal products are the most important source of food poisoning infection or Salmonellosis for man (Lewis, 1992). The farm animal may be infected from many sources: animal-to-animal (especially with host-adapted serotypes), transmission from breeding flocks to newly-hatched chicks via infected eggs, contaminated animal feeds amd contaminated environment (dust, infected rodents, insects, water etc) (WHO, 1983). Infact the chain of infection begins with the animal in its natural habitat.

The vehicles of transmission include meat and meat products, poultry, egg and egg products, milk and milk products, fish and marine products, cereals and vegetables, water and drugs and therapeutic agents of animal origin (Silliker, 1980; Bryan, 1981; WHO, 1983). Infection of animals occur on the farm in large part because of feed contamination. Shipping and lairaging result in increased infection rate leading to contamination of carcasses during slaughter and processing. Contaminated products cause disease as a result of inadequate cooking or cross-contamination in the Kitchen environment (Bryan, 1981). When brought into the kitchen, contaminated raw products may cross-contaminate working surface, kitchen and utensils. Once a contaminated environment has been established in a kitchen or other food preparation area, working surfaces and utensils serve to spread the contamination to other foods. If there is inadequate refrigeration, the problem is compounded by the rapid

multiplication of the bacilli at room temperature. A further complication is the frequent inadequate thawing and cooking of contaminated frozen foods (WHO, 1983). Infact Salmonella outbreaks as a result of inadequate microwave cooking has been reported (Evans et al., 1995).

The aerosol route has also been implicated in some outbreaks and it may be more important than previously suspected. The aerosol route has also been implicated in several laboratory acquired cases (WHO, 1983).

The various mode of transmission in animals and humans could be:

A. (i) Direct from pet animals (dogs, cats, rabbits and monkeys) and laboratory animals to man (Kauffmann, 1966).

(ii) Direct from man-to-man especially human excreters in a hospital environment (Bowmer, 1964).

(iii) Direct transmission from animal to animal. This could be venereal as the organism has been isolated from ram and Lamb testicles (Jack, 1968).

B. (i) Indirect transmission through drugs such as Carmine dye capsules (Lang et al., 1967), oral vaccine (Tigertt, 1959) and pancreatin (George, 1976).

C. (i) Through contamination of food for human consumption, for example animal carcasses (Grau and Smith, 1976; Kwaga et al., 1985a).

(ii) Through contamination of food by handlers who may be excreters (Gilbert and Roberts, 1987).

(iii) Contamination of manufactured food by equipments.

This could be human food or animal feed (Bensink and Roland, 1979).

(iv) Contamination of animal feed by the use of infected by products (horn meals, meat, bone meal and blood meals) (Rosted et al., 1978).

(v) Contamination of pasture by the spread of sewage sludge as manure (Wray, 1975).

(vi) Contamination of drinking water by sewage (John et al., 1965).

Salmonella organisms can gain access to meat at any stage during butchering (Nabbut and Al-Nakhli, 1982).

Although other modes of transmission occur, human salmonellosis results almost always by organisms of animal origin and this fact must be borne in mind in any control measures (Anderson, 1972).

.1.9 Diseases Caused in Man and Animals:

Jawetz et al. (1989) and Lewis (1992) have suggested that the signs and symptoms of Salmonellosis be classified into four clinical syndromes. They are, gastroenteristis, bacteraemia with or without extra intestinal localization, typhoid like patterns (enteric fevers) and the carrier state, which is either asymptomic or convalescent. Of these, gastro enteritis is the most common clinical picture, being present in about 70% of cases (Saphra and Winter, 1957). It frequently follows consumption of contaminated food. Symptoms may range from mild diarrhoea to a fulminant form with rapid

dehydration. The disease is self-limiting and last from one to four days (Macdonald et al., 1954).

The disease in animals has a trend similar to what prevails in humans. It is a mild dullness, inappetence, fever and diarrhoea as clinical signs (Hunter et al., 1976). In severe outbreaks, abortion is observed in infected pregnant animals (Hunter et al., 1976). Salmonellosis in young chicks can result in fatality of upto 80% if infected in the first three weeks of life. Chicks that survive become long term carriers (Bowmer, 1964).

Salmonellosis has a relatively stable incidence in the environment for a number of reasons:

(a) Mutation is common amongst members of this group Enterobacteriaceae (Shigella, Salmonella, Escherichia species and paracolon organisms). Thus the possibility of emerging new species (Black et al., 1960).

(b) They are of widespread distribution among animals and humans. This makes control measures difficult (Black et al., 1960).

(c) Temporal and permanent carrier states are easily developed after convalescence (Ames and Robins, 1943).

(d) Localization in body tissues is common (Goubler et al., 1942). Cases of Salmonella osteomyelitis relapsing after 24 years have been documented (Black et al., 1960).

(e) The organisms develop resistance to a number of a antibiotics (Aserkoff and Rennett, 1969).

(f) Acquisition of a resistance factor from enteric organisms like E. coli has been reported (Gast and Stephens, 1986).

In Nigeria, various manifestations of human salmonellosis described include: healthy carriers, gastro enteritis with severe diarrhoea, enteric fever, suppurative lesions, urinary infections and empyema (Collard and Sen, 1960; Spiller, 1967).

2.2.0 Reservoir and Host Range:

The intestinal tracts of animals are the major source of the salmonellae in nature. Approximately 1% to 3% of all domestic animals are infected with these organisms. Domestic livestock reservoirs of salmonellae include chickens, turkeys, cattle, swine, horses and sheep (Litchfield, 1980). However, salmonellae are ubiquitous and are widely distributed in animals, birds, reptiles, fishes, insects, man and the environment (Morse, 1978). The major reservoir of salmonellae are live animals (Rexach et al., 1994) and salmonellosis is recognized as one of the most important zoonotic diseases (Linton, 1983).

Salmonellae have been isolated from house hold pets including cats and dogs (Morse, 1978; Kwaga et al., 1989), and also from a wide range of arthropods such as flies, cockroaches, fleas and ticks (Lewis, 1992). Andrews et al., (1975) isolated salmonellae from diverse sources such as Moroccan food snails and frog legs. Frogs and a wide range of wild and zoo animals including bats, birds, rats, mice, squirrels, rabbits and mink, elephants, lions, lizard and snakes have been incriminated (NAS, 1969; Hubbert et al., 1975).

Salmonella may be isolated also from the environmental sources including surface waters, sewage effluents, sewage sludge and agricultural products contaminated by polluted water (Edel *et al.*, 1977). Animals may derive infections from any of these sources but also by the ingestion of compounded feeds in which contaminated animal and vegetable protein is incorporated (WHO, 1983). In many countries the occurrence of *Salmonella* is ubiquitous and this contributes to a very complicated epidemiology (WHO, 1983).

Of the 2339 or more *Salmonella* serotypes recognized, about 100 have been isolated regularly from food, animals and man. Not all, however, behave alike and some are host adapted. For instance, *S. dublin*, an important cattle pathogen is encountered rarely in other species (occasionally in sheep). It cause a limited number of infections in man, chiefly by drinking raw bovine milk, which can be severe especially in debilitated patients. Cattle derive infections from each other, by licking faecally contaminated surfaces or by drinking milk from an infected cow (Osborne *et al.*, 1977, Hickman-Brenner and McWhorter-Murlin 1994).

In contrast to terrestrial animals, fish and shell fish do not carry members of the *Enterobacteriaceae*, unless they encounter polluted water. Fish and Shellfish caught in the open sea do not contain coliforms or Salmonellae while those caught close to shore in polluted waters may contain these organisms (Litchfield, 1980).

The cycle of transmission of salmonellae has been described as feed stuffs -> animals -> food stuffs -> man with a cycle of perpetuation through use of contaminated animal processing by-products as a component of feeds (WHO, 1983). This points out that host-specific serotypes will spread more rapidly than non-host specific types as a result of large increases in number in the infected animal and extensive environmental scattering of the heavily contaminated liquid faeces (Litchfield, 1980).

The relative frequency of Salmonella as a cause of food borne diseases varies from country to country and depends on factors such as dietary habits, hygienic standards in food production and service establishments and animal husbandry practices. In the United States of America, about 40% of reported food-poisoning cases are due to Salmonella, while in England and Wales, it is about 80%. These percentages may reflect, in part, differences in the surveillance programmes and isolation methods practices in each country but are, no doubt, due mainly to differences related to the predominant source of animal protein in the human diet (WHO, 1983).

The lack of regular surveillance data in developing countries, however, makes it difficult to evaluate the real magnitude of the Salmonella problem. Although some extensive outbreaks from common sources of food or water have been reported (Essafi et al., 1968; Jegathesan, 1980).

In developing countries, food borne salmonellosis is also a widespread disease and the dangers presented by this

infection will probably increase with the industrial production of food (Abdussallam, 1980). Strains of Salmonella have been isolated frequently from meat carcasses imported from South American countries (Van Schothorst and Kampelmacher, 1967), from cattle imported from middle eastern countries (Nabbut et al., 1968), from different local foods in the Middle East countries (Nassir and Rihab, 1979) and also from meat and meat (poultry) products in some developing countries (Ndzinge et al., 1981; Kwaga et al;. 1985a). The role of animals as reservoirs of infection for man in Nigeria is well known (Collard and Sen, 1960; Okubadejo et al., 1971). Salmonellae have been isolated from the following sources by various workers in Nigeria, cattle (Collard and Sen, 1956; Plowright, 1957; Johnson, 1958; Corke et al., 1975; Olayemi, 1978; Kwaga et al., 1984, 1985a), poultry (Falade and Ehizokhale, 1981), pigs (Sen and Collard, 1957b; Falade et al., 1983), sheep and goats (Johnson, 1958; Ojo, 1972; Corke et al., 1975; Falade, 1978; Tabukum, 1990), dogs (Kwaga et al., 1989), Lizards (Collard and Montefiore, 1956), Wildlife (Falade and Durojaiye, 1976), environment (Addo and Diallo, 1981., Osiyemi and Agbonlahor, 1983). Many of the serotypes that have been isolated from animals have also been isolated from man including clinical cases (Collard and Sen, 1958; 1960).

2.2.1 Epidemiological Aspects of Salmonellosis in Man:

Salmonellosis remains as one of the main causes of food borne illnesses worldwide (Baird-Parker, 1990). It is worldwide in distribution but it is grossly under reported because of the large number of carriers and asymptomatic excretion (Edel et al., 1977). The reported number of cases of Salmonellosis have continued to increase during the past 2 decades (Baird-Parker, 1990), especially with a marked increase in infections caused by S. enteritidis. Salmonellosis in human is today one of the most important infection. The ever increasing world trade in foods and feeds as well as mass processing, new methods of distribution and change of food habits are factors which are in close relation to the Salmonellae problem (Edel et al., 1977).

Different serotypes of Salmonella are dominant in different parts of the world (Katouli et al., 1993), but these patterns may change in a relatively short time because of the movement of people, foods and feeds. The number of infections attributable to S. enteritidis increased from 1,700 during 1976 to 8,340 during 1990, representing a fivefold increase during that period that was especially apparent in the New England and Mid-Atlantic regions of the United States. By 1990, S. enteritidis surpassed S. typhimurium to become the most predominant Salmonella serotype among human beings and other animals (CDC, 1987; 1990).

The prevalence of individual serotypes of salmonellae associated with human infections varies with time,

geographical isolation and a wide range of additional parameters. Nevertheless certain serotypes of S. enterica tend to dominate over long period (example are Enteritidis and Typhimurium). Some remain notable by their persistence of low incidence such as Infantis whereas a third group, typified in the UK by Virchow, Hadar and Agona have each achieved temporary prominence within a 5-10 year period and then declined (Rankin et al., 1995). The mechanisms that underlie these patterns of incidence remain largely unknown although the consistently dominant serotypes harbour plasmids that enhance virulence in animal models and are serotype-specific (SSP) (Woodword et al., 1989).

The distribution of Salmonella types in tropical Africa is quite different from that found elsewhere. In general no one serotype occurs in overwhelming numbers and there is a great diversity of infecting types (Spiller, 1967; Kwaga et al., 1985b). The age of individuals may affect distribution because salmonellosis is mainly a disease of children although other age groups especially the elderly may be affected (AL-Qarawi et al., 1995). Rice and Loewenstein (1979) reported that the highest incidence of salmonellosis is found in children less than 5 years of age and this is perhaps because children with diarrhoea are more likely than adults to have a stool culture taken. A study carried out by Al-Qarawi et al., (1995), confirms that salmonellosis affect all age groups except children under 1 year of age. There are no sex or race association but the socio-economic status of individuals

reflected in food and sanitary habits may affect distribution. Sickle-cell disease in blacks may pre-dispose to infection and disease (Hubbert et al., 1975).

In temperate countries, the disease exhibits a seasonal pattern occurring mostly in the warm summer months (Gangarosa, 1978).

The progressive cellular immune defects associated with human immunodeficiency virus (HIV) infection have led to an increased frequency of opportunistic infections with intracellular bacterial pathogens, including non-typhoidal Salmonella species (Levine et al., 1991). Recurrent episodes are characteristics of Salmonella infection in the AIDS population and are mainly associated with S. enteritidis or S. typhimurium (Levine et al, 1991). Despite the higher rates of recurrence by these Salmonella serotypes, it has not been clearly demonstrated whether this recurrence is usually due to re-infection or relapse (Mayer and Hanson, 1986).

There is increased risk of exposure among those who work in abattoirs, poultry processing plants and those in contact with animal and their products. Although Salmonellosis is world wide, its importance is related to other diseases, availability of diagnostic services, reporting laws, animal husbandry, food preparation and geographic differences in serotypes distribution (Hubbert et al., 1975).

2.2.2 Salmonellae in Feeds

The contamination of animal feeds has been recognized as a primary source of infection in animals. Contaminated feeds have resulted in great numbers of clinically healthy carriers and to a considerable degree, contamination of foods of animal origin, the environment including effluents and surface waters, insects, birds and rodents (Edel et al., 1977). Contamination of feeds originate primarily from feed ingredients of animal origin such as meat meal, meat scraps, bone meal and fish meal (Edwards and Galton, 1967; Bryan, 1981).

Some evidence exist which indicate that salmonellae especially exotic serotypes have a pathway of infection from feeds to animals and poultry, to meat and poultry products and then to man (Collard and Sen, 1958; Silliker, 1982). Clark et al. (1973) have shown that the best evidence comes when a new and exotic serotype of Salmonella (example, S. agona) is introduced into an area where it was previously rare or unknown. Prior to 1970, only two isolation of S. agona from man had been reported in the United States. In 1970, there were four isolations, then there was a rapid increase to 44 in 1971, to 252 in 1972 and 864 in 1973. By the latter year the organism had spread all over the country. Meanwhile, in 1969 and 1970, S. agona was reported in turkeys and a shipment of Peruvian fish meal. Later it was found repeatedly in poultry and poultry feeds containing Peruvian fish meal. During 1973 and 1974, most of the isolations came from swine, dogs, zoo animals, red meats and other non-poultry sources, suggesting

a recycling of infection through domestic vehicles (Clark et al., 1973).

Later, Sojka et al. (1975), concluded that the role of imported animal feedstuffs in bovine salmonellosis does not seem to be of great importance from the evidence available to them in England and Wales. However, hopes of eradicating salmonellosis in human beings and in animals will never materialize without elimination of salmonellae from animal feedstuffs (Bryan, 1981).

2.2.3 Environmental Consideration of Salmonellosis:

Salmonellae have accomplished the ultimate goal in the ideal host-parasite relationship by adapting to a very wide host range and to the environment. They have great propensities to exist in the extra-host environment for considerable periods and may multiply under favourable conditions in the environment (Morse and Duncan, 1974). Environmental factors exert a profound impact on the occurrence, epidemiology, diagnosis, prevention, therapy and control of salmonellosis (Morse and Duncan, 1974).

Faeces of infected animals and man readily contaminate human food, animal feeding stuffs, rivers and other water supplies, sewage systems, surface waters and soil, any of which may act as vehicles of infection to other animals and man (Bowmer, 1964). Treated sludge can contain variable numbers of salmonellas, depending on the amount entering the system and on the efficiency of the treatment system. Another

imput of Salmonellas into soil is from cattle, approximately 10% of which excrete these organisms in their faeces (Zibilske and Weaver, 1978) which is eventually used in agriculture.

When salmonellae are exposed to environmental stress many bacteria remain viable when enumerated by the direct viable count (DVC) of Kogure et al. (1979), but become non-culturable and cannot be counted on agar plates. This so-called "viable but non-culturable" state has been reported for many bacteria including salmonellas (Davis and Evison, 1991). Salmonellas undergo optimal growth in nutrient rich environments such as the gut or foods and so may be under considerable stress when introduced into soil in which nutrient are limited (Turpin et al., 1993).

Some food industry wastes which can contain salmonellas are also applied to agricultural land. However, survival of salmonellas in soil depends on many factors including temperature, moisture, soil microflora, sunlight, soil type and pH (Davis and Evison, 1991).

2.2.4 Salmonellae in Cattle:

Cattle at any age may become infected with various Salmonella organisms and give rise to either clinical or sub-clinical infection (Kwaga, 1984).

S. dublin shows a considerable degree of host specificity, it is primarily a pathogen of cattle (Gibson, 1961).

In areas where salmonellosis is endemic, S. dublin is the predominant serotype in calves, because it is the serotype also predominant in older cattle (Anon, 1991). Calfhood infection is associated with the presence of active carriers among the adult cattle (Gibson, 1961).

Although S. typhimurium and S. dublin are the most common serotypes encountered in cattle, several serotypes have been identified and among those occurring frequently are S. newport, S. enteritidis, S. heidelberg, S. anatum and S. panama (Edwards and Galton, 1967).

Bovine salmonellosis was first reported in Nigeria by Mettam in 1943 (Khan, 1970). Other early reports on the isolation of salmonellae from cattle in Nigeria were those by Collard and Sen, (1956), Ployright, (1957) and Johnson, (1958). Kwaga et al. (1984) reported the isolation of Salmonellae from 809 rectal swabs of cattle in Nigeria. There was a higher isolation frequency from calves than adult cattle, and more from intensive than from nomadic system. S. bergen was the most commonly encountered serotype in that study. From work done in Nigeria and other countries in Africa, it appears that there is a greater variety of types of salmonellae in cattle in the tropics compared to those in temperate countries (Collard and Sen, 1956; Spiller, 1967). In cattle, the sources of organisms will normally be either faeces or foetus, foetal fluids and placenta or materials contaminated by these materials (Hinton, 1971).

2.2.5 *Salmonella* in Animal by—Products:

The occurrence of salmonellae in animal by-products and wastes from meat packing plants in many parts of the world has been documented extensively (Vauderpost and Bell, 1977). Environmental conditions in rendering plants are important factors in the transmission of *Salmonella*. For example a variety of serotypes have been isolated from environmental swabs taken during various stages of processing (Wray *et al*., 1991) and from flies in the processing plant as well as from the finished products (Litchfield, 1980). Freshmeat and improperly processed meat products have long been recognized as a source of *Salmonella* infection in humans. Both beef and pork products can be contaminated with salmonellae at the processing plant or at retail and food service outlets (Bryan, 1978b). In the past few years, there have been a number of food borne outbreaks of salmonellosis attributed to inadequately pre-cooked beef (CDC, 1977).

Salmonellae may survive for extended periods in luncheon meats or fermented or non-fermented sausage products for extended periods in refrigerated storage (Litchfield, 1980). Salmonellae are spread to carcasses and cuts of meat during abattoir processing and are primarily found on surfaces of raw meat and poultry (Bryan, 1978). Varying rates of isolation of salmonellae from meat have been reported in different countries (Collard and Sen, 1956; Kwaga *et al*., 1985a). The prevalence of *Salmonella* in meat varies considerably, although

red meat and poultry do provide main vectors for its distribution (Goepfert, 1980). Watson, (1975) reported that in England from 1960-1970, the incidence of Salmonella in meat and its products as vehicles of infection responsible for human outbreaks rose from 47% to 71% during the 10 year period and within that period, Salmonella food-poisoning accounted for 73% of all recorded incidents of salmonellosis.

Ready-to-eat beef products have different appellations in different areas. Roasted beef ("Suya") and dried beef ("Kilishi") are two of such beef products commonly consumed in Nigeria. "Suya" is prepared and sold in most parts of Northern Nigeria, but mainly around hotels, and recreational venues in the Southern parts. Although beef is the commonly used raw meat for "Suya" preparation, mutton, camel and goat meat are also used occasionally (Adesiyun, 1984). Salmonella have been isolated from "Suya" in Nigeria by (Ndambi, 1992). However, reports on isolation of salmonellae from "Suya" and "Kilishi" in Nigeria is scanty.

Milk and dairy products such as cheese and ice cream have accounted for the many food borne outbreaks of salmonellosis. Salmonella have been isolated on many occasions from naturally contaminated dry milk products and these organisms grow readily in milk and related dairy product (Subramanian and Marth, 1968). S. typhi and other salmonellae may survive for extended periods in cheese. Also, survival of salmonellae has been demonstrated in cultured dairy products (Litchfield, 1980). The role of home-made ice cream as a vehicle of S.

enteritidis phage type 4 infection have been reported (Morgan et al., 1994).

Salmonellosis outbreaks have been attributed to confectionery and confectionery ingredients such as chocolate and desicated coconut (D'Aoust, 1978). It is `Known' that fermented milk is a source of many zoonotic diseases such as brucellosis and salmonellosis. It is therefore necessary to monitor the level of contamination of "nono" in order to establish the wholesomeness of the product (Zaria et al., 1992). The isolation of pathogenic microorganisms from raw milk and fermented milk ("nono") have been reported (Zaria et al., 1992).

2.2.6 Antibacterial Agents and Drug Resistance in Salmonella:

Despite the large volume of literature on the chemotherapy of salmonellosis, only a relatively small proportion of antibacterial substances exert action of practical significance against salmonellae. The success of chemotherapy depend no less upon a high standard of farm hygiene than upon the subsequent development of carrier animals (Buxton,1960). Although therapy using chloramphenicol is indicated with systemically complicated salmonellosis, antibiotics therapy of uncomplicated Salmonella gastro-enteritis is often ineffective and therapy probably lengthens duration of faecal excretion of salmonellae (Gibson, 1961). Several drugs have been found to be effective against Salmonella in vitro, but chloramphenicol would probably remain

the drug of choice (Neu et al., 1971). Hence effective control on the veterinary use of chloramphenicol would seem to be needed if resistance to this important drug is to be minimized (Timoney, 1978). The increase in the incidence of drug resistant salmonellae in animals and man is attributed to the development of the intensive system of cattle rearing with its attendant large scale indiscriminate use of antibacterial drugs in feeds, prophylaxis and therapy (Ojo, 1972; Anon, 1982). The prolonged use of small amounts of drugs encourages the emergence of resistant types of many organisms and emergence of transfer factors capable of infecting Salmonella and other organisms (Sojka et al., 1974). Continuous feeding of chlortetracycline to domestic animals complicates the problem of salmonellosis in animals. This may increase the risk of direct or food chain exposure to man (Dey et al., 1978).

The antibiogram of isolates of Salmonella from human beings and animals in the North-Eastern United States were found to be similar (Timoney, 1978). The incidence of multiple resistance in S typhimurium from human in England and Wales was more than doubled over an 8 year period (Threlfall et al., 1993).

Transferable drug resistance is the transfer of antimicrobial resistance from one bacterium to another by cell-to-cell contact and has been referred to as infective drug resistance, contagious drug resistance, multiple drug resistance, transmissible drug resistance or R-factor

resistance (Baldwin, 1970). Infective resistance can be transferred *in vivo* between organisms of the same species e.g. *E. coli* and organisms of different species *E. coli* and *Salmonella* species (Williams, 1967). Chromosomes are involved in resistant mutants, while in infective drug resistance, the material is located in the cytoplasm. It may be single or multiple and practically all available antibacterial drugs are involved. The donor and recipient cells must have contact (conjugate) so that hereditary material controlling infective resistance called R-factors, pass through the conjugation tube so formed from donor to recipient (Williams, 1967). R-factors are self replicating and consist of resistance transfer factor (RTF), which mediates transfer of resistance by potentiating conjugation. Resistance determinants (R-determinant) convey material responsible for the particular drug resistance involved (Williams, 1967). Without RFT's, the R-determinant cannot be transferred by conjugation; without R-determinant, the RFT's have no drug resistance to transfer. R-factors are transferred within one to three minutes after the conjugation pair is formed and the resistance expressed within ten to twenty minutes (Watanabe, 1963). The theories on the mechanism of R-factor action on drugs include non-specific alteration of cell membrane permeability to drugs, production of substance that inactivate drugs (Smith, 1966) and production of penicillin-destroying enzymes (Stanier *et al.*, 1989). Transferable drug resistance can occur in the presence or absence of antimicrobial drugs (Baldwin, 1970).

2.2.7 Isolation and Identification of Salmonellae

Salmonella species play an important role among the pathogenic micro-organisms causing food-borne disease. Besides food-borne salmonellosis, this group contains the agents that cause typhoid and paratyphoid fever. Methods used routinely for isolating and identifying Salmonella rely on enrichment in non-selective media (pre-enrichment), selective enrichment in selective broth and culturing on differential mean (Blackburn, 1993), biochemical and serological identification (Manafi and Willinger, 1994). Successful isolation of Salmonellae can be a complex multifactorial procedure (Harvey and Price, 1979). For large surveys or during investigation of epidemics, fresh faecal swabs may be inoculated directly in enrichment both. The efficiency of rectal swabs over faecal specimens for recovering of salmonellae has been questioned. Inspite of its deficiencies, the value of rectal swab in large surveys and epidemiological investigations cannot be over-looked (Edwards and Ewing, 1972; Ewing, 1986).

Until recently, pre-enrichment was used only when one believed that a process, such as heating, freezing or drying had injured the salmonellae. However, it is now recommended that all samples (except raw meat) be placed in a pre-enrichment non-selective medium to restore injured salmonellae cells to a stable physiological condition (Andrews, 1985). Pre-enrichment media vary widely. The following media have been recommended by various organisations; Lactose broth,

trypticase soy broth, reconstituted non-fat dry milk, nutrient broth and buffered peptone water (Andrews, 1985).

Enrichment of sample in some type of broth, either nutrient or selective is superior to direct plating. This is true particularly for samples from carriers, who may be excreting very small numbers of salmonellae. Most of the enrichments developed are highly selective and they serve the two fold purpose of permitting the salmonellae to multiply while reducing or inhibiting growth of competitive organisms, which might other wise overgrow salmonellae in laboratory media (Kent et al., 1981). The inhibition facilitate the isolation of Salmonella on different plating media (D'Aoust, 1981; Ewing, 1986). Although numerous selective enrichments have been developed for isolating Salmonella, selenite cystine broth and tetrathionate broth remain the most widely used (Andrews, 1985; Stone et al., 1994). Also selenite F broth and tetrathionate brilliant green broth are commonly used (Ewing, 1972, Harvey and Price, 1979). However, the medium used is determined by the choice of the bacteriologist (Harvey and Price, 1979). If a single enrichment must be selected for general purposes, selenite broth is the medium of choice (Ewing, 1986). Elevation of incubation temperature markedly affects productivity of selective enrichment broths by suppressing growth of competing micro-organisms. This results in an almost pure culture of Salmonella and marked reduction in enteric contaminants (D`Aoust, 1981).

Selective plating agar media are used to restrict the growth of non-salmonellae organisms while providing an indicator system that imparts colour to the colonies or the surrounding medium, which makes such media differential (Kent et al, 1981). Some selective agents added are intended to inhibit the growth of interfering bacteria while permitting unhindered salmonellae to grow. Selective agents used include bile salts, heavy metals, antibiotics and sulphonamide. Differentiation of salmonellae from other organisms on plating media is commonly based on fermentation of carbohydrates usually Lactose and sucrose which most salmonellae do not ferment (Moats, 1981). Numerous plating media have been used for the isolation of salmonellae and those that are highly selective are necessary for the isolation of salmonellae from faecal material and food products, since many other Gram negative organisms are frequently present and are not suppressed sufficiently on differential media (Edwards and Galton, 1967). The most widely used plating media for the isolation of Salmonella are bismuth sulphite agar, brilliant green agar, brilliant green sulphadiazine agar, salmonella - shigella agar, desoxycholate citrate agar, xylose lysine desoxycholate agar (Edwards and Ewing, 1972; Moats, 1981; Kent et al., 1981). But Hektoen enteric agar (Stone et al., 1994) and MacConkey agar have also been used (Kent et al., 1981). Plating media such as brilliant green sulphadiazine agar and bismuth sulphate agar were developed primarily for Salmonella other than S. typhi and for S. typhi respectively (Kent et al.,

1981). However, the choice of plating media varies from one laboratory to another and is largely dictated by the experience of the personnel and the facilities of the laboratory (Harvey and Price, 1979). The incubation of selective media at 37°C is ideal (Harvey and Price, 1979). There are several variations of methods currently used for the isolation of salmonellae. These variations in enrichment media and enrichment incubation, plating media and incubation temperature impedes comparison of isolation rates. There is a need for the standardization of isolation methods for adequate comparison of findings (Edel and Kampelmacher, 1968).

Biochemical tests are used to eliminate non-salmonella cultures, and to provide a tentative identification of Salmonella at the genus level (Kent et al., 1981). Although an extensive number of biochemical tests are available for characterization of cultures, it is not necessary to employ all the tests unless an atypical strain is encountered (Ewing, 1972). If the overall biochemical reactions of an isolate are characteristic of Salmonella it should be labelled as such pending further tests (Ewing and Ball, 1977). Commercial biochemical Kits, such as API, Enterotube, or Minitek systems may be used for the presumptive identification of food-borne Salmonella, as an alternative to the conventional biochemical tube system (Kent et al., 1984).

Serological techniques are used for definitive identification of the Salmonella culture. To positively identify the serotype of a Salmonella isolate both the Somatic

(O) flagella (H) antigens must be determined (Kent et al., 1981) It should be noted that any serological results obtained prior to biochemical identification must be considered as presumptive identification only (Difco, 1977).

Several attempts have been made during the past 30 years to devise rapid Salmonella detection methods for the examination of foods and food products (Swaminathan et al; 1985) and clinical specimen. Since then several methods have been devised, which include, fluorescent antibody (FA) techniques, enrichment serology (ES) techniques, membrane filter-disc immuno immobilization technique, colorimetric method employing lysine iron cysteine neutral red (LICNR) broth, radiometric method, Felix 0-1 bacterio-phage, Hydrophobic grid membrane filtration (Swaminathan et al., 1985), DNA-hybridization (Gopo et al., 1988), and polymerase chain reaction (PCR) (Stone et al., 1994). Also enzyme immuno assays (EIAS) which depend upon the assumption that an antibody (or an antigen) can be linked to an enzyme without significantly affecting the activity of either entity (Flowers, 1985;). Also, new commercial media have been introduced for the rapid detection of salmonellas using fluorogenic and chromogenic substrates. Various studies with the MUCAP test (Biolife, Italy) intended for screening of Salmonella species have been reported (Betts and McGoldnick; 1990). Also Rambach agar (Merck, Germany) has been evaluated for rapid detection of Salmonella (Heizmann, 1993), SM.ID agar (Biomerieux, France) was also evaluated (Poupart et al., 1991).

Recently, Safarik et al., (1995) used the immuno magnetic separation (IMS) techniques to separate and concentrate specific target bacterial cells from a mixed suspension without loss of viability. The technique uses paramagnetic beads coated with appropriate antibodies to capture the target cells from a mixed cell suspension. However, Mansfield and Forsythe (1993) tested the IMS technique using Dyna beads Anti-salmonella with 60 food stuffs spiked with one of five serovars. They demonstrated that the IMS procedure was as efficient as the 3 conventional enrichment broths. This eliminated the overnight enrichment incubation and hence reduced the detection period by one day. Commercial analytical techniques are more rapid (2-3 days) but not very sensitive (Rose et al., 1991).

PCR technology has revolutionized many facets of molecular medicine and microbiology over the past few years and the development of rapid and sensitive PCR methods for detection of infectious microbial agents from clinical or food specimens has been of great interest to many microbiologists (Persing, 1991). A PCR assay for the detection and identification of Salmonella serogroups A, B, C_1 and D has been described (Persing, 1991).

Other rapid detection techniques include, nucleic acid based technique, such as restriction fragment length polymorphism (RFLP) followed by hybridization with gene-specific probes (Knight et al., 1990). All the nucleic acid based techniques depend on the genotypes of the organisms

concerned and not on their phenotypic expression (Koh-luar et al, 1996).

B. ESCHERICHIA COLI

2.2.8 Historical Background:

Escherichia coli was first described in 1885 by Escherich who was studying diarrhoea in infants. In 1933, Goldschmidt applied a serological approach in trying to identify epidemic strains but was not successful (Gross, 1992). In 1943, Bay carried out a survey during an outbreak of enteritis in infants in a London hospital and incriminated a serological type he named Bacterium coli neapolitum. In 1947 and 1948, Giles et al recognized 2 different strains in successive epidemics of diarrhoea amongst babies in Aberdeen, they were designated Aberdeen alpha and Aberdeen beta. In 1946, Taylor et al recognized an important epidemic strain D433. Later E blattae was first described among bacteria isolated from the gut of the cockroach (Ewing, 1986; Gross, 1992) and it has not been reported from human clinical specimen (Gross, 1992).

2.2.9 Definition:

The genus Escherichia is a member of the family Enterobacteriaceae. The genus consists of a group of related species in the tribe Escherichieae and the other genus in the tribe is Shigella (Ewing, 1986). Escherichieae are primarily intestinal parasites widely distributed in man, mammals and

birds. They are pathogenic for man and animals, including birds and fishes (Gross, 1992).

The genus Escherichia is composed of motile or non-motile bacteria that conform to the definitions of the family Enterobacteriaceae and the tribe Escherichieae. Members of this tribe ferment lactose, salicin, glucose and adonitol. Inositol is not utilized and lysine and ornithine are decarboxylated by the majority of cultures, acid is formed from sodium mucate and sodium acetate is frequently utilized as a sole source of carbon (Ewing, 1986). The type species of this genus Escherichia is E. coli (Gross, 1992). E. coli grow well on non-selective media forming smooth, colourless colonies of 2-3mm in diameter in 18 hours on nutrient agar, large red colonies on MacConkey agar, there may be haemolysis on blood agar. They grow over a wide range of temperature (15-45°C). Some strains are more heat-resistant than other members of the family and will survive heating to 60°C for 15 minutes or 55°C for 60 minutes (Gross, 1992).

2.3.0 Classification:

According to Farmér et al., (1984) and Gross, (1992), the genus Escherichia is divided into five species namely E. coli, E. hermanni, E. blattae, E. vulneris and E. fergusonii. These were delineated by means of DNA relatedness studies by Brenner (1978). Studies of DNA - DNA reassociation show that E. coli and Shigella organisms form a single genetic species. Certain species which are non-motile, anearogenic and often

ferment lactose late or not at all have caused difficulties in classification. They were at one time included in the so-called "Alkalescens - Dispar" group but these strains are now considered to be atypical E. coli. Some workers believe that an intermediate group between Escherichia and Shigella should be established and that an important criterion for inclusion in it should be the ability to cause dysentery-like diseases (Gross, 1992).

2.3.1 Antigenic Structure:

In 1947, Kauffmann published an antigenic scheme for E. coli which was based upon his earlier work and that of his associates, Knipschildt and Vahlne. This scheme, which consisted of 25 somatic (O) antigen groups, 55 capsular antigens and 20 flagellar (H) antigens, was an extension of an earlier one devised by Kauffmann in 1944. Since 1944, many investigators have studied the antigens of E. coli and as a result of their efforts, the numbers of delineated O, K and H antigens have been extended greatly (Ewing, 1986).

Presently, more than 164 O-antigens (Cheesbrough, 1991) and over 50 H-antigens (Gross, 1992) are known. There is no comprehensive scheme that consist of all known serotypes of E. coli. However, two partial schemata exist: (a) scheme that includes most of the serotypes known to be associated with infantile diarrhoeal disease (Ewing, 1962) and the scheme presented by Ørskov et al., 1977). However, hundreds of serotype are known (Ewing, 1986).

Somatic (O) antigens are the heat-stable antigens which are composed of phospholipid-polysaccharides complexes. Analysis of the O antigens generally reveal polysaccharides (60%), lipid (20%-30%), and hexosamine (3.5% to 4.5%). It is the nature of the terminal groups and the order in which they occur in the repeating units of the polysaccharides chain that render specificity to the numerous kinds of O-antigens. These antigens are resistant to alcohol and dilute acid. Agglutination reactions as in tube tests, occur relatively slowly and aggregates are granular. O-antigens are subject to smooth(S) to rough(R) variations, and form variation. Some are subject to lysogenic conversion (Ewing, 1986). Numerous cross-reactions occur between individual E. coli O-antigens and between these and the O-antigens of organisms belonging to other genera of the family Enterobacteriaceae. E. coli produce O-antigens that may be neutral or acidic and in most cases these acidic component are hexuronic acids (Gross, 1992).

The capsular or fimbriae antigens when present in sufficient quantity, inhibit the agglutination of living and unheated bacterial suspension in O-antigera. The K-antigens form a class that may be sub divided according to their physical and chemical characteristics. Most of the varieties of K-antigen are not destroyed by heat. However some of their characteristics may be altered by heat treatment at various temperatures for specified periods of time (Ewing, 1986). In the past, these antigens were divided into three classes (L, A and B - antigen) according to the effect of heat on the

agglutinability, antigenicity and anti-body-binding power of bacterial strains that carry them (Gross, 1992). In modern usage the term K-antigens refers to the acidic polysaccharide capsular antigens and those of E coli can be divided into two groups (group I and II) that largely correspond to the old A and L antigens (Gross, 1992).

More recently, a number of filamentous protein structures resembling fimbriae have been described in E coli. These cause a mannose-resistant haemagglutination and there is good evidence that they play an important part in the pathogenesis of diarrhoeal disease and in urinary tract infection. They include the K88 antigen found in strain causing enteritis of pigs, the K99 antigen found in strain causing enteritis of calves and lambs and the colonization factor antigens (CFAs) found in enterotoxigenic E coli of human origin (Gross, 1992).

The flagellar antigens are heat-labile antigens that occur in the flagella of the bacteria. They are protein in nature and are composed of flagellins. The amino acid content and the order in which these amino acids occur in the flagellins determine the specificity of the many different H-antigens of Enterobacteriaceae. Flagella are inactivated slowly by alcohol. Flagellar agglutination occurs very rapidly, and the aggregates formed are loosely knit and floccular (Ewing, 1986). All the H-antigens are usually monophasic but rare diphasic strains have been reported. Also there are only a few significant cross-reactions between them and with the H-antigens of the other members of the

Enterobacteriaceae (Gross, 1992). However, in the motile members of the genus Escherichia, loss variation may occur (HO to O) (Ewing, 1986).

2.3.2 Serological Identification of E. coli:

Serology in probably the most useful way of subdividing E coli (FAO, 1979). Complete Serotyping depends upon the determination of the somatic (O) antigens, O-antigen factors, flagellar (H) antigens, H-antigen factors and capsular or envelope (K) antigens (Ewing, 1986).

2.3.3 Typing Methods:

The choice of typing methods for the differentiation of bacterial strains depends not only on the species involved but also on available laboratory resources. For E coli; the two-tier biotyping scheme of Crichton and Old (1982), comprising four primary and six secondary tests can be applied in any laboratory to provide excellent subspecific discrimination of strains as "biotypes". Because types are stable even after storage on non-selective medium for months (Crichton and Old 1982), the scheme can be used for both prospective and retrospective analysis (Crichton and Old, 1980) and it's flexibility allow extension by addition of any new tests that become available.

Recently, generally applicable molecular methods based on nucleotide sequence variation in the chromosomal DNA of the isolates have been developed and used in epidemiological investigations. These method which include identification of the pattern of restriction endonuclease digest of the genome

by pulsed field gel electrophoresis (Arbeit et al., 1990) or detection of the restriction fragment length polymorphism (RPLP) at specific loci, such as fimbrial adhesion or ribosomal RNA operon (Stull et al., 1988) have all been used with some degree of success. Other methods such as plasmid analysis, phage typing (Frost et al., 1993), Lipopoly-saccharide (LPS) polymorphism (Chart et al., 1993) and verotoxin genotyping have also been used with some degree of success (Freeman et al., 1995).

Ribotyping has been successfully used to evaluate the relatedness of certain E. coli isolates (Tarkka et al., 1994). So far phage typing has defined over 80 types and a potentially highly discriminating index is based on bacteriophage restriction fragment length polymorphism (X-RFLP) (Freeman et al., 1995). Also pyrolysis mass spectrometry (PyMS) is a highly discriminatory, rapid and inconsumable costs relatively cheap method for inter-strain comparisons of micro-organisms which has the additional advantage of being able to examine larger numbers of isolates at a time (Freeman et al., 1995). However, the epidemiological value of information provided by any bacterial typing system depends on identification of typing markers that are stable as well as highly discriminatory at the subspecific level (Crichton et al., 1993).

2.3.4 Diseases Caused by E. coli:

E. coli is wide spread in the intestinal tracts of mammals and birds (Gross, 1992). The fact that most strains probably had their origin in faeces, it's presence particularly in small numbers does not necessarily mean that the food contains faecal matter (Collins and Lyne, 1976). Certain strains are pathogens in man and animals (Gross, 1992). So far water, humans, animals and animal by-products and other foods have been the reservoir of these organisms and the organisms are transmitted from these sources to the human host (Chapman et al., 1989; Ojeniyi, 1989; Kohli et al., 1993).

It should be noted that strains that are pathogenic are the root cause of both septic infection and diarrhoea (Gross, 1992).

(i) **Urinary tract and Septic infections;**

Urinary tract infections (UTI) remain a common clinical problem and recurrent infections in susceptible patients present an especially difficult treatment challenge (Stamm et al., 1991). E. coli is commonly implicated in infections of the urinary tract and is by far the most common cause of acute uncomplicated UTI outside hospitals. E coli also causes neonatal meningitis and septicaemia as well as sepsis in operation wounds and abscesses in a variety of organs (Gross, 1992). Also E coli that cause UTI often originate in the gut of the patient and the infection is thought to occur in an ascending manner. There is evidence that the ability of E. coli to infect the urinary tract is associated with fimbriae that specially mediate adherence to the uroepithelial cells

(Gross, 1992). Our current understanding of the natural history of UTIs in women is that uropathogenic bacteria, such as E coli from the intestinal flora, sequentially colonize the perineum, vagina, urethra and bladder (Stamm et al., 1991).

UTI is much commoner in females than in males since the shorter, wider female urethra appears to be less effective in preventing access of the bacteria to the bladder. Also there is high incidence in pregnant women. This can be attributed to impairment of urine flow due partly to hormonal changes and partly due to pressure on the urinary tract. Since most UTIs are thought to be caused by organisms originating from the patients own bowel, each infection is generally regarded as sporadic. However, the prevalence of various serotypes of E. coli in UTIs varies with geographical location and this fits well with the view that E. coli that causes such infections are specific pathogens for the urinary tract (Gross, 1992). So far, the large intestine serves as a reservoir for uropathogenic E. coli and for other Enterobacteriaceae that cause UTI (Kunin, 1987). Colonization of the large intestine precedes the spread to the vaginal and paraurethral areas and ascending infection of the urinary tract. The individual who carries uropathogenic E. coli in the large intestine should therefore run a greater risk of developing UTI than those who harbour other E. coli strains (Plos et al., 1995)

(ii) **DIARRHOEA**

E. coli may cause acute enteritis in young humans piglets, calves and lambs. Acute enteritis in humans of

all ages occur mainly in the tropics and this include travellers diarrhoea; a dysentery-like disease and haemorrhagic colitis or "bloody diarrhoea" (Gross, 1992). Strains that cause diarrhoea fall into four groups, with different pathogenic mechanisms. (Levine, 1987; Gross, 1992). These strains include; Enteropathogenic E. coli (EPEC), Enterotoxigenic E. coli (ETEC), Entero-invasive E. coli (EIEC) and Verocytotoxin-producing E. coli (VTEC).

(i) **EPEC:**

EPEC strains cause infantile enteritis especially in tropical countries (Gross, 1992). Out breaks often occur in hospitals and may have a high mortality, but such outbreaks have become uncommon in industrialized countries (Gross, 1992). The ability of EPEC strains to cause diarrhoea has been confirmed by oral administration of the organisms to babies and adults, but the mechanism by which they do so is incompletely understood. Most strains do not produce heat-stable enterotoxin (ST), heat-labile enterotoxin (LT) or vero-cytotoxins (VT) and are non-invasive (Gross, 1992). Although EPEC is still very common in communities with poor hygiene, it is of little importance in areas with good standards of hygiene (Gross, 1992).

(ii) **ETEC:**

These are strains that produce a heat-stable enterotoxin (ST) or a heat-labile enterotoxin (LT) or both. In

addition, they possess colonization factors that are specific for the host animal species and enable the organisms to adhere to the epithelium of the small intestine (Gross, 1992).

ETEC strains are uncommon in geographical regions where hygiene and nutrition are good, but occasional outbreaks of diarrhoea and sporadic cases have been reported. In the developing countries, diarrhoeal diseases are a major cause of death in children under 5 years of age. Also ETEC are commonest causes of travellers' diarrhoea (Gross, 1992). ETEC diseases occurs mostly with those travelling from areas of good hygiene and temperate climate to areas with lower standards particularly in warmer countries (Gross, 1992). In African countries ETEC have been found in 31-75% of peace corps volunteers with diarrhoea (Gross, 1992). The sources and mode of spread of ETEC infection in warm-climate countries are not well understood but it seems likely that water contaminated by human or animal sewage plays an important part in the spread of infection (Gross, 1992).

(iii) EIEC:

EIEC strains cause illness identical to Shigella dysentery in patients of all ages. The most common serogroup is 0124. The epidemiology and ecology of EIEC is poorly studied, but there is no evidence of an animal or environmental reservoir. Surveys suggest that they are responsible for about 5% of all diarrhoeas in areas

of poor hygiene (Gross, 1992). In UK and USA, outbreaks are occasionally described, especially in schools and hospitals for the mentally handicapped. Infections are usually foodborne but there is also evidence of cross-infection (Gross, 1992).

(iv) **VTEC**

VTEC strains are an important public health problem. E. coli 0157 is by far the most common serogroup found in human VTEC infections (Ostroff et al., 1987). VTEC 0157 was first recognized as a human pathogen in 1982 (Morgan et al., 1993). It causes a wide spectrum of clinical disease in humans, including non-bloody diarrhoea, thrombotic thrombocytopoenic purpura (Karmali, 1989), haemorrhagic colitis (HC) and haemolytic uraemic syndrome (HUS) (Wright et al., 1994). VTEC 0157: H7 is the most common cause of HC and HUS in North America and Western Europe and it is also the most frequent cause of outbreaks in day care centres (Spika et al., 1986). HC, HUS and other illnesses associated with E.coli 0157. H7 have been reported with increasing frequency during the past decade (Ostroff et al., 1989). According to Griffin and Tauxe, (1991) between 2-7% of persons infected with E. coli 0157:H7 develop HUS, which is characterized by the association of renal failure, thrombocytopenia and haemolytic microangio-pathic anaemia. HUS occurs in all age groups but is more common in infants and young children and is a major cause of renal failure in

childhood (Rowe et al., 1991). Also children and the elderly appear to be most at risk for serious outcomes (Griffin and Tauxe, 1991), with a case-fatality rate of 35% reported among the elderly in one outbreak.

2.3.5 E. COLI IN FOODS:

Foods of bovine origin such as raw beef, unpasteurized milk and yoghurt have been incriminated in most traceable outbreaks of disease associated with E. coli 0157:H7 (Morgan et al., 1993). Other foods and beverages have also been incriminated (Besser et al., 1993). Exposure to under cooked ground beef is a predominant risk factor identified in both epidemics and sporadic instances of E. coli 0157:H7 (Bryant et al., 1989), although outbreaks have been reported after ingestion of unpasteurized milk, cheese, improperly refrigerated cold cuts, exposure to contaminated water and through person-to-person spread (Duncan et al., 1986). Outbreaks and sporadic cases of HUS have been reported in several countries, particularly the USA and Canada where beef products and unpasteurized cows' milk have been cited as vehicles of infection (Frost et al., 1993). VTEC 0157 was first identified as a human pathogen in 1982 following outbreaks of bloody diarrhoea associated with a "Fast-food" restaurant chain in the United States (Riley et al., 1983). E.coli 0157 is difficult to isolate from food as the infecting dose may be very small (Davis and Bryan, 1995). So far only ground beef has been considered a high risk food because

contaminated surface meat is thoroughly mixed in when it is ground. In a survey in Ontario, Canada, E. coli 0157:H7 was isolated from 21 of 200 beef cattle as well as 39 of 200 culled dairy cattle (Clarke et al., 1988) In the UK, verocytotoxin-producing (VT$^+$) E. coli 0157, the most common serogroup associated with illness in man have been isolated from cattle (Chapman et al., 1989), but the organism has been isolated only rarely from food (Chapman et al., 1993) and its epidemiology remains unclear.

In Nigeria, although some reports exist on the enterotoxigenicity of E. coli strains isolated from animals Adetosoye et al., 1976), information is not available on food isolates (Shehu and Adesiyun, 1990). E. coli has been isolated from water as a faecal contaminant and from various foods and food products of animal origin including market raw milk (Adesiyun et al., 1983; Alabi and Adesiyun, 1986). The organisms have also been implicated in a number of foodborne and waterborne outbreak worldwide (Rosenberg et al., 1977). However, only a report exist on the study of E. coli contaminants on "Suya" a roasted beef product (Igene, 1983). Also E.coli has been isolated from locally fermented milk ("nono") (Shehu and Adesiyun, 1990; Zaria et al., 1992).

2.3.6 ANTIBACTERIAL AGENTS AND DRUG RESISTANCE IN E.COLI:

The normal commensal aerobic flora in particular E. coli of healthy populations is increasingly recognized as an important reservoir of antibiotic resistance gene (Levy et al.,

1988). This is of considerable concern firstly, because commensal gut bacteria may themselves cause endogenous infections and secondly because the resistance determinants may be transferred to any incoming pathogens complicating the treatment of the subsequent infection (Mamun et al., 1993).

Since the introduction of antimicrobial agents, they have been successfully used to prevent and treat bacterial diseases in man and other species. The availability of antibiotics means that many previously severe infections can now be treated. In addition, antibiotics are used for growth promotion in animal husbandry and in agriculture for crop protection (Nijsten et al., 1994). As antibiotics are not only very effective, but also remarkably safe drugs, this safety may have provoked liberal, even lavish, use in man and other animals. The use of antibiotics however, leads inevitably to emergence of resistance in the endogenous bacterial flora of treated persons and animals alike, against the antibiotics used or to other drugs (Neu, 1983). These enteric micro-organisms may colonize other persons or animals and transfer resistance plasmid to their faecal flora. Consequently the environmental bacterial population may be contaminated (Lester et al, 1990).

Farm workers can directly become colonized by resistant bacteria due to close contact with animals and their faeces (Ojeniyi, 1989).

Some antibiotics, example the aminoglycoside antibiotic apramycin, has been used extensively in veterinary medicine

since the early 1980s (Johnson et al., 1995). Apramycin has not however been used in humans, although early studies indicated that bacterial resistance to apramycin was rare (Ryden and Moore, 1977). There have been subsequent reports of the isolation of apramycin resistant organisms particularly E. coli and Salmonella species from farm animals in the UK (Hunter et al., 1992) and France (Chaslus-Dancla et al., 1986). It is not known if the presence of antibiotic resistance plasmids in toxigenic E. coli has an effect on pathogenicity, although antibiotic resistance is considered to hinder treatment (Hunter, 1993).

Some studies have been reported on antibiogram of E. coli strain from animals in Nigeria (Ojo and Moomaw, 1972; Falade and Oluwadiya, 1975; Shehu and Adesiyun, 1990), but little is known about those isolates from locally prepared foods (Shehu and Adesiyun, 1990).

However, it should be noted that, the widespread use of antibiotic prophylaxis has been criticized both on the grounds of drug toxicity and because of the possibility that the development and spread of drug resistance might be encouraged among a variety of enteropathogenic organisms (Gross, 1992).

2.3.7 ISOLATION AND IDENTIFICATION OF E. COLI:

E.coli plays an important role among the pathogenic microorganisms causing both septic infection and diarrhoea (Gross, 1992). Some strains are responsible for UTI (Levine, 1987).

Routinely used methods for isolating and identifying E. coli from stool rely on direct streaking on a less inhibitory differential media such as MacConkey agar, Eosin methylene blue agar and Endo agar. In the case of food samples, FAO (1979) recommended the use of lauryl sulphate tryptose broth as enrichment broth for E. coli. Some other modified enrichment broth have been used successfully. These include: EC broth containing novobiocin (Bennett et al., 1995), buffered peptone water containing vancomycin, cefsulodin and cefixime (Chapman et al., 1993). The incorporation of novobiocin in EC broth and vancomycin, cefsulodin, cefixime in buffered peptone water help to reduce the numbers of contaminating cells to multiply to levels detectable on culture media. Also the combination of vancomycin, cefsulodin and cefixime with buffered peptone water has been effective for isolating the organism from carcass meat samples (Chapman et al., 1993). Bernett et al., (1995) recommended the use of sorbitol. MacConkey agar incorporated with cefixime and tellurite for the isolation of E. coli 0157. The medium increases the rate and ease of isolation of E. coli 0157. However, the detection of E. coli 0157 present in low numbers in environmental samples is a problem due to the sensitivity of enrichment and direct culture methods currently available (Wright et al., 1994).

After enrichment and selective plating, FAO (1979) recommended a short set of biochemical reactions used to isolate E. coli. These biochemical reactions include: triple

sugar iron, methyl red, Voges-Proskauer, indole production, urease production, cytochrome oxidase, lactose, maltose, sucrose, mannitol fermentation and lysine and ornithine decarboxylation.

The increased test time arising from the use of enrichment has led to the development of commercial rapid procedures for detection of E. coli (Padhye and Doyle, 1992). The development of commercial rapid ELISA procedures for the detection of E. coli 0157 and immuno-magnetic separation system has helped to reduce total enrichment time and improve the sensitivity of detection (Wright et al., 1994). Also ELISA and immuno-blotting have been used to examine sera from patients with HUS for antibodies against E. coli 0157 (Chart et al., 1991). When the intermediate ELISA values are obtained (Chart et al., 1991), immunoblotting is used to confirm the presence of antibodies, specific for E. coli 0157 Lipopoly-sacchrides. However, a more rapid immunoblotting method that can be used to detect serum antibodies to E. coli 0157 LPS in one-day has been described (Chart et al., 1994). ELISA has been used for the detection of E. coli 0157:H7 in meat (Johnson et al., 1995).

Some other rapid methods have been devised for the detection, enumeration and identification of E. coli. Most methods in current use are based on selective media, B-galactosidase activity and the ability to convert lactose to acid and gas, these method requiring 24-48 hours of incubation (Vilamu and Vives-Rego, 1994).

Several methods based upon the expression of B-galactosidase (B-GAL) and B-glucuronidase (B-GUD) for the enumeration of E. coli have been reported (Sarhan and Foster, 1991). More recently, commercial system based on the detection of constitutive B-GUD and B-GAL have been described (Clark and EL-Shaarawi, 1993). Gale and Broberg, (1994) used a commercial gene probe assay kit for rapid Most Probable Number (MPN) enumeration of E. coli in drinking water. Flow cytometry (ECM) which has great potential as a rapid automated tool for ecological studies of microorganisms (Edwards et al., 1993) has been used as a rapid tool for the detection of E. coli (Porter et al., 1995).

CHAPTER THREE

MATERIALS AND METHODS:

3.1.0 STUDY AREA:

These studies were conducted in the Zaria area (comprising Samaru, Sabon Gari, Shika and Tudun-Wada).

Most of the animals are slaughtered at the main Zaria abattoir in the Muslim tradition. There is no antemortem and post-mortem inspection. The meat from cattle is pooled in water-filled drums and conveyed by car to the market.

3.1.1 SPECIMEN COLLECTION:

All specimens were collected in duplicate because two different organisms, requiring different media were to be isolated. Bile and lymphnodes were collected from slaughtered cattles at the abattoir. Rectal swabs were collected from cattle herds in Zango and Jos road. Raw meat, Kidney and fermented milk (nono), roasted meat ("Suya") and dried meat ("Kilishi") were purchased from the market into sterile containers. Fresh milk were purchased in Shika village and human stool collected at Ahmadu Bello University Teaching Hospital, Zaria, University Health Centre (Sickbay), Samaru and Jama'a Clinic, Samaru. All specimens were transfered to the laboratory for analysis.

3.1.2 ESTIMATING BACTERIAL NUMBER IN RAW MEAT, KIDNEY, "SUYA" "KILISHI", FRESH MILK AND FERMENTED MILK ("NONO")

(I) **Total aerobic count:**

About 2g of each ground raw meat and kidney were mixed in 18ml of buffered peptone water, 2g of "Suya" and "Kilishi" each were blended in 18ml of buffered peptone water making a 1:10 dilution. About 1ml of each specimen (rawmeat, kidney, suya, kilishi, freshmilk and fermented milk (nono) were placed in separate sterile tubes containing 9ml of 0.1% peptone water. Each specimen was serially diluted up to 10^{-4} dilution and 0.1ml of each placed on separate plate count agar (Oxoid) and spread with a sterile glass spreader until the entire surface was covered wih the inoculum. The plates were incubated at $37^{\circ}C$ for 18-24 hours. After incubation, the colonies of each plate was counted and the number of colonies on each plate was multiplied with the dilution factor to obtain colony forming units per gram or per millilitre of specimen (CFU/g or Ml).

(II) **Total coliform count:**

About 0.1ml of 10^{-2} dilution of each specimen (mentioned above) was placed on separate eosin methylene blue agar plate and spread with a sterile glass spreader until the entire surface was covered with the inoculum. The plates were incubated at 37 C for 18-24 hours. After incubation, the colonies growing on each plate were counted and the number of colonies on each plate was multiplied with the dilution factor to obtain colony forming units per gram or per millilitre of specimen (CFU/g or Ml).

3.1.3 PROCEDURES FOR CULTURAL DETECTION OF SALMONELLA AND ESCHERICHIA COLI:

3.1.4 PRE-ENRICHMENT AND SELECTIVE ENRICHMENT

All specimens (except raw meat and stool) were pre-enriched in 1% buffered peptone water.

(I) **Raw meat and Kidney:**

Fifty raw meat and kidney specimens, each weighing about 400g were purchased from two local markets located in Sabon Gari, and Samaru,Zaria. Each specimen was packed according to local practice and taken to the laboratory immediately for microbiological studies. About two 200g of each specimen was swabbed thoroughly over the entire surface and the swab placed in 5ml selenite F broth. Another portion (about 200g) was swabbed thoroughly over the entire surface and the swab placed in 10ml Lauryl sulphate tryptose broth (LST). The selenite F broth and Lauryl sulphate tryptose broth were incubated at 37^{0}C for 18-24 hours.

(II) **Lymph nodes and bile**

A total of fifty lymph nodes and bile were collected from animals slaughtered at the Zaria abattoir. At least two mesenteric lymph nodes were collected from each animal. In the laboratory, lymph nodes were de-contaminated by immersing for 5 seconds into boiling water. Excess fat and fascia were trimmed off using a pair of sterile scissors and forceps. The lymph nodes were then cut into tiny pieces using sterile scissors and forceps. Each specimen was ground using sterile mortar and pestle and about 1g of each lymph node was placed in 10ml selenite F broth. 1ml of bile was also placed in 9ml

selenite F broth. Another 1g of each lymph node and 1ml of bile was placed in 10ml and 9ml lauryl sulphate tryptose broth respectively. The selenite F broth and lauryl sulphate tryptose broth were incubated at 37°C for 18-24 hours.

(III) **"Suya" and Kilishi"**

Fifty specimens of "Suya" and fifty specimens of "Kilishi", weighing about 25g each were purchased from hawkers and processors' stands at various locations in Sabon Gari, Tudun-Wada, Shika and Samaru. Each "Suya" and "Kilishi" specimen was packed as is practice locally and taken to the laboratory immediately for microbiological studies. About 25g of each specimen of "Suya" and "Kilishi" was blended with 225ml of 1% buffered peptone water and incubated for 18-24 hours at 37°C. After incubation, 1ml of each pre-enrichment culture of "Suya" and "Kilishi" were transfered to 9ml of selenite F broth and another 1ml to lauryl sulphate tryptose broth. The broths were incubated at 37°C for 18-24 hours.

(IV) **Fresh and fermented milk:**

A total of one hundred specimens of fresh and fermented milk each measuring about 25ml were purchased from milk-maids in Sabon Gari, Tudun-Wada, Samaru market and from Shika village. Each specimen was placed in a sterile universal bottle and taken to the laboratory immediately. After shaking thoroughly, 1ml of each fresh and fermented milk was pipetted into 9ml of selenite F broth and another 1ml into 9ml of lauryl sulphate tryptose broth. All the enrichment broths were thoroughly shaken and incubated at 37°C aerobically for

18-24 hours. The pH of the fermented milk samples were determined using a pH meter (PYE UNICAM Model 292 MK 2).

(V) **Rectal Swabs**

Rectal swabs were obtained from 100 cattle from herds in Zango and along Jos road. Two swabs were taken from each animal; one was placed in 10ml of selenite F broth and the other in 10ml lauryl sulphate tryptose broth. They were both taken to the laboratory and incubated at 37°C for 18-24 hours.

(VI) **Human Stool:** One hundred stool specimens were collected from patients who were test positive for S. typhi infection by the Widal test at the following clinics: University Health Center (Sick bay), Samaru, Jama's Clinic, Samaru and Ahmadu Bello University Teaching Hospital, Tudun Wada. About 1g of each stool sample was suspended in 1ml of sterile peptone water and allowed to stand for the debris to sediment. After sedimentation, 0.5ml of the supernatant fluid was transfered into 5ml of selenite F broth and another 0.5ml of each specimen was placed in lauryl sulphate tryptose broth. The enrichment broths were incubated at 37°C for 18-24 hours.

3.1.5 **SELECTIVE PLATING:**

All specimens enriched in selenite F broth were plated on desoxycholate citrate agar (DCA) (Oxoid) and salmonella-shigella agar (SSA) (Oxoid) for isolation of Salmonella. Similarly, all specimens enriched in lauryl sulphate tryptose broth were plated on eosin methylene blue agar (Oxoid) for isolation of

E. coli. The media were prepared and utilized according to the instruction of the manufacturers. The plated media were incubated at 37°C for 24-48 hours. After incubation all cultures that appeared colourless on desoxycholate citrate agar and creamy with black spot at the center were then picked and subjected to other tests. On the other hand, all cultures that appeared blue-black with transmitted light and with greenish metallic sheen by reflected light were picked and subjected to other tests.

3.1.6 PRELIMINARY DIFFERENTIATION OF SALMONELLA AND E. COLI

All typical colonies on desoxycholate citrate agar and salmonella-shigella agar were transfered onto triple sugar iron agar (TSI), Lysine iron agar (LIA) and then onto urea agar. Colonies that showed typical Salmonella species reactions (alkaline/acid with or without hydrogen sulphide production on TSI, alkaline/alkaline with hydrogen sulphide production on LIA and urease negative) were stored at 4°C for further biochemical characterization. Also, typical colonies on eosin methylene blue agar were transfered onto triple sugar iron agar (TSI) and urea agar. Colonies that showed typical E. coli reactions (acid/acid without hydrogen sulphide production and urea negative) were stored on nutrient/agar slants at 4°C for further biochemical characterization.

3.1.7 BIOCHEMICAL CHARACTERIZATION:

All isolates suspected to be Salmonella and E. coli were biochemically characterized using standard techniques (Cowan, 1981). All isolates giving reactions characteristic of Salmonella and E. coli as given below were considered to belong to genus Salmonella and Escherichia. The typical characteristic reactions of Salmonella are indole negative, citrate positive, methyl red positive, Voges-Proskauer negative, lysine decarboxylase positive, gelatin liquefaction negative, motile in molility test medium, produce an alkaline reaction in the butt of lysine iron agar and hydrogen sulphide, produce and acid butt and an alkaline slant of triple sugar iron agar with hydrogen sulphide, ferment glucose, xylose, dulcitol, rhammose, mannitol and maltose but fail to ferment lactose and sucrose. They are also salicin negative, esculin negative and phenylalanine deaminase negative. The typical characteristic reactions of Escherichia coli are indole positive, methyl red positive, Voges-Proskauer negative, gelatin liquefaction negative, citrate negative, ferment lactose, maltose, glucose and mannitol.

3.1.8 SEROLOGICAL TESTING:

SOMATIC "O" ANTIGENS ANALYSIS:-

Isolates suspected to be Salmonella were serologically tested using Salmonella polyvalent "O" group A-S antiserum according to the instructions of the manufacturer (Wellcome Ltd, England). All isolates were streaked on blood base agar

slant and incubated at 37°C for 18-24 hours. A glass slide was marked into two sections. A drop of the polyvalent antiserum was placed on one side using the dropper provided and on the other side a drop of 0.85% sodium chloride (NaCl) was placed. A portion of growth of each isolate was picked with a sterile loop and mixed thoroughly in the saline (negative control) and another portion mixed thoroughly with the appropriate antiserum. The slide was rocked gently for about three minutes and agglutination where occurred were recorded from slightly positive (+) to highly positive (4+).

3.1.9 DETECTION OF ENTEROPATHOGENIC E COLI;

All the 59 isolates of E. coli were streaked on a nutrient agar slant and incubated at 37°C for 18-24 hours and on eosin methylene blue agar plate and incubated at 44°C for 24 hours. Isolates that were able to grow on eosin methylene blue agar at 44°C were selected as enteropathogenic E. coli (FAO, 1979).

3.2.0 SUSCEPTIBILITY OF ESCHERICHIA COLI AND SALMONELLA ISOLATES TO ANTIMICROBIAL AGENTS:

ANTIMICROBIAL AGENTS USED:

Antimicrobial agents and concentration used were: Ampicillin (25µg), Gentamicin (10 µg), Augmentin® (Ampicillin - Clavulamic acid) (30µg), Nalidixic acid (30µg), Nitrofurantoin (50µg), Streptomycin (25µg), Tetracycline (100µg), Cotrimoxazole (25µg) (Mast Diagnostics Multidiscs) and Chloramphenicol (30µg) (Oxoid).

SUSCEPTIBILITY TEST:

The tests were conducted by the disc diffusion method of Bauer et al., (1966). A few colonies of each Salmonella and E. coli culture were inoculated into 5ml of Mueller-Hinton broth and incubated at 37°C until the turbidity approximated that of the baruim sulphate standard (0.5). 0.2ml of each broth culture was inoculated to each Mueller-Hinton agar (Oxoid) plate and each inoculum was spreaded over the entire surface of the agar with a separate sterile glass spreader. The plates were allowed to dry for 15 minutes at room temperature and the antimicrobial agent discs were then placed on the inoculated Mueller-Hinton agar plates with a sterile forcep. The discs were pressed gently with the forcep to ensure complete contact with the medium. The petri dishes were kept for a further 15 minutes and then inverted and incubated at 37°C for 16-18 hours. After incubation, the zones of complete inhibition were measured to the nearest millimeter and interpreted based on the table of interpretation of zone diameters of test cultures provided by the disc manufacturers.

3.2.1 MINIMUM INHIBITORY CONCENTRATION (MIC) DETERMINATION

This was done using the dilution techniques as described by Cheesbrough (1985). The MIC for Ampicillin, Gentamicin, Streptomycin and Chloramphenicol were determined for some of the Salmonella isolates using the doubling dilution technique.

One capsule each of Ampicillin (250µg) and Chloramphenicol (250µg) was emptied into separate conical

flask containing 50ml of sterile distilled water to give a concentration of 5000µg/ml and then allowed to dissolve completely. From the 5000µg/ml, 0.6ml of each antibiotic was transfered into separate tube containing 9.4ml of sterile distilled water to give a concentration of 300µg/ml each. From the 300µg/ml of each antibiotic, a doubling dilution was carried out to a final concentration of 150µg/ml, 75µg/ml and 37.5µg/ml. 0.2ml of each concentration was added into each inoculated tube containing 9.8ml of broth and 0.2ml of Salmonella culture to give a final concentration of 6µg/ml, 3µg/ml,1.5µg/ml, and 0.75µg/ml. Also 1g of streptomycin was dissolve in 10ml of sterile distilled water to give a concentration of 10^5µg/ml. From the 10^5µg/ml, 0.1ml was transfered into a tube containing 9.9ml of sterile distilled water to give a concentration of 1000µg/ml. From the 1000µg/ml, 1ml was transfered into another tube containing 9ml of sterile distilled water to give a concentration of 100µg/ml. From the 100µg/ml, a doubling dilution was carried out to a final concentrations of 50µg/ml and 25µg/ml. 0.2ml of each concentration was added into each inoculated tube containing 9.8ml of broth and about 0.2ml of Salmonella culture to give a final concentration of 2µg/ml, 1ug/ml and 0.5µg/ml. Same procedure was applied to Gentamicin (80mg/2ml). All tubes were well shaken and incubated at 37^0C for 18-24 hours. After incubation all the tubes were examined and the lowest concentration of the antibiotic where no growth occured was considered to be the MIC.

Tetracycline (250µg), streptomycin (1g), Ampicillin (250µg) and Gentamicin (80mg/2ml) were also used to determine the minimum inhibitory concentrations for the enteropathogenic E. coli isolates using the dilution technique above.

CHAPTER FOUR

RESULTS

4.1.0 ESTIMATED BACTERIAL NUMBERS:

Table 1 shows the estimates of bacterial numbers in 6 different categories of specimens analysed. The range of aerobic plate and coliform counts of raw meat products were about 5.0×10^6 - 4.95×10^7 CFU/g and 1.0×10^6 - 5.05×10^7 CFU/g of specimens respectively. For the processed meat products ("Suya" and "Kilishi"), the range of aerobic plate and coliform counts were 2.0×10^6- 6.3×10^7 CFU/g and 1.0×10^6 - 5.0×10^7 CFU/g of specimens respectively. The range of aerobic plate and coliform counts of Fermented milk ("Nono") were 5.0×10^5 - 4.95×10^6 CFU/ml and 1.05×10^5 - 4.85×10^6 CFU/ml of specimen respectively, with a pH range of 4-5.2. For fresh milk, the range of aerobic plate count was 5.0×10^5 - 6.0×10^6 CFU/ml and range of coliform count was 1.0×10^5 - 4.5×10^6 CFU/ml of specimen.

4.1.1 ISOLATION FREQUENCY:

Table 2 shows the recovery frequency of E. coli and Salmonellae in the different categories of specimens analysed. E. coli was present in all except in rectal swab and "Kilishi", with frequency occurence ranging from 1% to 30%. Salmonella was isolated from raw meat 3(6%), lymph nodes 4(8%) and human stool 3(3%). Neither E. coli nor Salmonella was isolated from "Kilishi" and rectal swab. Of the 59 E. coli isolates, 19(32.2%) were enteropathogenic, isolated from human

stool 10(53%), fresh meat 2(11%), lymph nodes 2(11%), bile 3(16%) and fresh milk 2(11%).

Table 4.1: Estimates of bacterial numbers in 6 different categories of specimens analysed.

	Aerobic Plate Count CFU/g OR ML			Coliform Count CFU/g OR ML		
Specimens	Mean X10^7	Range	*Standard Range	Mean x10^7	Range	Standard Range
Raw Meat	2.1	5.0x10^6-4.7x10^7	2.0x10^6-5x10^6	1.2	5.5x10^6-2.4x10^7	Not Exceed 5.0x10^3
Kidney	2.0	5.5x10^6-4.95x10^7	2.0x10^6-5x10^6	1.9	1.0x10^6-5.05x10^7	Not Exceed 5.0x10^3
"Suya"	2.1	2.0x10^6-6.3x10^7	NA	0.93	1.0x10^6-5.0x10^7	NA
"Kilishi"	2.1	5.5x10^6-4.9x10^7	NA	1.3	1.0x10^6-5.0x10^7	NA
"Nono"	0.19	5.0x10^5-4.95x10^6	NA	1.4	1.05x10^5-4.85x10^6	NA
Fresh Milk	0.21	5.0x10^5-6.0x10^6	1x10^5-2x10^6	1.6	1.0x10^5-4.5x10^6	Not Exceed 5

*Standard Ranges From FAO (1979)

NA = Not available

Table 4.2: Recovery frequency of Escherichia coli and Salmonellae in Specimens Analysed

Specimen	Number of Specimens Analysed	Number(%) Positive for Salmonella	Number(%) Positive for E. coli	Number(%) Positive for Enteropathogenic E. coli
Rectal Swab	100	0 (0)	0 (0)	0 (0)
Stool	100	3 (3)	16 (16)	10 (53)
Raw Meat	50	3 (6)	9 (18)	2 (11)
Kidney	50	0 (0)	2 (4)	0 (0)
Lymphnode	50	4 (8)	10 (20)	2 (11)
Bile	50	0 (0)	15 (30)	3 (16)
"Suya"	50	0 (0)	2 (4)	0 (0)
"Kilishi"	50	0 (0)	0 (0)	0 (0)
Fresh Milk	100	0 (0)	4 (4)	2 (11)
"Nono"	100	0 (0)	1 (1)	0 (0)
Total	700	10 (1.4)	59 (8.4)	19 (32.2)

4.1.2 SEROLOGICAL TESTING:

All the isolates biochemically identified as Salmonella agglutinated in Salmonella polyvalent "O" group A-S antiserum.

4.1.3 ANTIMICROBIAL SUSCEPTIBILITY TESTING FOR SALMONELLA AND E. COLI

The susceptibility of isolates of Salmonella and E. coli to antimicrobial agents are shown in Table 3. All ten isolates of salmonellae were tested for susceptibility to nine antimicrobial agents. None of the isolates were sensitive to Tetracycline and Nalidixic acid, while all the isolates were resistant to Ampicillin, Cotrimoxazole and Chloramphenicol. None of the isolates was resistant to all the drugs. On the other hand, only 35 out of a total of 59 E. coli isolates were tested. Only 1(2.8%) was resistant to all the drugs, and all the 35 isolates were resistant to Cotrimoxazole and Nitrofurantoin.

Table 4 and 5 shows the resistance pattern of Salmonella and E. coli respectively. Six patterns of resistance were recorded for Salmonella. The most common pattern of resistance irrespective of the source of Salmonella was Cotrimoxazole - Streptomycin - Nitrofurantoin - Augmentin - Gentamicin - Ampicillin - Chloramphenicol occuring in 5 isolates. The other patterns occured in one isolate each. On the other hand, out of the 35 isolates of E. coli tested for susceptibility, only the patterns of the enteropathogenic E. coli have been considered. However, ten patterns of resistance were recorded, with the most frequent patterns of resistance irrespective of the source of the enteropathogenic E. coli isolates been, Ampicillin - Augmentin - Nalidixic acid - Nitrofurantoin - Tetracycline - Cotrimoxazole, Augmentin - Nitrofurantoin - Streptomycin - Tetracycline - Cotrimoxazole, Ampicillin - Augmentin - Nitrofurantoin - Streptomycin - Cotrimoxazole and Ampicillin -

Nitrofurantoin - Streptomycin - Tetracycline - Cotrimoxazole occuring 4, 3, 3 and 3 times respectively. The others occurred one time each.

Table 6 shows the number and percentage of Salmonella isolates with multiple drug resistance. Of all the Salmonellae tested, 5, 2, 2, 1 and 0 were resistant to 7, 6, 5, 4 and 3 or less than 3 drugs respectively.

4.1.4 MINIMUM INHIBITORY CONCENTRATION (MIC) FOR SALMONELLA AND ENTEROPATHOGENIC E. COLI ISOLATES

The MIC was considered as the lowest antibiotic concentration that prevented growth . Table 7 shows the MIC of 5 commonly used antibiotics resistant to Salmonella and enteropathogenic E. coli isolates.

Table 4.3: Suspectibility of 10 Salmonella and 35 E. coli Isolates to Antimicrobial Agents

	Salmonella	E. coli
Antimicrobial Agents Tested	Number(%) of Isolates Resistant	Number(%) of Isolates Resistant
Ampicillin	10 (100)	24 (68.5)
Gentamicin	9 (90)	10 (28.6)
Augmentin	7 (70)	10 (28.6)
Nalidixic acid	0 (0)	18 (51.4)
Nitrofurantoin	8 (80)	35 (100)
Streptomycin	8 (80)	20 (57.1)
Tetracycline	0 (0)	25 (71.4)
Cotrimoxazole	10 (100)	35 (100)
Chloramphenicol	10 (100)	ND

Key: ND = Not Determined

Table 4.4: Resistance Patterns of *Salmonella* to Antimicrobial Agents Tested

Isolates	Resistance Pattern
M3	Ts, S, Ni, AUG, GM, AP, C
M6	Ts, S, Ni, Gm, AP, C
M24	Ts, GM, AP, C
L2	Ts, S, Ni, AUG, GM, AP, C
L14	Ts, S, Ni, AUG, GM, AP, C
L25	Ts, S, Ni, AP, C
L34	Ts, S, Ni, AUG, GM, AP, C
S38	Ts, S, Ni, AUG, GM, AP, C
S49	Ts, S, AUG, GM, AP, C
S100	Ts, Ni, GM, AP, C

Key

M = Raw Meat,
L = Lymphnode,
S = Stool,
Ts = Cotrimoxazole,
Ni = Nitrofurantoin,
AUG = Augmentin,
GM = Gentamicin,
S = Streptomycin,
AP = Ampicillin,
C = Chloramphenicol,
T = Tetracycline,
NA = Nalidixic acid.

Table 5.5: Multiple Drug Resistance Patterns of 19 Isolates of enteropathogenic *E. coli* Tested

Drug Resistance pattern	Number of Isolates Resistant	% of Isolates Resistant
AP, AUG, NA, Nİ, T, Ts.	4	21.0
AUG, Nİ, S, T, Ts.	3	16
AP, AUG, Nİ, S, T, Ts.	3	16
GM, NA, Nİ, T, Ts.	1	5
AP, GM, Nİ, S, Ts.	1	5
AP, GM, AUG, NA, Nİ, S, T, Ts	1	5
AP, Nİ, S, T, Ts.	3	16
AP, Nİ, T, Ts.	1	5
GM, Nİ, S, T, Ts.	1	5
GM, AUG, NA, Nİ, Ts.	1	5

key
Ts =Cotrimoxazole
Ni = Nitrofurantoin
AUG = Augmentin
GM = Gentamicin
S = Streptomycin
AP = Ampicillin
T = Tetracycline
NA = Nalidixic acid

Table 4.6: Number and percentage of Salmonella isolates with Multiple Drug Resistance

Drug Susceptibility	Number of Isolates	%
Resistant to all drugs	0	0
Resistant to 1 drug	0	0
Resistant to 2 drugs	0	0
Resistant to 3 drugs	0	0
Resistant to 4 drugs	1	10
Resistant to 5 drugs	2	20
Resistant to 6 drugs	2	20
Resistant to 7 drugs	5	50

Antimicrobial agents tested
Ts =Cotrimoxazole
Ni = Nitrofurantoin
AUG = Augmentin
GM = Gentamicin
S = Streptomycin
AP = Ampicillin
T = Tetracycline
NA = Nalidixic acid

Table 4.7: MIC of 5 Antibiotic Against Salmonella and Enteropathogenic E. coli Isolates.

Antibiotics Tested	MIC For Salmonella (ug/ml) n = 10	MIC For Enteropathogenic E. coli (ug/ml) n = 19
Ampicillin	3	6
Gentamicin	0.8	1.6
Streptomycin	2	3
Tetracycline	ND	6
Chloramphenicol	1.5	ND

Key:

ND = Not determined
n = Number of isolates tested

CHAPTER FIVE

DISCUSSION

5.1.0 Estimated Bacterial Numbers

Total Aerobic Plate and Coliform Counts:

In this study, the aerobic plate and coliform counts for raw meat, kidney and raw milk were higher than those of the standards specified by FAO (1979).

The high aerobic plate and coliform counts recorded for the various samples of meat and meat products, could have resulted from contamination during processing and handling, of the carcasses in the case of unprocessed meat. With the processed meat products in addition to contamination by processors during handling other sources of contamination may come from trays used for hawking, environment, added spices, processing surfaces and the keeping of these products without covering at the retail outlets for long periods. On the other hand, contamination of milk and milk products could have resulted from the unhygienic collection of raw milk, processing and handling of fermented product ("Nono"). The locally fermented milk ("nono") is usually prepared under very unsatisfactory hygienic conditions. The contamination of this product starts right from collection of the raw milk as the udder of the cow is not washed or disinfected before collection. Bankole (1991) reported the isolation of some

micro-organisms from calabash washed with water after use the previous day and kept ready for the next day's milking.

In this study, the calabash used for milking may be another source of contamination of the raw milk and hence the fermented product ("nono"). The addition of unboiled well water and other miscellaneous products such as the Adansonia digitata seeds extracts to the fermented milk ("nono") to increase volume and an attempt to improve the taste and colour (Shehu and Adesiyun, 1990) is also a possible source of contamination in this study. Other possible source of contamination include the environment and the calf which is allowed to stimulate lactation before collection of milk.

While the high aerobic counts of bacteia in foods and food products could be a reflection of unhygienic food handling practices, it does not provide a good basis for predicting the the presence of pathogenic microbes in the food (Holland, 1979). A high coliform count on the other hand is an index of coliform contamination and therefore an indicator of probable presence of other enteric pathogens such as Salmonella and Shigella species and E. coli.

5.1.1 Recovery Frequency of Salmonella and E. coli in Specimens Analysed

The intestinal tracts of animals are the major sources of E. coli and Salmonella (Litchfield, 1980). However, the failure to isolate both Salmonella and E. coli from rectal swabs in this study agree with the report of Ewing (1986) who

opined that rectal swabs are not the best specimens for the isolation of enteric pathogens.

The recovery of Salmonella in all unprocessed meat samples except kidney samples is an indication of contamination during processing and handling of the carcasses in the abattoir and during marketing. Experimental work has shown that salmonellae are recoverable from finger tips 3 hours after contamination (Pether and Gilbert, 1971). Thus, the butchers may not only contaminate other carcasses but could get infected by accidentally eating without washing hands. Tabukum (1990), reported isolation of Salmonellae from the floor of the slaughter area. Hence the floor can also be a possible source of contamination of the carcasses since most of the operations are done on the ground. Other possible sources of contamination of meat and meat products include, the mode of slaughtering cattle, the vehicle used for conveying the meat to the market and at the point of sale. However, slaughtering of Salmonella - infected animals is difficult to avoid (Addo and Diallo, 1981).

In the study area, some observations were made which are likely the cause of or contribute to the contamination of carcasses and meat. Carcasses are always submerged in large drums of water which may increase the chances of cross-contamination. Furthermore, knives used for evisceration were used on other carcasses without any washing or disinfection. The carcasses together with butchers are transported to the market in the same open vehicles and at the point of sale

customers handle the meat indiscriminately. All these increase the chances of contamination with microbes especially the enterobacteria. Again, it is common to find stray dogs, chicken, ducks roaming the abattoir premises and feeding on intestinal contents, blood and condemmed offals. Salmonella have been isolated from dogs (Kwaga et al; 1989). Hence dogs could be a possible source of infection to those living around the abattoir or to the members of the household. They could also be a source of contamination to the carcasses, meat and premises of the abattoir.

The isolation of salmonellae and E. coli from lymph nodes may be attributetd to carrier status of the animals. Mesenteric lymph nodes are situated around the large intestine, hence are consumed with the offals. Mesenteric lymph nodes are believed to be significant reservoir of salmonellae which are readily transferable to meat and meat products (Samuel et al; 1979). The isolation frequency for salmonellae (8%) in the present study is lower than the 13.7% isolation frequency recorded by Kwaga et al; (1985) in the same location. The lower frequency seen in this study may be as a result of changes in the incidence of Salmonella in Zaria. However, the public should be enlightened on the dangers involved in consuming improperly cooked lymph nodes. Samuel et al; (1979) found that salmonellae are irregularly distributed within individual lymphnodes and with some nodes yielding high numbers of Salmonella than others from same animal and others not yielding Salmonella.

The failure to isolate Salmonella from bile in the present study, does not necessarily means that the animals were free of Salmonella. Falade and Durojaiye (1977) also reported similar result. In the present study, the isolation frequency of E. coli from bile was 30%. The consumption of these organisms in bile together with liver or for medicinal purposes will result in a serious gastro-intestinal infection.

No Salmonella was isolated from either "Suya" or "Kilishi". Similarly, only two E. coli isolates were obtained from "Suya" and none was recovered from "Kilishi". It should be noted that Salmonella species can be present in "Suya" and "Kilishi", but their growth could be inhibited by various factors. The roasting temperatures for "Suya" are higher than 60°C and "suya" remains exposed to such heat for about five minutes. Furthermore, most "Suya" samples are coated with ground groundnut. During roasting the oil from the groundnut traps heat, further raising the temperature. Thus such increases should be adequate to eliminate most bacteria including salmonellae and E. coli. Also, salt concentration of 5% and above is inhibitory to some bacteria including salmonellae and E. coli (WHO, 1983). Hence, the inability to isolate Salmonella from "Suya" and "Kilishi" may not be unconnected with the high temperatures and salt concentrations involved in their preparation. Also the low moisture content of the products could have prevented the growth of Salmonella and E. coli.

No Salmonella was isolated from either fermented milk ("nono") or raw milk. Similarly, E. coli was recovered from only one fermented milk and from four raw milk samples respectively. E. coli strains are ubiquitous in nature and therefore gain access to milk and dairy products during processing an handling (Cruickshank, 1970). To prevent consumers from milk-borne infection by Salmonella and E. coli, the processors should be educated on sanitary practices during milk collection, pasteurization and proper handling of milk during processing. The presence of E. coli in a fermented milk ("nono") sample may be as a result of contamination from either the animals, the environment or the personnel during processing and handling. Another source of contamination could be from the unboiled well water that the milk maids reportedly add to their milk during the processing of "nono".

In the present study, Salmonella and E. coli were recovered from 3% and 16% of human stool specimens examined respectively. *Salmonellosis has been recognized as a public* health problem throughout the century with an increase in the number of reported cases (Rice and Loewenstein, 1979). In Nigeria, diverse serotypes have been associated with several syndromes including gastro-enteritis, enteric fever, suppurative bone lesions, urinary tract infection and empyema (Collard and Sen, 1960).

Although E. coli has long been recognized as a normal inhabitant of the gastro-intestinal tract of man and animals,

on several occasions it has been responsible for acute gastro-enteritis and diarrhoea (Goullet, et al., 1994).

5.1.2 Sensitivity of Isolates of Salmonella Species and E coli to Antibiotics

The results obtained from this study suggest that multiple antibiotic resistance is wide spread among the local strains of Salmonella species and E. coli. These observations agree with the report of Kwaga (1984) who attributed the development of drug resistance to frequent usage of drugs in veterinary practices at sub-optional concentrations.

It is also very significant to note that, Chloramphenicol, Ampicillin and Gentamicin were completely resisted by all the Salmonella isolates. This is of serious concern because, these drugs are still considered the most efficacious and are therefore, still recommended for the treatment of salmonellosis in both animal and man. There is therefore, an urgent need to reverse this notion in the light of more current findings with regards to the sensitivity pattern of each particular isolate of Salmonella.

It was also found that, all the isolates of E. coli were resistant to cotrimoxazole and Nitrofurantoin, a finding similar to that reported by Shehu and Adesiyun (1990). Similarly, a high percentage of resistance has been reported among food isolates of Staphylococcus aureus (Kwaga and Adesiyun, 1984) as well as those from animals and human beings

(Adekeye, 1979). This may reflect the misuse or abuse of antimicrobial agents.

The MIC results obtained in this study are within the range of dosage required for treatment purposes because the maximum tolerance dose of Ampicillin, Tetracycline, Gentamicin, Chloramphenicol and Streptomycin are 1600µg/daily, 3200µg/daily, 3000 to 5000µg/daily, 1600µg/daily and 750,000µg/daily respectively (Wade and Reynold, 1978). The MIC obtained in this research may be different from that obtained by some other workers, since different laboratories using the same organisms, the same media and the same antibiotics may produce strikingly different results (Woolock and Mutimer, 1983; Kwaga, 1984). It is essential to monitor the MIC of drugs especially those used in therapy so as to provide a guide to dose administration of these drugs.

CONCLUSION AND RECOMMENDATION

From the findings of the present study, one can conclude that:

(1) The aerobic plate and coliform counts of raw meat, kidney and raw milk were higher than the standards recommended by FAO (1979). The counts for "Suya" and "Kilishi" and "nono" were also relatively high, however no standards are available for these products to compare.

(2) Bovine rectal swab yielded no isolate of Salmonella and E. coli, while human stool contamination with salmonellae and E coli were low. The presence of enteropathogenic E. coli in

human stool was high, may be because of drinking highly contaminated water or eating contaminated food

(3) The extent to which raw milk is contaminated with Salmonella and E. coliin samples analysed is low. Only one E coli was isolated from fermented milk, this is because of the acidic nature of the product. While only few animals in the population examined were carrying salmonellae and E.coli in their mesenteric lymph nodes.

(4) No Salmonella was recovered from bile and kidney, while few E. coli were isolated from bile.

(5) No Salmonella was isolated from the processed meat product and only two E. coli were isolated from "suya".

(6) Resistance to Chloramphenicol, Ampicillin and Gentamicin was found to be widespread amongst local isolates of Salmonella. Also resistance to Cotrimoxazole, Tetracycline and Nitrofurantoin are widespread amongst local isolates of E. coli. The MIC of Chloramphenicol, Ampicilin, Gentamicin, Steptomycin for Salmonella and Tetracycline, Ampicillin, Gentamicin and Streptomycin for E. coli are within standard range required for treatment.

Considering the problems discussed above, it can be recommended that:

(1) To improve the quality of fermented milk ("nono") in order to make it safer for consumption, there is need to educate the Fulani women on the need to boil the fresh milk from which "nono" is made and the well water used for preparation to kill any pathogen present, coupled with the use of clean utensils,

cleaning of the environment and disinfection of the udders of lactating cows. There is need also to discourage adulteration of "nono" with Adansonia digitata seed extracts inorder to reduce the likelihood of contamination.

(2) In Nigeria, slaughtering processes varies from abattoir to abattoir. At the Zango abattoir, slaughtering is usually done in the traditional way (Halah Method). Hence the following should be taken into consideration;

(a) Antemortem and post-mortem inspection of the animals before slaughtering should be reinstituted.

(b) The use of disinfectants and washing of hands, knives after flaying and eviscerating of each animal should be encounraged.

(c) Proper separation of carcasses and the offal should be well stressed.

(d) Stray dogs, ducks and chicken should be kept away from the abattoir premises by adequately fencing the abattoir.

(3) Tetracycline, Nalidixic acid and Gentamicin, Augmentins should be prescribed to patients suffering from any infection caused by Salmonella and E. coli respectively.Carriers should not be permitted to handle food and they should be screened periodically.

(4) More work need to be done so as to establish standard aerobic plate and coliform counts for nono, "Suya" and "Kilishi".

(5) There is a need, to set up a Reference Laboratory for complete serological analysis of the most frequently occurring pathogens infood and environmental sources.

(6) There is a need to construct a data-base station equiped with reference strains and documentation of epidemics.

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