ANTIBIOTIC RESISTANCE OF SOME GRAM NEGATIVE ORGANISMS FROM BEEF AND "TSIRE" SOLD IN AHMADU BELLO UNIVERSITY, ZARIA, NIGERIA.

BY

RILWAN HASSANA

P13SCMC8026

A DISSERTATION SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY, ZARIA

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF A MASTER DEGREE IN MICROBIOLOGY

DEPARTMENT OF MICROBIOLOGY,

FACULTY OF LIFE SCIENCES

AHMADU BELLO UNIVERSITY,

ZARIA, NIGERIA

FEBUARY, 2017

DECLARATION

I declare that the work in this Dissertation entitled ANTIBIOTIC RESISTANCE OF SOME GRAM NEGATIVE ORGANISMS FROM BEEF AND "TSIRE" SOLD IN AHMADU BELLO UNIVERSITY, ZARIA, NIGERIA has been carried out by me in the Department of Microbiology, Ahmadu Bello University, Zaria. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree or diploma at this or any other institution.

_____	_____	_____
Name of Student	Signature	Date

CERTIFICATION

This Dissertation entitled ANTIBIOTIC RESISTANCE OF SOME GRAM NEGATIVE ORGANISMS FROM BEEF AND "TSIRE" SOLD IN AHMADU BELLO UNIVERSITY; ZARIA, NIGERIA by HASSANA RILWAN meets the regulations governing the award of the degree of M.Sc. Microbiology of the Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

_____ _____

Chairman, Supervisory Committee Date
Professor C.M.Z. Whong

_____ _____

Member, Supervisory Committee Date
Professor I.O Abdullahi

_____ _____

Head of Department Date
Professor I.O. Abdullahi

_____ _____ Dean,

School of Postgraduate Studies Date
Professor Kabir Bala

ACKNOWLEDGEMENTS

In the name of Allah the most gracious the most merciful, who guides us on to the right path; all praises are due to Allah SWT. All respect and regards to the Holy Prophet (PBUH). It is a great pleasure for me to express my profound gratitude to my respected research supervisor, Prof. C.M.Z. Whong for his guidance throughout the course of my research work. I would like to express my sincere appreciation to the co-supervisor Prof. I.O. Abdullahi, Department of Microbiology, Ahmadu Bello University, Zaria for his valuable contributions towards the success of this research. May Almighty Allah reward them abundantly.

My appreciation also goes to the entire academic and non-academic staff of the Department of Microbiology Ahmadu Bello University Zaria. My appreciations also go to Mallam Shitu and Mallam Danlami as well as Mr Y.Y Palla of the Faculty of Veterinary Medicine, for their support in the success of this work.

I also have to express my profound gratitude to my parents; Alh. Rilwan Muhammad Inuwa and Haj.Hajara Umar for their moral and financial support towards the successful completion of my studies. May Allah reward them all and make paradise their final abode.

My gratitude also goes to my siblings Aunty Aisha, Yaya Jamilu, Usaini, Najaatu, Sumayya, Abdullahi, Habiba, Hassana and Usaina, my relatives, colleagues and course mates for their care and concern throughout the course of this research.

Finally, I am also indebted to and recognise the support and contributions of my beloved husband Alh Rabiu Salisu, my children Habiba, Salisu, Fatima Zahra and others too numerous to mention for their valuable contribution and support throughout the period of the study. I am very grateful to you all.

DEDICATION

This work is dedicated to Allah S.W.T, Who gave me the strength and ability to endure the difficulties encountered.

ABSTRACT

The use of antimicrobials in animal feed formulation has given rise to resistance noticedin species like *Citrobacter* spp, *Enterobacter aerogenes,Escherichia coli, Klebsiella* spp, *Proteus* spp, *Salmonella*sppand*Shigella* spp, which are commonly associated with food borne diseases. This study was aimed at detection, characterization and determination of the antibiotic resistance of some Gram negative organisms from raw beef and "Tsire" sold in Ahmadu Bello University Zaria, Nigeria. A total of 240 raw beef and processed beef ("Tsire") samples were collected and assayed for bacteria contaminants using standard cultural and biochemical methods. Susceptibility pattern of isolates to conventional antibiotics was also determined. Proximate composition of raw beef and "Tsire" revealed the various proportions of moisture, protein, fats, carbohydrate and ash content. "Tsire" had higher value than raw beef in all the parameters except in moisture. The total bacteria counts for the raw beef samples according to sales location were $2.61x10^{4}\pm4.89x10^{3a}$ (Faculty of Art), $4.57x10^{4}\pm8.3x10^{3ab}$(Pepsi Garden), $3.57x10^{4}\pm6.89x10^{3ab}$ (Community Market) and $5.24x10^{4}\pm9.41x10^{3b}$ (Sculpture Garden) while the bacteria counts for the "Tsire" samples according to sales location were $1.87x10^{4}\pm2.78x10^{3a}$ (Faculty of Art), $1.74x10^{4}\pm4.1x10^{3a}$ (Pepsi Garden), $1.37x10^{4}\pm1.77x10^{3a}$ (Community Market)and$1.59x10^{4}\pm3.49x10^{3a}$ (Sculpture Garden). Statistical analysis of the mean bacterial load showed no significant difference ($p>0.05$). A total of 16 isolates were isolated namely *E. coli* (7 strains), *Enterobacter sakazakii* (3 strains), *Hafnia* alvei (4 strains), *Klebsiella oxytoca* and *Tatumella* ptyseos. All the isolates (100%) were susceptible to Imipenen, Nitrofuratoin and Chloramphenicol,81.25,69, 56, 50, 43.75, and 38% were susceptible to Gentamycin,Ampicillin, Tetracycline, Sulfamethoxazole/Trimethoprim, Amoxicillin Clavulanate, and Ciprofloxacine respectively. While31, 44,50, 37.5, 88% were resistant to Ampicillin, Tetracycline, Sulfamethoxazole/Trimethoprim, Amoxicillin Clavulanate and

Erythromycin respectively. Polymerase Chain Reaction detection of *tetA* gene in Tetracycline resistant isolates revealed that only three (18.75%) of the Tetracycline resistant isolates possess *TetA* gene. The presence of these bacteria in meat should receive particular attention, because their presence indicates public health hazard and gives warning signals for the possible occurrence of food borne intoxication/infection.

TABLE OF CONTENTS

CHAPTER TWO

CHAPTER FIVE

CHAPTER SIX

LIST OF TABLES

Table **Title** **Page**

LIST OF FIGURES

Figure **Title** **Page**

LIST OF PLATES

Plate **Title** **Page**

LIST OF APPENDICES

Appendix **Title** **Page**

CHAPTER ONE

1.0. INTRODUCTION

1.1. Background of the study

Food-borne pathogens are the leading cause of illness and death in developing countries costing billions of dollars in medical care and social costs (Fratamico *et al*., 2005). Changes in eating habits, mass catering complex and lengthy food supply procedures with increased international movement and poor hygiene practices are major contributing factors. Contaminated raw meat is one of the main sources of food-borne illness (Bhandare *et al*., 2007; Podpecan *et al*., 2007). Meat is the main edible part of domestic animals; however, recent definition includes species, as well as fish, shellfish, poultry and exotic species such as frogs, (Nakai and Moddler, 2000). Similarly, meat refers to animal tissue used as food, mostly skeletal muscles and associated fat but it may also refer to organs including lungs, livers, skin, brains, bone marrow, kidney and a variety of other internal organs as well as blood. Recent increase in the consumption of meat and its products arises from reasons including high protein contents, vitamins, minerals, lipids and savoury sensation (Nychas *et al.*, 2008).

A number of studies have reported outbreak of infections due to consumption of contaminated food and poor hygiene and in most of the cases, data are loosely based on laboratory isolates which do not reflect the actual ratio of food-borne infections. However, a few community-based reports provide evidence of several outbreaks caused by *Salmonella*, *Shigella*, *Escherichia coli* and *Listeria* spps in different parts of the world (Zweifer *et al*., 2008). It is not inevitable to prove a direct role of drug resistance in bacteria contaminating food items with increased clinical cases of resistant infections but the presence of such bacteria in food items and their related environment could play a role on the spread of

antimicrobial resistance among food borne pathogens (Farzana *et al*., 2009). There are a lot of human health consequences in consuming contaminated foods ranging from protracted illness to death and patients with impaired immunity are at greater risk (Zweifer *et al*., 2008).

"Tsire" is a popular, traditionally processed, ready-to-eat Nigerian meat product, which may be served or sold along streets, in club houses, at picnics, parties, restaurants and within institutions (Igene and Mohammed, 1983). It is a mass consumer fast food. Its preparation and sales along streets are usually not done under strict hygienic condition because they are still done locally. "Tsire" is roasted meat of either mutton, beef, or goat that is roasted around a burning charcoal in which the meat pieces are stacked on wooden sticks and spiced with peanut cake, spices, vegetable oil, salt or other flavourings (Alonge and Hikko, 1981). The prepared "Tsire" when being sold are usually packaged in newspapers and sometimes in cellophane or nylon bags. Most of the stages of "Tsire" preparation, materials used in its preparation and packaging, the handlers and the surrounding environment can serve as source of contaminants to the meat product (Igene and Mohammed, 1983).

Generally meat including "Tsire" is excellent in supplying high quality protein, vitamins and minerals salts such as iron and zinc (Kramiliah *et al.,* 1973). Meat is ideal for the growth of a wide range of spoilage bacteria (May *et al.,* 2003) which accounts to a great extent why it is perishable. Its high ultimate pH generally makes it very susceptible to microbial growth even under the best handling or manufacturing conditions and practice (Hedrick *et al.,* 1994).

The processing operations of meat to produce these products preserve and increase the shelf life in addition to improving the palatability and food value of the meat. The consumption of "Tsire", klishi, balangu, kundi and dambunama has extended to other parts of the country (Inyang *et al.,* 2005). In Nigeria, "Tsire" sales in cities and small towns are prominent. In "Tsire" preparation, use of lean meat is necessary since fat becomes rancid during the drying

process. “Tsire” preparation process in Nigeria lacks hygienic control and the risk of foodborne infections is very high. Some researchers elsewhere had noticed sporadic cases of gastroenteritis and symptoms of infection after consumption of “Tsire” which indicated that the product indeed constitute a food safety risk (Odusole *et al.*, 2003; Inyang *et al.*, 2005).

Emergence of antibiotic resistant pathogen is one of the most serious threats to public health in the 21st century (Willey *et al*., 2007). Multidrug resistance is emerging worldwide at an alarming rate among a variety of bacterial species, causing both community-acquired and nosocomial infections (Nordman *et al*., 2012). This may have arisen from extensive use of antibiotics in agriculture such as for treatment of infections, growth enhancement and prophylaxis in food animals at low concentration (Burgos *et al*., 2005).

The prevalence of these drug and multi-drug resistant bacteria in food animals and their products may serve as a potential transfer route of antibiotic resistant bacteria and resistant genes into human food-chain and environment. This has the potentials to pose a health threat to lives (Tansuphasiri *et al*., 2006). Some food products may be wholesome prior to processing. For instance, chicken is wholesome while the chicken is alive. However, the sterility can be compromised by unhygienic handling and some contaminants from the environment and in the process of cutting and packaging. (Ingham, 2001). Animals are known to constitute a vast reservoir of enteric bacteria with the general problem of drug resistance and environmental contamination through organic wastes and vectors (Adeleke and Omafuvbe, 2011; Olaitan *et al*., 2011; Samuel *et al*., 2011; Bahrndorff *et al*., 2013). Most of the contaminants of food animals originate from the alimentary tracts, respiratory tracts and or external surfaces of either life animal or the handlers.

In raw beef and “Tsire” (a spicy, barbecued, smoked or roasted meat product) for instance, the possible contaminants come from the carcass itself, the handlers and or even from the

spices used in the preparation. According to Edema *et al.* (2008) all "Tsire" processors prepare the "Tsire" at their wooden stalls located by the roadsides. The surroundings are considered unhygienic given that garbage and dirty wastes litter the food processing environment with open gutters nearby, all of which attracted houseflies. Slabs and trays used for cutting and smoking were noted to be inadequately cleaned.

1.2 Statement of Research Problem

Contaminated raw meat is one of the main sources of food-borne illnesses (Bhandare *et al.*, 2007, Podpečan *et al.*, 2007). Raw meat available in open-air local retail shops without appropriate temperature control is purchased by approximately 23% households in developing countries (Anjum *et al.*, 2004). The risk of the transmission of zoonotic infections is also associated with contaminated meat. International food management agencies, especially the World Health Organization (WHO), the Food and Agriculture Organization (FAO) and the International Hazard Analysis Critical Control Point (HACCP) Alliance have already provided guidelines to member countries about safe handling procedures such as HACCP and Good Manufacturing Practices (GMPs). In many situations international or national trade in livestock or other animals may be the major threat (Anon, 2008). Many animals, especially poultry and pigs, may also be infected but show no clinical sign (Wray and Wray 2000). Such animals may be important in relation to the spread of infection between flocks and herds and as causes of human food poisoning. In the latter case, this can occur when these animals enter the food chain thus producing contaminated food products (Wray and Wray 2000).

Proper steps should be taken to prevent contamination during transport or storage (International Organization for Standardization (ISO), 1996; 1999). Because of the large variety of food and feed products there is no single sampling method appropriate to all

products. Therefore different methods specific to the product should be used and it is usually more effective to carry out process monitoring than mass testing of individual feed ingredients and finished feed (Anon, 2008).

1.3 Justification of the Study

Food borne diseases are among the most serious problems affecting public health and development worldwide (WHO, 1994). Bacteriological quality assessment of raw beef and "Tsire" is very critical. Even though epidemiological evidence on outbreaks of "Tsire"-borne diseases is scarce, there are indications that "Tsire" could be contaminated to unsafe level with air flora and other microorganisms from handlers, equipments, utensils like trays, spoons and knives.

Although, various studies had been conducted on raw meat and "Tsire" in different parts of Nigeria, a close investigation however reveals that not so much data is available addressing the presence of Gram negative bacteria as contaminant of "Tsire". Hence this work is important not only in providing baseline information that will inform the public on meat consumption but also in providing useful data that will enhance further research.

1.4 Aim of the Study

The aim of this study was to isolate and characterize some Gram negative organisms from raw beef and "Tsire" locally sold in Ahmadu Bello University, Zaria main campus, Samaru Zaria, Kaduna State Nigeria.

1.5 Objectives of the study

The specific objectives of this study were to:

1. determine proximate composition of raw beef and “Tsire”.
2. determine the total bacterial counts of raw beef and“Tsire”.
3. isolate and characterise some Gram negative organisms from raw beef and“Tsire”.
4. determine the antibiotic susceptibility pattern of the isolates.
5. detect*tet*Agenes for tetracycline resistance in the isolates using PCR.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1. *Enterobacteriaceae*

The family *Enterobacteriaceae* comprised the coliform and faecal coliform bacteria (Sleigh and Duguid 1989). These bacteria are intestinal; as normal microflora of man and other animals, but with a greatest potential of inhabiting any other part of the body as saprophytes obligates or opportunistic pathogens (Dodds and Gillie 1984). Many also multiply in vegetation, sewage and surface waters around man's environment. The organisms are also allochtonous to water and vegetation under natural conditions (Neal, 1995). Their occurrence is considered a parameter for evaluating the quality of drinking water as reported by the World Health Organization (WHO 1987; WHO 1991). It was established that these pollution – indicator bacteria consist of disease-causing genera of importance to public health (Amstrong *et al.*, 1981).

The typical genera of this large heterogenous family include *Citrobacter* spp, *Edwardsiella* spp, *Enterobacter aerogenes*,*Erwinia*spp, *Escherichia coli, Klebsiella, Proteus* spp.*Salmonella, Serratia,* spp, *Shigellae, Vibrio* sppand *Yersinia*. *Shigella, Salmonella* and *Vibrio Cholera* aswell *Vibrio parahaemolyticus* are obligate pathogens; *Proteus* and *Klebsiella* which inhabit mainly the urinogenital andrespiratory tracts are opportunistic pathogens. *Escherichia coli* and *Enterobacter aerogenes* are normal microbiota, becomingpathogenic under very exceptional circumstances (Cheesbrough, 1984; Sleigh, 1989; WHO 1991; Exner and Martins 1995).

2.1.1 The Microbiology of *Enterobacteria*

These organisms are Gram-negative, non – spore-forming bacilli of size 1 – 3 x 0.4-0.7µM. Members of the group are capable of profuse growth aerobically and anaerobically on simple laboratory media such as peptone water without the addition of blood or serum. They are circular, convex, smooth colonies of up to 2mm in diameter after overnight incubation on MacConkey agar. They are oxidase-negative and produce acid and/or gas fermentatively from glucose or lactose. They reduce nitrates to nitrites. The medically – important ones are the *Arizona* spp, *Citrobacter* spp, *Enterobacter aerogenes,Escherichia coli, Klebsiella* spp, *Proteus* spp, *Salmonella,Shigella* spp(Stokes and Ridgeway 1980; Dodds and Gillie, 1984; Sleigh and Duguid, 1989).

2.1.2. Antigenic Structure of *Enterobacteriaceae*

Enterobacteriaceae have a complex antigenic structure. They are classified by more than 150 different heat-stable somatic O (Lipopolysaccharide) antigens, more than 100 heat-stable K (Capsular) antigens and more than 50 H (Flagella) antigens. In *Salmonellatyphi*, the capsular antigens are called vi antigens (Jean, 1976; Earnest *et al.*, 1987). O antigens are the most external parts of the cell wall consisting of repeating units of polysaccharides with unique sugars. Antibodies to O antigens are predominantly IgM. Each genus has specific O group but a single organism may possess several O antigens. Most *Shigella* share one or more O antigens with *Escherichia coli* and may cross – react with some *Providencia*, *Klebsiella* and *Salmonella* species. Occasionally, O antigens may be associated with specific human disease, e.g., specific O types of *Escherichia coli* are found in diarrhoea and urinary tract infection (Earnest *et al.*, 1987). Example O157:H7 emanating as an emerging pathogen (Bennett and Laurence, 1989). K antigens are external to O antigens on some but not all *Enterobacteriaceae.* Some are polysaccharides including the K antigens to *Escherichia coli,*

others are proteins. K antigens may interfere with agglutination by O antisera, and they may be associated with virulence, e.g., *Escherichia coli* strains producing KI antigens are prominent in neonatal meningitis and K antigens of *Escherichia coli* cause attachment of the bacteria to epithelial cells prior to gastro-intestinal or urinary tract invasion. *Klebsiella* sppforms large capsules containing polysaccharides (K antigens) covering the somatic O or H antigens and can be identified by capsular swelling tests with specific antisera.

2.2. Enterobacterial Pathogensis

The harmful effects caused by enterobacterial pathogens to man and animals result after successful invasion and final establishment in the host system resulting in immunological interactions manifested by a specific disease type presentation. This is mostly a consequence of strict and opportunistic pathogen action (WHO, 1987).

2.2.1. Gastroenterists

Gastroenterists is defined as the inflammation of the mucous membrane of the gastro-intestinal tract due to bacterial infection. Other incriminated aetiological agents of Gastroenterists are the protozoa such as *Entamoeba histolytica, Giardialamblia,* enteric viruses and helminthes. It may also be due to toxins and allergic reaction (Stokes and Ridgeway, 1980; Earnest *et al.*, 1987; Luois, 1987). Gastroenteritis is one of the disastrous communicable infections occurring all over the world more especially in developing countries where the standard of hygiene and economy is very low (Jacob *et al.,* 1970). It was maintained that poor environmental sanitation including low control of flies facilitated the introduction of enterobacteria into foods and water. A warm humid climate with lack of adequate storage facilities (refrigeration and smoking) act as a factor for the growth of these organisms. “Well-oral” is the route of transmission mainly. Both adults and children suffer from the disease but infants are most susceptible due to probably immature immunological

responses and malnutrition especially in non-breast fed infants. This was reiterated to be due to their slow rate of mucosal epithelial regeneration hence low production of local IgA. As a consequence, gastroenteritis is probably responsible for the killing of more children throughout the world than any probably responsible single disease.

2.2.2. Yersiniosis

This is a disease caused by *Yersinia enterocolitica.* It is characterized by enterocolitis and acute septicaemia withdiarrhoea, common in Europe and the United States of America.It present up to 1-3% type gastroenteritis but with nocomparable data in the developing countries. A study in Victoria,Australia, reported that the pathogenic organism existsfrequently in environmental waters. Generally, the clinicalmanifestations include hyper secretion of toxins leading totravellers' diarrhoea, profuse diarrhoea, vomiting, muscularcramps, loss of electrolytes (Na+, K+, Ci- etc) leading tosuppression of urine with consequent loss of body fluids andthus, dehydration and collapse in many case (Cheesbrough, 1984; Collee *et al,.*1989). Morbidity andmortality is high due to the case of the spread of thepathogen through contaminated food and water as a result ofignorance on the dangers associated with poor environmentaland personal hygiene (Indakwo and Abu, 2004).

2.2.3. Shigellosis

The infection type, shigellosis, is also known as bacillary dysentery (PHC 1995). It is an acute infection of the colon occurring throughout the world and spreads by contaminated food and water. Most severe amongst its types is the dysentery due to *Shigella dysenteriae* followed by *S. flexineri, S. boydii* and that of *S. sonnei* is the least. The incubation period is one week after which vomiting, high fever, profuse diarrhoea with blood in stool abruptly set in. Dehydration and sometimes collapse and death within a few hours result. But *S. sonnei* presents milder symptoms with slight fever, diarrhoea and vomiting, colic, abdominal pain

but without any serious constitutional symptoms. However, recovery is attained within a week. This disease calls for treatment with tetracycline, ampicillin, sulphonamides and streptomycin (Pelczar *et al.,* 1993; Kano State Ministry of Health 1996; Mukhtar *et al.,* 2001). *Shigella* infection accounted, in 1973, for up to 39, 3121 cases with up to 29 deaths in Nigeria as reported by the World Health Organization (WHO, 1987).

2.2.4. Typhoid and Paratyphoid Fevers

These are due to *Salmonella typhi* and *S. paratyphi* A, B (*S. schottmuelleri*) and C *(S. Hirschfield*). The former is muchmore severe and has claimed more lives than the latter (Thrum and Gericke, 1994).Typhoid and paratyphoid B occur worldwide while paratyphi Ais common in the East and C is rare. The disease is prevalentin countries with low standard of sanitation. One local studyreported up to 5% incidence in children (Mukhtar *et al.,* 2001). The clinical picture is marked after an incubation period of about two weeks with onset of headache (10 day for typhoid), myalgia and tiredness, cough and fever with about 0.5^0C rise per day to reach up to 40^0C in the first week with accompanying febrile illness. Characteristic rashes consisting of a few crop spots appear, particularly on the abdomen or chest for a few days, which can easily be over-looked (Earnest *et al.,* 1987). Constipation may be pronounced initially, culminating in diarrhoea by the second week with severe attack to take patient into "COMAVIGIL". It characterized by drowsiness, confusion, muttering to one-self, plucking at beddings, delusion, irritability and other psychotic reactions as well as anaemia. In the third and fourth weeks, however, improvement sets in gradually. Diagnosis is achieved using blood culture by employing selective media such as Desoxychlolate citrate agar (DCA) or serologically by Widal test (WHO, 1987). Chloramphenicol applied parentally and/or orally, tetracycline and metronidazole are the drugs of choice after carrying out sensitivity test (Kano State Ministry of Health, 1996).

2.3. Immunity to *Enterobacteriaceae*

Specific antibodies develop in systemic infection but it is uncertain whether they last in human. Antibodies against the core glycolipid of *Enterobacteriaceae* are associated with the protection against the haemodynamic squealer of bacteraemia due to Gram – negative rods and also reduce the feverish response and augment intravascular clearance of certain organism (Deborah *et al.,* 1995; Stokes and Ridgeway, 1980)**.**

2.4. Diagnosis of Enterobacteria

Specimens helpful for diagnosing enterobacterial infectionsinclude faeces, urine, pus, blood, sputum, spinal fluid andmaterials from localized site of the disease process. Forepidemiological purposes, drinking water and foods are alsogood materials for investigation. These are cultured on bloodagar for rapid preliminary identification. Triple sugar Iron agar(TSI), Desoxycholate Citrate agar (DCA), Wilson and BlairsBrilliant Green Bismuth Sulphate (BBS) agar, Xylose LysineDesoxycholate, Bile Salt agar (TCBS) are selective for*Salmonella, Shigella, Proteus, Citrobacter* and *Vibrio cholera*respectively (Jean, 1976; Cheesbrough, 1984; WHO, 1987). Biochemical tests such as Indole, Methyl,Voges-Proskaeur and Citrate (IMVIC test) as well as specificserological test can be included for detailed identification (Duguid, 1968).

2.4.1. The Problem of Antibiotic Resistance in Enterobacteria

The ability of *Enterobacteriaceae* to produce disease is well established. As well, information on antibiotic susceptibility especially for aquatic and seemingly wild isolates is being reported. However, the pattern of sensitivity to amoxicillin, carbenicillin, chloramphenicol, co-trimoxazole, erythromycin, ofloxacin, gentamicin, streptomycin, ciproxim, cephalexin and tetracycline were found to be in the region of 40% to 90%. However *Pseudomonas* species

showed a high resistance to co-trimoxazole and streptomycin. With ampicillin, the situation was discouraging; for example, it was seen to be resisted by almost all the organism at greater frequency with the exception of *Proteus* spp. In general, multiple drug resistance was evidenced. Nevertheless, carbenicillin, co-trimoxazole, ofloxacin, chloramphenicol, gentamicin and metronidazole could still be reliably be employed as their efficacy and potency on *Enterobacteriaceae* was shown to be high. With co-trimoxazole, the sensitivity was also lower than for the other much more active antimicrobials mentioned earlier. The high popularity of the drug among the population in the common name "Septrin" might have accounted for that. It could thus berecommended that while trying to treat enterobacterial infectionschemotherapeutically, multiple drug therapy should beemployed, so that chemotherapeutics such as chloramphenicol,co-trimoxazole, gentamicin, tetracycline and doxycycline canbe combined appropriately to achieve greater efficacy andpotency (Marie *et al.,* 1997). Antibiotic resistance which is especially notorious with *Enterobacteriaceae* has become a serious menace to clinicians and laboratory agents (Hug 1980; WHO, 1987). Worldwide resistance of *Shigella* sppto sulphonamides, tetracyclines streptomycin, chloramphenicol and ampicillin is well known (Jazrawi and Hindawi, 1986). Resistance of *Yersinia enterocolitica* to antibiotics was also reported (Yusha'u *et al.,* 2008). Multiple antibiotic resistance had also been reported in Bangladesh with up to 5-30% incidence rates (Hug, 1980).

Enterobacterial isolates resistant to penicillin, trimethoprim, sulphamethoxazole, tetracycline and chloramphenicol have been reported elsewhere. Transferable drug resistance was detected in 69% *Escherichia coli,* 60% *Klebsiella,* 67% *Proteus* and *Citrobacter* strains. The increased incidence of drug resistance has created difficulty in treating urinary and gastro-intestinal tract infections strikingly (Kendal, 1980; Yusha'u *et al.,* 2007; Yusha'u *et al.,* 2008). It could be stated therefore, that the existence of antibiotic resistance, and among the

family *Enterobacteriaceae* severally limits the possibilities of antibacterial therapy worldwide. Drug resistance is favoured by the extensive use of antibacterial drug in our communities.

Antimicrobials /antibiotics are obtainable outside of recognized treatment centres, and taken without medical authorization or supervision. This led to an inappropriate use and then being taken at wrong dosages and for an insufficient length of time to kill these organisms. Laboratory facilities and trained personnel are often not available to facilitate isolation of pathogens to and perform sensitivity tests.

Guidelines regarding the selection of drugs and information about drug resistance are not perfectly communicable in many of those presenting antimicrobials especially in rural areas. Control procedures in hospital are often inadequate resulting in the spread of infectious diseases and resistant strains. It has to be borne in mind however, that enterobacterial organisms that were not resistant before, could still be introduced into environmental waters by wild animals and even human rural dwellers that were not necessary exposed to drugs (Hilde – Kruse and Hening, 1994; Exner and Martins 1995). The resistance factors are retransferred to them as they come across the resistant ones in such water (Kendal, 1980).

2.5. Food Borne Diseases

The battle against foodborne diseases is facing new challenges due to the globalization of the food market, climate change and changing patterns of human consumption as fresh and minimally processed foods are currently preferred (Schelin *et al*., 2011). As food is biological in nature, it is capable of supporting the growth of microorganisms and foodborne diseases result from the ingestion of contaminated foods and food products (Guyader and Atmar,

2008). More than 250 different types of viruses, bacteria, parasites, toxins, metals, and prions are associated with foodborne diseases in humans (Schmidt *et al*., 2009). Although viruses are more responsible for more than 50% of all foodborne illnesses; generally hospitalizations and deaths associated with foodborne infections are due to bacterial agents. The infections range from mild gastroenteritis to life-threatening neurologic, hepatic, and renal syndromes caused by either toxin from the "disease-causing" microbe, or by the human body's reaction to the microbe itself (Teplitski *et al*., 2009).

Foodborne diseases remain a major source of morbidity and mortality in the general population, mainly in susceptible groups, such as infants, the elderly and the immune-compromised (WHO, 2011). According to the World Health Organization (WHO), up to 1.5 billion cases of diarrhoea and more than three million deaths that occur in children every year are as a result of food and water contamination (WHO, 2007), In the United States of America (USA) it is estimated that, foodborne diseases result in 76 million illnesses, 325,000 hospitalizations and 5,000 deaths each year (Mead *et al*., 1999; WHO, 2007). In France, it is estimated that these pathogens cause 10,200 - 17,800 hospitalizations yearly (Vaillant *et al*., 2005). The developing world are not spared, in South East Asia, approximately one million children below the age of five years die each year from diarrheal diseases due to contaminated food and water (WHO, 2000). Several devastating foodborne outbreaks have been reported on the African continent; in 2004, Kenya experienced an acute aflatoxicosis outbreak which was attributed to maize whereas in 2007, Angola registered 400 cases of bromide poisoning, associated with the use of sodium bromide as cooking salt (WHO, 2005). The prevalence of antimicrobial resistance among foodborne pathogens is reported to have increased (Yucel *et al*., 2005; Nyenje *et al*., 2012a), probably as a result of selection pressure created by the use of antimicrobials in animals.

Food diseases are divided into three types: 'Infection', 'intoxication', and 'intermediate' (Schmidt *et al*., 2009). 'Infection' is caused by the oral ingestion of viable microorganisms in adequate amounts to build up infection and the commencement of symptoms is normally delayed, reflecting the time required for an infection to develop. Examples of food infections are enteric viruses, *Salmonella*, *Campylobacter* and *Vibrio* species. 'Intoxication' on the other hand, is caused by the ingestion of toxins that have been pre-formed in the food. Therefore, there is no necessity for live organisms to be present and the onset of the symptoms is rapid. Examples are *Bacillus cereus* and *Staphylococcus aureus*. The 'intermediate' food poisoning occurs when live bacteria are ingested and subsequently produce a toxin in the host, as in the case of *Clostridium perfringens* food poisoning (Teplitski *et al*., 2009).

2.5.1. Aetiology, Pathogenicity and Epidemiology of Foodborne Illnesses

2.5.1.1 Bacterial agents

Foodborne bacterial agents are the leading cause of severe and fatal foodborne illnesses. More than 90% of food-poisoning illnesses are caused by species of *Staphylococcus, Salmonella, Clostridium, Campylobacter, Listeria, Vibrio, Bacillus,* and Entero-pathogenic *Escherichia coli* (Nyenje *et al*., 2012b).

Salmonella* spp:Salmonella enterica* is a Gram-negative bacillus (Akoachere *et al*., 2009a); it is a common cause of human bacterial gastroenteritis worldwide, and food animals are important reservoir for non-typhoidal *Salmonella* spp. (Skov *et al*., 2007). Human salmonellosis are mostly caused by *Salmonella* serovar *typhimurium* and serovar *enteritidis* (Galanis *et al*., 2006), although prevalence of other serovars especially serovar *schwarzengrund* in Denmark and USA has also been reported (Oslen *et al*., 2001; Vugai *et al*., 2004). Mostly, salmonellosis is asymptomatic but in symptomatic cases, fever, diarrhoea, abdominal cramps and nausea may be experienced often controlled within a week. However, the organisms may be excreted in the faeces for many weeks after symptoms subside (Food

and Drug Administration/Center for Food Safety and Applied Nutrition) (FDA/CFSAN, 2003).

Salmonellosis represents an important public health problem globally. *Salmonella* can enter the food supply chain to cause illness in three main ways: food animals harbour the bacteria in their intestines, making meats, poultry, eggs, and milk important vehicles for salmonellosis (Liu *et al*., 2011). It can also be introduced into the environment, through manure and litter subsequently, contaminating farm produce in particular fruits and vegetables which are eaten raw or with minimal cooking. Cross-contamination can also occur in food service environments or homes, often between raw poultry and ready-to-eat products (McEntire *et al*., 2004). Nevertheless, most studies have documented foods of animal origin as the major vehicles for salmonellosis, which man nurtures through mishandling (Zhao *et al*., 2001; Akoachere *et al*., 2009a). The Center for Disease Control (CDC) estimates that 75% of *Salmonella enteritidis* cases result from the consumption of raw or undercooked eggs; this is because the microorganism is localized inside eggs, making thorough cooking imperative (Food and Drug Administration /Center for Food Safety and Applied Nutrition) (FDA/CFSAN, 2003).*Salmonella* can colonise the avian reproductive tract, persist in the ovary and oviduct and survive in hen's eggs (Gantois *et al*., 2009). Worthy to note is the fact that there is an increasing trend of salmonellosis outbreaks associated with fresh produce and many such crops are produced in the developing countries where manure is frequently used as a natural fertilizer and some studies suggest that some *Salmonella* spp. have now evolved to attach to and colonise vegetables (Klerks *et al*., 2007; Franz and van Bruggen, 2008).

Campylobacter* species:Campylobacter* species are Gram-negative, non-spore forming rods. Most species require a micro-aerobic atmosphere for optimal growth; however, some species grow aerobically or anaerobically. An atmosphere containing increased hydrogen appears to

be a growth requirement for other species such as *C. sputorum, C. concisus, C. mucosalis, C. curvus, C. showae, C. rectus, C. gracilis,* and *C. hominis* (Nachamkin, 2003).

Campylobacters are a leading cause of bacterial enteritis worldwide and can be transmitted directly from animal to person, through ingestion of faecally contaminated water, food, or by direct contact with animal faeces or contaminated environmental surfaces. Species of the organism are primarily zoonotic, with a variety of animals implicated as reservoirs of infection, including a diverse range of domestic and wild animals and birds (Butzler, 1984; Altekruse *et al*., 1999). In addition, water and the environment play a significant but poorly understood role in the epidemiology of campylobacteriosis (Koenraad *et al*., 1997).

Several epidemiological studies in different countries have identified sources of *Campylobacter* enteritis in man to include animals, food, water, and milk products (Oporto *et al*., 2007; Esteban *et al*., 2008).

Although the majority of *Campylobacter* infections are self-limiting, complicated cases may warrant antimicrobial therapy. Antimicrobial susceptibility data show an increase in the number of fluoroquinolone-resistant and, to a lesser extent, macrolide-resistant *Campylobacter* strains causing human infections (Gibreel and Taylor, 2006; Anderson *et al*., 2006). Antimicrobial treatment is indicated for systemic *Campylobacter* infections in immune-suppressed patients and for severe or long-lasting infections (Allos, 2001). Erythromycin is considered the drug of choice for treating *Campylobacter* gastroenteritis, and ciprofloxacin and tetracycline are used as alternative drugs (Nachamkin *et al*., 2000); however resistance of these species has been reported to these antibiotics (Pezzotti *et al*., 2003; Oporto *et al*., 2009).

Isolates of *C. jejuni* and *C. coli* with resistance to various antimicrobial agents have been reported in both developed and developing countries (Hart and Kariuki, 1998; Van Looveren *et al*., 2001). A significant increase in the prevalence of resistance to macrolides among

Campylobacter spp. has been reported since the 1990s, and this is recognized as an emerging public health problem (Altekruse *et al*., 1999). It has been suggested that resistance to macrolides is mainly found in isolates of animal origin; especially *C. coli* from pigs and also *C. jejuni* from chickens (Van Looveren *et al*., 2001).

Escherichia coli: *Escherichia coli* is a Gram-negative rod, a member of the family *Enterobacteriaceae,* and a successful gut coloniser in many host species (Tarr *et al*., 2005). Classification of *Escherichia coli* strains is based on specific virulence factors and phenotypic traits; these include enteroheamorraghic *Escherichia coli* (EHEC) or Shiga toxin-producing *Escherichia coli* (STEC) strains that produce verocytotoxin or shiga-like toxin, the causative agent of haemorrhagic colitis (HC) and haemolytic-ureamic syndrome (HUS); enterotoxigenic *Escherichia coli* (ETEC) strains which produces enterotoxin causing diarrhoea; enteroinvasive *Escherichia coli* (EIEC) strains, the causative agent of dysentery-like illnesses; enteroaggregative *Escherichia coli* (EAEC), which do not secret heat-labile enterotoxins but adhere to mucosal cells in an aggregative pattern, and diffusely adherent *Escherichia coli* (DAEC) strains that adhere to the surface of epithelial cells (Tarr *et al*., 2005; Gyles, 2006; Wu *et al*., 2011). However, foodborne outbreaks have been particularly associated with EHEC and EAEC strains (Tarr *et al*., 2005; Wu *et al*., 2011).

Among the EHEC strains, *Escherichia coli* O157:H7 has been widely recognized as the major cause of foodborne illness (Saghaian *et al*., 2006). The first devastating outbreak of EHEC (*Escherichia coli* 0157:H7) occurred in Japan, in which 2,764 confirmed cases were reported; the source was radish sprouts (Michino *et al*., 1999). Ever since, outbreaks and sporadic cases have been reported globally; in most of these outbreaks, contaminated meat, meat products, unpasteurized milk and leafy green vegetables and fruits fertilized with contaminated animal manure was the source of contamination (Sartz *et al*., 2008; Center for Disease Control, 2011). In 2011, an unusual outbreak of enterohemorrhagic gastroenteritis

and haemolytic uremic syndrome (HUS) related to infections with shiga toxin-producing *Escherichia coli* O104:H4 (STEC O104:H4) occurred; first reported in Germany followed by France before spreading to other European countries and North America (Wu *et al*., 2011); raw tomatoes, cucumber and leaf salad were the source of contamination (Frank *et al*., 2011). This was the first and largest outbreak of infections due to *Escherichia coli* serotype O104:H4 worldwide with 3,167 enterohemorrhagic gastroenteritis and 908 HUS cases which claimed 50 lives (WHO, 2011).

2.5.2 Treatment of Food Borne Illnesses

Foodborne illnesses are mostly manifested as acute gastroenteritis and are usually self-limiting. However, in other, fluid replacement and supportive care may be essential, oral rehydration is indicated for patients who are mildly to moderately dehydrated; whilst in more severe dehydration, intravenous therapy may be administered (Mølbak, 2005).

Worthy of note is the fact that many anti-diarrheal agents have potentially serious adverse effects in infants and young children; therefore their routine use in this age group is discouraged (Center for Disease Control, 2004). For severe cases, extra-intestinal disease, or for immune-compromised persons, antimicrobial therapy is prerequisite and should be based on: clinical signs and symptoms; organism detected in clinical specimens; and antimicrobial susceptibility tests. Such information can also support public health surveillance of infectious disease and antimicrobial resistance trends in the community (Center for Disease Control, 2004; Gilbert *et al*., 2004; Foley and Lynne, 2008).

A number of drugs have been recommended for severe extra-intestinal foodborne disease; they include fluoroquinolones or third-generation cephalosporins, ampicillin, gentamicin, sulfamethoxazole/ trimethoprim (Mølbak, 2005, Akoachere 2009b). Since fluoroquinolones are reported to cause damage to the cartilage in children under the age of 16 years, third-

generation cephalosporins including ceftriaxone are an alternative therapy for this age group (Gilbert *et al*., 2004).

The treatment of severe cases of food poisoning (SFP and botulism) involves supportive therapy, with mechanical ventilation where pulmonary involvement is expected. Administration of immune globulin to neutralize circulating botulism toxin can also assist (Arnon *et al*., 2006). Suphamethaxazole also appears to reduce the incidence of *Salmonella* infections after transplant, although resistance has occurred in some *Salmonella* species (Safdar and Armstrong, 2003; Conter *et al*., 2009; Morvan *et al*., 2010).

Antimicrobial resistance is a major challenge in the management of severe foodborne illness; even though much of the resistance experienced in human medicine is accredited to improper use of antibiotics in humans, antimicrobial use in animals selects for resistant foodborne pathogens that may be transmitted to humans as food contaminants (Mesa *et al*., 2006). Worthy of note is the fact that most of these foodborne pathogens are of food-animal origin notably, *Campylobacter*, *Salmonella*, *Listeria*, and *Escherichia coli* O157.

Outbreaks of a multidrug resistance *Salmonella* typhimurium DT104 strain (Chloramphenicol, ampicillin, streptomycin, sulfonamides, chloramphenicol, and tetracycline) have been documented (Davis *et al*., 2007; Sofos, 2008). *Salmonella* spp resistant to nalidixic acid, gentamicin, kanamycin, sulfamethoxazole/trimethoprim and ciprofloxacin has also been reported (Savadkoohi and Kacho, 2007; De Paula *et al*., 2010).

2.5.3. Prevention and Control of Food Borne Diseases

Most of the foodborne pathogens are ubiquitous in nature; as such there is a possibility of cross–contamination between one or several products during processing. Preventive measures, such as the pasteurization of milk and dairy products, irradiation, healthier manufacturing processes, and attention to clean water have helped to minimize cases of

tuberculosis, typhoid fever, and cholera that were once common causes of foodborne illnesses (Linscott, 2011).

Internationally, countries are now adopting the Hazard Analysis and Critical Control Point (HACCP) system; endorsed by the Codex Alimentarius Commission as a tool that can help prevent known hazards and reduce the risks that may occur at specific points in the food chain. Since the approach enforces procedural governance and rigorous documentation practices, HACCP serves not only as a model to assess risk, but also as an effective means to communicate risk control (WHO, 2007). In addition, quality systems such as good agricultural practices (GAP) are recently recommended for farms, to provide basis for the development of best practices in the production of horticultural products (example fruits, vegetables, potatoes, salads, etc.) (Codex Alimentarious Commission, 1997; Kokkinakis and Fragkiadakis, 2006).

Other possible interventions include biological control, chemical control and breeding for resistance strains (Wagacha and Muthomi, 2008). At consumer's level, foodborne illnesses can be reduced by proper cooking of meat, poultry and eggs to temperatures that will kill bacteria; although, spores are heat resistant; steaming under pressure, grilling, roasting and frying of foods can destroy the vegetative cells and spores (Linscott, 2011). Additionally, refrigerating leftovers promptly and storing foods at recommended temperatures; avoiding cross-contamination of cooked and raw foods; washing of utensils and surfaces before and after use with hot, soapy water; and frequently washing hands and/or using gloves when preparing food are all recommended (Gashaw *et al*., 2008).

2.6 Antimicrobial Resistance

Antibiotic resistance is the ability of a bacterium or other microorganism to survive and reproduce in the presence of antibiotic doses that were previously thought effective against them (WHO, 2011). Resistance isoften associated with reduced bacterial fitness, and it has

been proposed that a reduction in antibiotic use (and,therefore, in the selective pressure to acquire resistance) would benefit the fitter susceptible bacteria, enablingthem to outcompete resistant strains over time (Levin, 2007).

Antibiotics are widely used in human and veterinary medicine, and have been essential for ensuring human and animal health. However, the emergence of bacteria resistance to commonly used antibiotics compromises effective treatment, resulting in the need for stronger drugs and more costly therapy. The spectre of antibiotic-resistant (ART) microbes is a worldwide concern due to the public perception of an incoming post-antibiotic era. Besides clinical therapy, antibiotics have also been used to improve aquaculture and agricultural production (Wang *et al*., 2012).

Certain antibiotics used for treatment or growth promotion in agriculture are also used for disease control in humans. Others select for cross-resistance in bacteria to antibiotics used in human medicine. Microbiological and clinical evidence is mounting that resistant bacteria or resistance determinants might be passed from animals to humans, resulting in infections that are more difficult to treat. With an increase in the prevalence and distribution of antibiotic-resistant infections in hospitals and the community, the question has been raised as to how this escalation of resistance could have been influenced by the use of antibiotics in livestock production (WHO, 1997).

Antibiotic resistant infections occur too often and with increasing frequency, interfering with the effective treatment of people and animals. Although antibiotics and antibiotic resistance are natural phenomena, the population of resistant bacteria has increased by the introduction of antibiotics into an environment. To preserve the effectiveness of antibiotics, it is critical to examine the uses of these drugs, in both humans and animals. This series of talking points

developed for use by dietetic and nutrition professionals will provide a background on the use of sub therapeutic antibiotics in animals (Center for Disease Control, 2008).

None the less, evidence is mounting that antibiotic-resistant enteric bacteria (for example, *Escherichia coli, Salmonella, Campylobacter* and *Enterococc*i) can be transferred from animals to man via the food chain or by direct contact, leading to the establishment of a community reservoir of resistance genes (van den Bogaard and Stobberingh, 1999). Therefore, protection of food supplies includes microbiological quality and safety of Commodities available for public consumption.

2.6.1. Origin of Antibiotic Resistance

Most cells in a naive, susceptible bacterial population causing an infection are susceptible to the antibiotic of choice for treatment, and are killed upon exposure to it; however there is always a minute sub-population of resistant cells that will be able to multiply at higher concentrations (Smith and Crabb, 2008).

If the antibiotic concentration is not sufficiently high to kill this sub-population then the environment is such that this population is selected. The accepted dogma is that these resistant cells arise by random mutations in bacteria and that occasionally these random mutations occur in a gene that affects antibiotic susceptibility. Resistant gene is then stably inherited by all daughter progeny if, as is usual, the gene is chromosomal. Sometimes the gene is on an extra chromosomal element alternatively, the bacterium acquires a resistance gene(s) from another bacterium. Evidence for the existence of resistance genes prior to exposure to antibiotics comes from examination of historic cultures from before the antibiotic era (Smith, 2002), from the examination of bacteria from populations in the process of exposure to antibiotic (Smith and Crabb, 2008) and by examining populations of bacteria in

locations remote from any antibiotic use such as farm animal species in isolation, wildlife and soil bacteria (Mare, 1998).

2.6.2. Classification of Antibiotic Resistance

Resistance to most traditional, regulatory-approved, or naturally-occurring food antibiotic agents are difficult to characterize because of the lack of a precise definition for such resistance. From a functional perspective, resistance correlates with failure of a given antibiotic treatment; whereas from a laboratory perspective, resistance is denoted through a "Minimal Inhibitory Concentration" (MIC) value that exceeds a threshold value, which may or may not be associated with a clinical outcome. A microorganism is resistant if it exhibits "significantly reduced susceptibility" when compared with that of the "original isolate" or a group of sensitive strains (Chapman, 1998). Resistance can result from mutations in housekeeping structural or regulatory genes, or alternatively, horizontal acquisition of foreign genetic information (Courvalin, 2005).

2.6.2.1 Innate Resistance

As is the case for a natural property of a microorganism, innate (intrinsic) resistance is chromosomally controlled (Russell, 1991). Innate resistance is related to the general physiology or anatomy of a microorganism and stems from pre-existing mechanisms or properties. This type of resistance is most likely responsible for differences in resistance observed among different types, genera, species, and strains of microorganisms in identical environmental conditions and concentrations (Gilbert and McBain, 2003).

Innate resistance may stem from the complexity of the cell wall, efflux mechanisms (means by which microbes pump antibiotics out of the cell or enzymatic inactivation of the antibiotic (Russell, 2001). Innate resistance is not considered an important clinical problem because

antibiotics were never intended for use against intrinsically resistant bacteria. There are certain circumstances in which antibiotics do not adversely affect bacteria that are generally susceptible to the particular agent. The efficacy of most food antibiotics and sanitizers is dependent upon and influenced by the conditions of its uses (IFT, 2002a).

2.6.2.2. Acquired Resistance

Acquired or extrinsic resistance results from genetic changes that occur through mutation of the antibiotic's target site within the bacterium or acquisition of genetic material encoding resistance via plasmids or transposons containing integrin sequences. Acquired resistance, the most common type of antibiotic resistance, has been well studied for antibiotics, but has not been well studied for food antimicrobial agents and sanitizers (Russell, 1991).

Acquisition of genes for β-lactamase (an enzyme capable of breaking down and inactivating β-lactam antibiotics [penicillins and cephalosporins]) and mutation of one of the subunits of DNA gyrase (the target of fluoroquinolones) are examples of this type of resistance. Another example includes resistance of some microorganisms to sanitizing compounds, such as quaternary ammonium compounds (QACs), as a result of the presence of plasmid-encoded efflux pumps that remove the QACs (Gilbert and McBain, 2003).

2.6.3. Adaptation of Antibiotics

For certain types of antibiotics, adaptation, may be demonstrated by exposing a microorganism to a stepwise increase in concentration of the substance. This type of resistance, however, is often unstable; the microorganism may revert back to the sensitive phenotype when grown in an antibiotic-free medium, termed "back-mutation" (Russell, 1991).

2.6.4. Antibiotic Resistance in Animals

Most concern about antibiotic resistance in animal isolates of bacteria is directed towards the enteric bacteria, *Escherichia coli, Salmonella,* thermophilic *Campylobacters and Enterococci* (Smith, 2005). Resistance to other antibiotics was detected as new agents were introduced for therapeutic and growth-promoting purposes (Anderson, 2000). More recently, feeding low doses of ampicillin to chickens was shown to select for high levels of resistance to that antibiotic (El-Sam *et al.,* 1993). In herds and flocks treated with tetracycline, aminoglycoside and sulphonamide, widespread resistance is seen (Sunde *et al*., 1998). Resistance is generally less prevalent in *salmonella*, but that resistance to tetracyclines, sulphonamides and streptomycin is quite widespread (Griggs *et al*., 1994).

2.6.5. Antibiotic Resistance In Relation To Food Safety

2.6.5.1 Antibiotic Use in Food Production

In food animals, antibiotics are predominantly used to treat respiratory and enteric infections in groups of intensively fed animals. They are used especially during the early part of ananimal's life – for example, in broiler chickens and in weaning pigs and calves. Antibiotics are also used to treat infections in individual animals caused by a variety of bacterial pathogens. In particular, antibiotics are often used to treat mastitis in dairy cows, common infections in cows with a high milk output (Smith, 2005). They may also be used for long periods at low levels to promote growth, increase feed efficiency, or compensate for unsanitary growing conditions on concentrated animal feeding operations (WHO, 2011).

2.6.5.2. Antibiotic Resistance as a Food Safety Problem

Food products of animal origin are often contaminated with bacteria, and thus likely to constitute the main route of transmitting resistant bacteria and resistance genes from food

animals to people. Direct contact with animals or the animal environment, however, may also be of significance, depending on the type of bacteria. Such foods as fruits and vegetables contaminated by animal waste or contaminated water may also constitute a transmission route (WHO, 2011). There is clear evidence of adverse human health consequences due to resistant organisms resulting from non-human usage of antibiotics. These consequences include infections that would not have otherwise occurred, increased frequency of treatment failures (WHO, 2003).

2.7. Transmission of Antibiotic Resistant Bacteria to Human

Antibiotic resistance bacteria spread from animal to human indirectly via food (e.g. by contamination of carcases during slaughter), or less commonly by direct contact (e.g. in farmers, abattoir workers) (JETACAR, 1999).

2.7.1. Major Food Vehicle of Antibiotic Resistant Bacteria

The presence of antibiotic resistant bacteria in foodstuffs of animal origin is becoming a matter of concern as these bacteria can be transmitted to humans through food supply. Therefore, protection of food supplies includes microbiological quality and safety of commodities available for public consumption. While such concerns most frequently address pathogenic microorganisms, that present immediate risks to human health, there is growing interest in commensal components of the microbiota associated with food (Hayes *et al.,* 2003; Martin *et al*., 2005).

2.7.2. Meat products

Antibiotic-resistant *Salmonella*, *Campylobacter*, *Escherichia coli* and multidrug-resistant *Staphylococcus* have been detected in many different types of retail meat and poultry products, as well as in farm animals and the farm environment (Bhargava *et al*., 2011).

Besides raw meats, they are also associated with processed meats (Franz *et al*., 1999). Heating of processed meats during production may confer a selective advantage to *Enterococci* because these bacteria are among the most thermotolerant of the non-sporulating bacteria. After surviving the heat processing step, *Enterococci* have been implicated in spoilage of cured meat products, such as canned hams and chub packed luncheon meats (Magnus *et al*., 2008). This is especially true where recontamination with competing bacteria is prevented, when products are heated after packaging in cans or in impermeable plastic films. The heat resistance of *Enterococcus* spp. in these products is influenced by components such as salt, nitrite, and meat tissue (Franz *et al*., 1999).

2.8. Common Antibiotic Residues in Food

The following drugs are considered as the most likely to be detected in meat: penicillin (including ampicillin), Tetracycline (including chlortetracycline and oxytetracycline), Sulfonamides (including sulfadimethoxine, sulfamethazine and sulfamethoxazole), Neomycin, Gentamicin, Flunixin, Streptomycin and Arsenicals. The hypothesis was that the greater the amount of a drug used, the more likely bacteria would develop resistance to it **(**Boothe and Arnold, 2003).

Results from the 2003 FSIS National Residue Monitoring Plan indicate that penicillin and sulphonamide drugs were most commonly detected at various levels in swine and cattle. Neomycin and gentamicin were also detected in a number of cattle, particularly calves. Other drugs detected in cattle and swine included tilmicosin, flunixin, and tetracyclines. Arsenicals were detected in poultry. Data from 1996 indicated the percentage of various residues accounted for by each drug: CAST (calf antibiotic sulfa test), 32%; penicillin, 20%; oxytetracycline, 10%; sulfamethazine plus sulfadimethoxine, 10%; tetracycline, 8%; gentamicin, 6%; neomycin, 3%; other, 7% (Paige *et al*., 1999).

2.9. Impact of the Use of Antibiotics in Livestock Production

Indiscriminate use of antibiotics may lead to antibiotic residues and antibiotic resistance. Antibiotics and antibiotic-resistant bacteria can be found in the air, groundwater, and soil around farms and on retail meat (Smith *et al*.,2005) and people can be exposed to these pathogens through infected meat, vegetables fertilized with raw manure, and water supplies contaminated by farm animal waste (Acar and Moulin, 2006). When resistant bacteria are themselves pathogenic or can transfer their resistance genes to pathogenic bacteria, adverse health effects can result (WHO, 1997).

Until very recently, controls on antibiotic use in animals focused almost exclusively on the control of residues in the tissues of treated animals. Concerns about residues revolve around allergic reactions and the possible adverse effects on the flora of the human gastrointestinal tract (selecting for resistance or transfer of resistance). For example penicillin residues in milk could provoke allergic reactions in sensitised individuals, but there were no other adverse effects associated with antibiotic residues (Swann, 2009).

Confirmed cases of allergy to substances in food are very rare, although adverse reactions to antibiotics have been linked to hypersensitivity and cases of chronic urticaria (Woodward, 1991). A rare fatal blood dyspraxia in individuals sensitised to chloramphenicol could also be triggered by chloramphenicol residues in food (Settepani, 2004).

2.9.1. The Control of Antibiotics Resistance

2.9.1.1. Responsible Use

Guidelines exist for responsible (proper, appropriate, prudent, or judicious) use of antibiotics in veterinary and human medicine, and are similar in the medical and agricultural sectors

(Phillips *et al*., 2004). Veterinary and animal producer organizations in many countries have developed and implemented responsible use principles or guidelines. These address use in various species, including poultry, swine, dairy and beef cattle, and sheep. International organizations, such as the OIE, WHO, also have developed or are developing principles or codes of practice to contain antibiotic resistance. The WHO published Global Principles for the Containment of Antimicrobial Resistance in Animals Intended for Food (WHO, 2000).

The OIE issued five documents concerning antibiotic resistance, including Guidelines for the Responsible and Prudent Use of Antimicrobial Agents in Veterinary Medicine. The other four documents deal with risk analysis methodology, monitoring of use quantities, surveillance programs, and laboratory methodologies (Acar and Rostel, 2001).

2.9.1.2. Preventing infectious diseases in animals

Antibiotics are probably the most valuable drugs in animal production, and maintaining their beneficial effect is therefore of utmost importance. Focus should be given to the continuous implementation of appropriate measures for disease prevention, to decrease the need for antibiotics. To minimize infection in food-animal production and decrease the volume of antibiotics used, efforts should aim to improve animal health, thereby eliminating or reducing the need for antibiotics for treatment or prophylaxis. This can be achieved by improving hygiene, biosecurity and health management on farms and preventing disease through the use of vaccines and other measures such as probiotics (beneficial bacteria found in various foods), prebiotics (non-digestible foods that help probiotic bacteria grow and flourish) or competitive exclusion products (intestinal bacterial flora that limit the colonization of some bacterial pathogens) (WHO, 2011).

2.9.1.3. Antimicrobial Peptides

Antimicrobial peptides are host-cell-produced compounds that have been identified in plants, animals, and insects. Extensive research has led to an increased understanding of the mechanisms of action of porcine antimicrobial peptides, but has not addressed the numerous practical aspects that are necessary to achieve regulatory approval or market place success (Zhang *et al.,* 2000).

Bacteriocins, pore-forming antibacterial proteins produced by microorganisms, bacteriocins have been investigated for their potential use in the control of certain zoonotic pathogens in the avian intestinal tract. One bacteriocin, nisin, has been approved for use in several food products (Cleveland *et al*., 2001).

2.10 "Tsire" Meat

"Tsire" is a spicy, traditional stick meat product that is commonly produced by the Hausas in Northern Nigeria from beef (Alonge and Hiko 1981), where rearing of cattle is an important pre – occupation and major source of livelihood for the people (Edema, *et. al*., 2008). This leads to the production of ready – to – eat beef products such as "Tsire", kilishi, balangu and kundi. "Tsire" is however the most popular as its consumption has extended to other part of the country (Inyang *et. al*., 2005).

It is produced from boneless meat, hung on stick and spiced with peanut cake, salt, vegetable oil and other flavourings followed by roasting around a glowing charcoal fire (Abdullahi, *et. al*., 2004). Even though meat from freshly slaughtered, healthy animals is supposed to have no, or very low microbial populations, laboratory evidence suggests that they could be contaminated to an unsafe level at the point of consumption (Umoh, 2001). The fact that there are sporadic cases of gastroenteritis and symptoms of food infection after consumption

of "Tsire" indicate the products indeed constitute a food safety risk (Odusote and Akinyanju, 2003; Inyang, *et. al*., 2005).

2.10.1. Preparation of "Tsire"

Usually in Nigeria, skinless, boneless flesh of cattle or chicken is used for commercial preparation of "Tsire" meat. Finely grounded roasted peanut cake, red pepper, salt, grounded ginger, grounded garlic, chunked fresh tomatoes and minced fresh onions are required on suitable conditions depending on the quality of "Tsire" meat required to be prepared (Jay, 2002; Judge *et al*., 2002).

The process of preparation involves a few steps, first is the grinding of peanut. The shell and the skin are removed from the peanut before grinding into fine powder using mortar and pestle or crushed with a rolling pin. If the powder is oily; it is wrapped with an absorbent paper and squeezed for a minute or two. Next, the grounded pepper, garlic, ginger are stirred into the peanut powder and mixed properly.

The meat is then cut into small sizes or thin sliced, dipped and rolled in a bowl containing the mixed peanut-spice and allowed to coat completely. The minced meat are then kept for thirty minutes or more for the peanut cake to stick to it after which the meat slices are threaded unto skewer and brushed with vegetable oil and roasted on the glowing charcoal fire for fifteen to twenty minutes. It is finally removed from the skewer and served hot in an old newspaper wrap with sliced onions, tomatoes and cabbage (Judge *et al.*, 2002).

2.10.2. Microbiology of Meat.

During slaughtering process, there is contamination of the sterile tissue with intestinal flora such as Gram-negative organisms which includes *Escherichia coli* as well as contaminants such as *Pseudomonas specie* and Gram-positive *lactic acid bacteria* and *Staphylococci* speciesassociated with humans, animals and their environment. Meat spoilage is usually

associated with Gram-negative proteoliticbacteria which literally decompose the protein with production of offensive odour (Hamman, 1997).

The addition of salt and drying of fresh meat have been an effective means to control the meat microflora and thus preserve the tissue for later consumption. The curing salt (sodium chloride or sodium nitrate or sodium nitrite) and subsequent proper handling methods, favours the growth of Gram-positive bacteria, primarily *Staphylococcus aureus* while inhibiting the proliferation of Gram-negative bacteria (Boles *et al.,* 2000).

There are also other types of common microorganisms apart from enteric organisms found in meat which are members of *Micrococcaceae* and *Staphylococcaceae* families. The predominant types are coagulase-negative *Staphylococci* that are salt tolerant and can also grow with or without oxygen. The most common strain belongs to the species of *Stapylococcus carnosus*, *S. xylosus* and *S.kocuria*. However, these organisms are harmless and do not present a microbial hazard. The most common lactic acid microorganisms found in fermented meat are various strains of Lactobacilli, Leuconostoc, Pediococci, Streptococci and Enterococci (Lawries, 2001).

The microbiological quality of meat depends on the physiological status of the animal at slaughter, the spread of contamination during slaughter and processing, the temperature and other conditions of storage and distribution. In fact, some of the microorganisms originate from the animal's intestinal tract as well as from the environment with which the animal had contact at some time before or during slaughter (Koutsoumanis and Sofos, 2004). Other organisms, including psychrotrophic bacteria, are recovered from hides and work surfaces within an abattoir as well as from carcasses and butchered meat at all stages of processing (Gill, 2005).

A wide range of micro-organisms coming from different sources are introduced onto carcass surfaces, which contain abundant nutrients and which have high water availability. Only a

few of the contaminants will be able to initiate growth, and only some of these will eventually spoil the meat by means of their biochemical attributes. Predominance of different groups of microorganisms on meat depends on the characteristics of the meat, the environment in which meat is stored as well as the processing that meat may undergo (Gill and Molin, 1991).

As earlier noted, a vast number of studies in meat microbiology have established that spoilage is caused by only a fraction of the initial microbial association that comes to dominate (Nychas *et al*., 2007).

2.10.3. Meat Spoilage

Meat has long been considered a highly desirable and nutritious food, but unfortunately it is also highly perishable because it provides the nutrients needed to support the growth of many types of microorganisms (Kolalou *et al.*, 2004). Due to its unique biological and chemical nature, meat undergoes progressive deterioration from the time of slaughter until consumption. Meat is spoilt when it loses its nutritive value, texture and brings out offensive odour thus rendering it unfit for human consumption. A number of factors could cause meat spoilage (Nester *et al.*, 2001). Most deterioration or spoilage of meat could be caused by bacteria, yeast and mould. When meat is not properly handled, it leads to microbial spoilage which makes it unfit for human consumption (Sokari *et al*., 1999).

2.10.3.1 Factors affecting meat spoilage

In general, the metabolic activity of the ephemeral microbial association which prevails in a meat ecosystem under certain aerobic conditions, or generally introduced during processing, leads to the manifestation of changes or spoilage of meat (Nychas *et al.*, 2008). These changes or spoilage are related to the (i) type, composition and population of the microbial

association and, (ii) the type and the availability of energy substrates in meat. Indeed the type and the extent of spoilage is governed by the availability of low-molecular weight compounds (e.g., glucose, lactate) existing in meat (Nychas *et al*., 2007; Nychas *et al.*, 2008). By the end of the phase changes and subsequently, overt spoilage is due to catabolism of nitrogenous compounds and amino acids as well as secondary metabolic reactions. The post-mortem glycolysis, caused by indigenous enzymes, ceases after the death of the animal when the ultimate pH reaches a value of 5.4–5.5 (Olaoye, 2010).

Afterwards, the contribution of meat indigenous enzymes in its spoilage is negligible compared to the microbial action of the microbial flora (Tsigarida and Nychas, 2001). A number of interrelated factors influence the shelf life and keeping quality of meat, specifically holding temperature, atmospheric oxygen (O_2), indigenous enzymes, moisture (dehydration), light and, most importantly, micro-organisms. All of these factors, either alone or in combination, can result in detrimental changes in the colour, odour, texture and flavour of meat.

Fresh meat has a shelf life of 1 day or less at ambient storage temperatures, 20-30°C (Lambert *et al*., 1991). Spoilage is said to be a state of a particular food in which it is offensive to consumers' senses, usually caused by metabolites of contaminant microorganisms (Paulsen and Smulders, 2003). Meat spoilage is not always evident and consumers would agree that gross discoloration, strong off-odours, and the development of slime would constitute the main qualitative criteria for meat rejection.

In general, spoilage is a subjective judgment by the consumer, which may be influenced by cultural and economic considerations and background as well as by the sensory acuity of the individual and the intensity of the change (Nychas *et al*., 2008). Spoilage of meat can be considered as an ecological phenomenon that encompasses the changes of the available

substrata, such as low molecular weight compounds, during the proliferation of bacteria that constitute the microbial association of the stored meat (Nychas *et al*., 2007). The prevailing of a particular microbial community of meat depends on the factors that persist during processing, transportation and storage in the market. Such may vary widely from one country to another as a result of differences in climatic conditions, coupled with possible varying levels in knowledge of food hygiene practices of the handlers. A lot of factors affect the growth of microorganisms on meat. These factors include temperature, pH, water availability, and presence of nutrients, moisture, acidity (intrinsic factors), gaseous requirement, and atmosphere of storage (extrinsic factors) (Nester *et al.*2001).

Temperature: Microorganisms have optimum, minimum and maximum temperature that they can grow. This ability of growing at low temperature provides opportunity for proliferation in contaminated meat products (Fraizer and Westhoff, 2000).*Pseudomonas* speciesgrow *at* temperature less than 20 and so are found (Dineen *et al*.1999).Psychrophiles have optimum temperature between 2and 7°C, mesophiles between 10 and 40°C and thermophiles from 43/66°C. Examplesofpsychrophiles are *Achromobacter, Alcaligens, Pseudomonas, Streptococcus, Salmonella,* Most yeast and Mould.

pH: Most bacteria grow optimally at above pH 7 and not well below pH 4 or above pH 9 (Abdul *et al.* 1995). But the pH of maximal growth is determined by the simultaneous operation of variables other than the degree of acidity or alkalinity. Proteolitic enzymes operate best near neutral pH 7.

Water availability: Water is required by microorganism so reducing water below the optimum level will prolong shelf life of meat. If meat is stored at a relative humidity below 95%, moisture will be lost from the surface. Since most spoilage bacteria can grow only on the surface, drying the meat surface will not favour their growth but *Staphylococcus aureus*

can grow in meat with 0.86%, which is lower than that of other spoilage bacteria (Nester *et al*., 2001). Moulds are able to grow in drier conditions than bacteria so that desiccation has a selective effect on microbial growth.

Nutrients: Microorganisms depend on nutrients from meat product or meat for survival. Meat contains protein, fat vitamins and phosphorus which support the growth of microorganisms. *Pseudomonas aeruginosa* synthesizes its vitamins and so cause spoilage even in a medium without vitamin. *Staphylococcus aureus* require about 6.5% of sodium chloride for growth and is usually found in salty meat products (Boles *et al*., 2000).

2.11. Livestock production, meat quality and consumer preference

Livestock production; primarily poultry, pig, goat, sheep and cattle is carried out mainly at subsistence level and this level of agriculture is the main feeder of the meat industry in the developing countries (Momoh and Ochaba, 2002). This type of production system is characterised by inefficiency in input-output relationships – the poor housing, nutrition, animal health and feed conversion efficiency that lead to the production of livestock that takes longer to mature and yield poor quality meat (AVMA, 2010).

According to Permentier *et al*. (2013), the common determinant of meat quality is the protein – fat ratio. Meat is composed of water, fat, protein, minerals and a small proportion of carbohydrate. The most valuable component from the nutritional point of view is protein and it is the type and amount that define the quality of any meat irrespective of the source or the state of preparation. Animal studies show that fat in meat is deposited subcutaneous - under the skin, around the organs like kidney and heart or between muscles – inter-muscular fat (Wood *et al*., 2008; FAO, 2013). Fat is an essential component of meat that impact on its juiciness, flavour and texture. Fat – mono-unsaturated or polyunsaturated in meat provides the body with essential fatty acids that cannot be synthesised by humans but saturated fat are

detrimental to human health and constitutes bulk of the fat in meat (FAO, 2013). Furthermore, Moloney *et al*. (2002) noted that increase in fat deposition in animals is generally accompanied by an increase in intramuscular fat concentration and that the degree of fatness is influenced by genotype, the weight of the carcass and how close the animal is to its full maturity when slaughtered. Therefore management of livestock to yield better quality meat is achieved by better understanding of how livestock body weight is gained, the distribution and composition.

Studies using consumer behaviour to investigate production systems abound – such as willingness to pay for organic products (Ogbeide, 2013; Ogbeide *et al*., 2014a and b), quality assessment (Verma and Gupta, 2004) and preference for particular products (Chryssohoidis and Krystallis, 2005; Latvala *et al*., 2012; Okunlola, 2012; Gracia and de-Magistris, 2013). Consumers' preference for quality meat can be influenced by their attitude towards health and the environment (Bhaskaran *et al*., 2006). Consumers are concerned about the contribution of the livestock sector directly and indirectly to climate change through the emissions of greenhouse gases such as carbon dioxide (CO_2), methane and nitrous oxide.

FAO (2014) noted that globally, the sector contributes 18 percent - 7.1 billion tonnes of CO_2 equivalent of global greenhouse gas emissions and nine percent of global CO_2. Concerns about human health are evidently considered in the meat market as consumers are keenly aware of the negative effects of the consumption of fatty meat. Science has also implicated the quality of the meat consumed as responsible for some chronic, long-term health problems that impact on consumers and government financially. Diets high in animal fats, which tend to contain high proportions of saturated fatty acid have implications for cardiovascular disease (KwaZulu-Natal Department of Health, 2001; Kearney, 2010; Baade *et al*., 2012; Youl *et al*, 2012). Consumers want quality meat and in part influence their preference. An assessment of meat quality can vary between individuals; cultural inclination of consumers

can also affect quality perception, all due to the objective and subjective nature of the quality attributes of meat upon which the consumers base their preference (Fortomaris *et al*., 2006). FAO (1992) and Permentier *et al*. (2013) reported that meat quality refers to the compositional quality (lean to fat ratio) and palatability quality (juiciness, tenderness, and flavour) of meat. This suggests that there are two major aspects of meat quality, compositional quality which is the objective attribute and palatability quality as the subjective attribute perceived by the consumer (FAO, 1992; Fortomaris *et al*., 2006). Consumers' first assessment of meat is based on the appearance which is the visual identification of quality based on colour, marbling, and water holding capacity. According to FAO (2013), meat of good compositional quality should have a normal uniform colour, it should have marbling throughout the cut (marbling is defined as small streaks of fat dispersed within the meat); which is an indication of tenderness and juiciness as well as flavour. Certain compositional qualities are heritable and can be directly influenced by livestock producers through appropriate livestock breeding and management programs (Curtis *et al*., 2007). Wood *et al*. (2008) noted that when fat is deposited between the fibre bundles of a muscle, it leads to higher accumulations of marbling, meat tenderness and improved flavour. Therefore, marbled meat is tender with improved flavour. Many consumers prefer marbled meat for steaks and other roasted meat dishes (Wood *et al*., 2008) due to its tenderness (easy to shear) and flavour.

Flavour is mainly determined at the cooking stage but is influenced by the quality of meat. It occurs when the denatured proteins on the surface of the meat recombine with the sugars present. The combination creates the "meaty" flavour and changes the colour; this process is referred to as "browning" or "Maillard" reaction". Martinez *et al*. (2007) reported that meat quality attributes such as flavour, tenderness, nutrition, and safety are not apparent to consumers until the product is consumed but studies have shown that consumers value them -

e.g. (Beriain *et al*., 2009) and most of these attributes are a function of good compositional qualities of meat.

Davis *et al*. (2007) reported consumers are now inclined toward healthier eating and food producers have responded by providing foods that meet or even exceed the consumers' expectations, with added healthy attributes and health claims.

CHAPTER THREE

3.0. MATERIALS AND METHODS

3.1. Study Location

The Main Campus of Ahmadu Bello University is located in Samaru, a suburban of Zaria in Kaduna State, Nigeria. Samaru is situated on latitude 112°12" N and longitude 07°37" E, at an altitude of 550-700 meters. It is about 13km from Zaria-city on the Sokoto road, 8km to Shika and 7km from Bassawa. The Samaru town is the fourth and the most recent addition to the Zaria suburban area made up of Zaria-City, Tudun-Wada, the Government Reservation Area (GRA), and Sabon-Gari. Samaru evolved from a small colonial farming settlement to become a large community, a melting-pot, often referred to as "the University village". It is cosmopolitan in nature, drawing and fusing people of divergent national and international backgrounds (Fig: 3.1).

Zaria metropolis is divided administratively into Zaria and Sabon gari Local Government Areas (L.G.As), each comprising of six district areas. Zaria LGA comprises Zaria city, Tudun wada, Gyelesu, Tukur Turkur, Wuciciri and Dutse Abba; while Sabon Gari comprises Hanwa, Muciya, Samaru, Bassawa and Bomo (Ministry of Economic Development, 1996).

Fifty to seventy five percent of the working populations of Zaria metropolis derive their livelihood from farming. Agriculture in Zaria can be divided into two types: Rainfall (from May to October) and irrigation farming in the dry season (from November to April). It is characterized by a tropical climate, a monthly mean temperature ranging from 13.8 to 36.70C and annual rainfall of 1,092.8mm. Dry season farming is the second most prevalent agricultural activity in Zaria with vegetables being the common produce, but in some cases

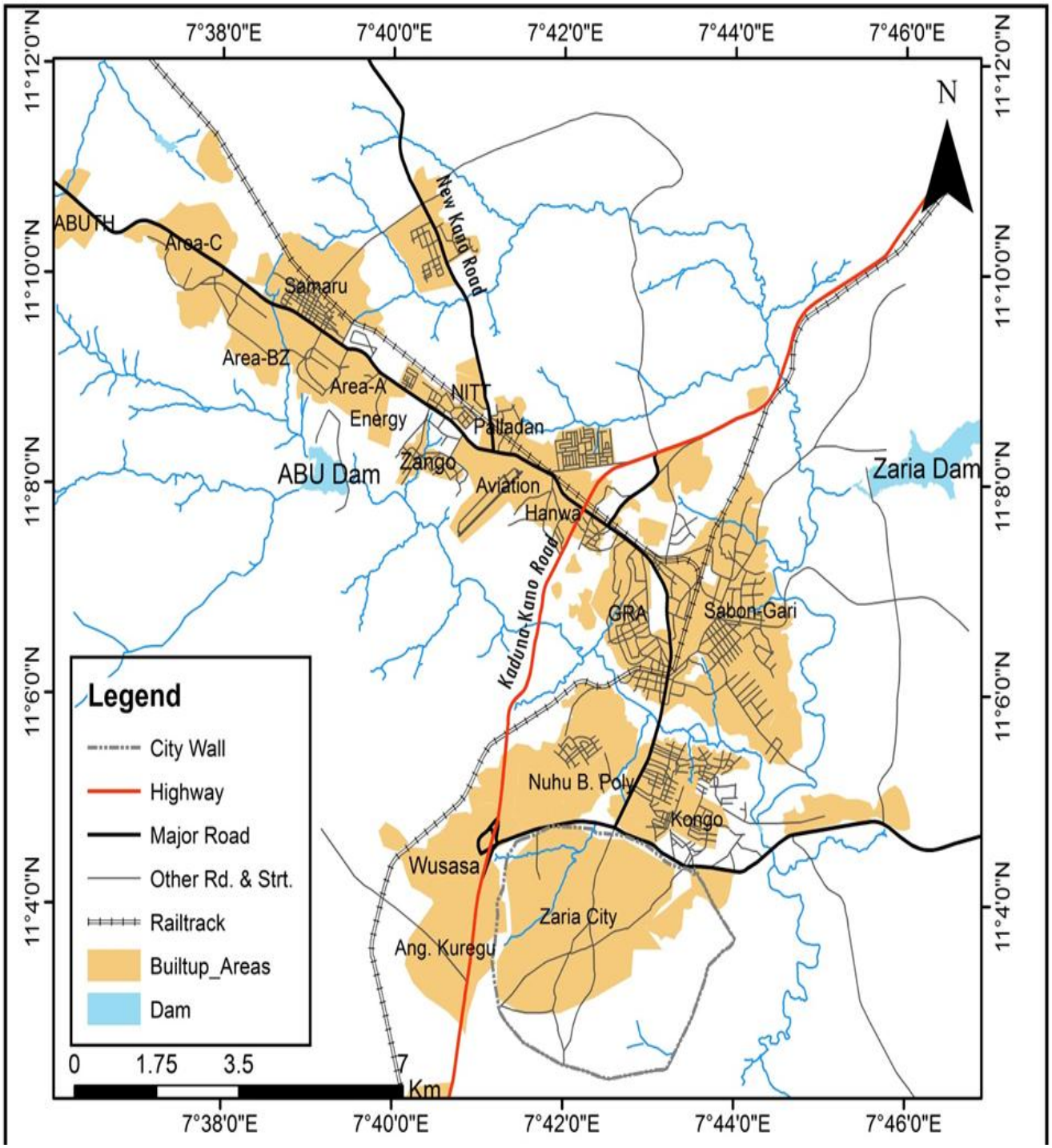


Figure 3.1: Map of Zaria Urban Area.

Source: Quickbird Imagery, 2013

fruits are sandwiched among cereal crops (Agbogu *et al*., 2006)

3.2. Sample Size Determination

The required number of samples was determined according to the formula described by Wan Muhammad *et al.* (2012).

Sample size, $n = \frac{Z^2 p(1-q)}{\Delta^2}$

Where =Z; the standard normal deviate at 95% confidence interval = 1.96

=(Δ); absolute precision = 5%

=P; anticipated population proportion with the characteristic of interest at the 100(1-α) %

=Confidence interval = 2.3% (Ogbonna *et al.*, 2012)

Sample size, $n = (1.96/0.05)^2 \times 0.02(1-0.02)$

$=1536 \times 0.02 \times 0.98$

$= 30.10 \equiv 30.$

Thirty (30) raw beef and "Tsire" samples were collected randomly from each of the four "Tsire" spots in A.B.U Samaru main campus i.e. Pepsi Garden "Tsire" spot, Sculpture Garden "Tsire" spot, Community Market "Tsire" spot and Faculty of Art "Tsire" spot. In all, 240 samples were collected.

3.3. Study Design and Sampling Method

A cross sectional design involving convenience sampling method was employed in the study. Each of the four "Tsire" spots was visited once a week, five samples each of both raw beef and "Tsire" were purchased from each of the sampling area

3.4. Collection of Samples

A total of 240 samples were screened for some Gram negative organisms. These included: 120 raw beef and 120 "Tsire" Samples were bought and appropriately labelled as they were usually sold to the consumers (wrapped in aluminium foils) and then placed in an ice-packed flask and transported to Microbiology Department laboratory, Ahmadu Bello University Zaria for analysis. Raw beef was collected in the morning while the "Tsire" was sampled in the evening based on availability.

3.5. Samples Analysis

3.5.1 Proximate analysis of raw beef and "Tsire"

Moisture content, Ash content, Fibre, Fat (Lipids), Protein and carbohydrate of the samples were determined according to methods described by AOAC (1990)

3.5.1.1 Moisture content (AOAC 1990)

Aluminium foil dishes were washed and dried to a constant weight in an oven at 100°C. These dishes were later removed and cooled in a desiccator and weighed (W1). Two (2g) of the grounded samples was placed on the weighed moisture dish (W2). The dish containing the sample was kept in an oven for 3 h, the sample was removed and cooled in the desiccator and weighed W3

% Moisture content was calculated as: $W2 - W3 \quad X\ 100$

$$\frac{}{W2 - W1}$$

Where W1= Weight of cooled Aluminium foil dish

W2= Weight of moisture dish containing 2g of the sample

W3= Weight of dish containing dried and cooled sample

3.5.1.2 Ash content

Crucibles were rinsed and dried in the oven. After drying; they were cooled in the desiccator and weighed (W1). Two (2g) of the ground (pulverised) sample was placed in the crucibles and weighed (W2). They were now transferred into the muffle furnace at 550°C for 5 h, then removed and cooled in the desiccator and weighed (W3).

% Ash content was calculated as: $\frac{W2 - W3}{W2 - W1} \times 100$

Where W1= Weight of clean dried and cooled empty crucibles

W2= Weight of crucible containing 2g of the sample

W3=Weight of crucible containing the sample transferred into the Muffle furnace

3.5.1.3 Fibre Content

Two (2g) of the sample was placed in a beaker containing 1.2ml of H_2SO_4 per 100ml of solution and boiled for 30 minutes. The residue was filtered and washed with hot water, and transferred to a beaker containing 1.2g of NaOH per 100ml of solution and boiled for 30 minutes. The residue was washed with hot water and dried in an oven and weighed (C2). The weighed sample was incinerated in a furnace at 550°C for 5 h, removed and allowed to cool, and weighed (C3).

% of Fibre content was calculated as: $\frac{C2 - C3}{W} \times 100$

Where C2= Weight of washed and dried residue of the sample

C3= Weight of incinerated and cooled residue of the sample

W= Weight of the sample

3.5.1.4. Lipids (Fat) Content

A 250ml clean boiling flask was dried in oven, then transferred into desiccator and was cooled, and then an empty filter paper was weighed and labelled (W1). Two (2g) of the sample was weighed into labelled filter paper (W2), the boiling flask was filled with petroleum spirit, and then the soxhlet apparatus was assembled and refluxing was allowed for 9 h and it was then removed and transferred to an oven to dry and it was now transferred from the oven into a desiccator and allowed to cool then weighed, W3.

% of fat content was calculated as: $\frac{W2 - W3}{W2 - W1} \times 100$

Where W1= Weight of clean dried boiling flask with filter paper

W2= Weight of labelled filter paper containing 2g of the sample

W3=Weight of dried and cooled boiling flask containing the sample

3.5.1.5 Protein Content

Digestion

Two (2g) of sample was weighed into Kjeldahl flask and then catalyst (copper) was added and 15ml conc. Sulfuric Acid H_2SO_4.The fume cupboard was heated until solution assumed a green colour,any black particles showed at the mouth and neck of the flask was cooled and washed down with distilled water.After cooling, the digest was transferred with several washings into 100ml beaker with distilled water.

Distillation:

The Markham distillation apparatus was steamed through for 15 minutes before use, 100ml erlmmeyer flask containing 10ml of Boric indicator was placed under the condenser and 10ml of the digested sample was pipetted into the body of the apparatus via the small funnel aperture; and washed down with distilled water followed by 10ml of 40% NaOH solution.

It was then steamed through for 5 minutes to collect ammonium sulphate. The receiving flask was removed and the tip of the condenser was washed down into the flask

Titration

The solution was titrated in the receiving flask using N/100 (0.01N) hydrochloric acid and the Nitrogen content was calculated and hence the protein content of the sample.

NB: Blank was run always along with the sample. The % protein was calculated as: final reading – initial reading- blank (0.2) X standard number of Nitrogen (1.4) divided by initial weight (0.5) X standard number of protein (6.25).

3.5.1.6 Carbohydrate (CHO) Content by Difference

By difference, in this method carbohydrate content was obtained by calculations having estimated all other fractions by proximate analysis and subtracting the sum from 100 as follows

100 – (% moisture + % Ash + % Protein + % Fat)

3.5.2 Pre-treatment of Samples

Ten grams of each raw beef and "Tsire" sample was weighed using a weighing balance and mashed in a sterile laboratory mortar and pestle and placed in a 100ml conical flask containing 90ml of 0.1% buffered peptone water then the mixture homogenized by thorough shaking. One millilitre of the homogenate was used to carry out serial dilution up to 10^3 concentrations (Ogbonna *et al.*, 2012).

3.5.3 Total Viable Count.

Total viable count was carried out on each sample.Plate Count Agar (PCA) (Biotech Lab. Ipswich, UK) sterilized by autoclaving at 121°C for 15 minutes was inoculated. One millilitre from dilution 10^2 and 10^3 were transferred aseptically into sterile Petri-dish. To each dilution 15 ml of sterilized plate count agar was added. Inocula was mixed well with the medium and allowed to solidify. The plates were then incubated in an incubator at 37°C for 24 h. Discrete colonies were counted using digital colony counter (Labtech, New Delhi, India) and the count was multiplied by the dilution factor. Counts were expressed as CFU/g.

3.5.4. Isolation of Some Gram Negative Organisms

3.5.4.1. Pre-enrichment

Aliquots of 0.1 ml of dilution 10^2 and 10^3 were transferred to 10ml Rapaport-Vassiliadis soy (RVS) broth and incubated at 37°C for 24 h.

3.5.4.2 Isolation of *Salmonella* species

From the pre-enrichment a loopful of the broth was streaked onto *Salmonella-Shigella* Agar plate using sterile wire loops and incubated at 37°C for 24 h.

3.5.4.3. Characterization of Bacterial Isolates

Identification of bacteria was carried out using standard microbiological/biochemical methods (Brown, 2005). The identification battery included Gram staining, oxidase, citrate, urease, indole, motility and Tripple Sugar Iron test.

Gram Staining Technique

A smear of the colony was made on a clean glass slide and fixed. The fixed smear was covered with crystal violet for 60 seconds, and then the crystal violet stain was rapidly washed off with clean water, all the water was tipped off. The smear was then covered with Lugol's iodine for 60 seconds, and washed off. And then the smear was decolorized rapidly with acetone-alcohol and washed off immediately. The smear was covered with safranine for two minutes and washed off, then placed in a draining rack for the smear to air dry, and the smear was examined microscopically with oil immersion objective.

Oxidase test

A piece of filter paper was placed in a sterile petri-dish and 2 drops of freshly prepared oxidase reagent was added to the filter paper, a loop of the test organism was picked and smeared on the filter paper. Oxidase producing organisms would oxidize the phenylenediamine to a deep purple colour within a few seconds (Cheesbrough, 2009).

Urease Test

Urea agar slants were prepared according to Manufacturer's instructions (Merck Germany). The urea slants were inoculated by streaking the surface of the slants with sterile wire loop. The organisms were incubated at 37°C for 24 h.

Sugar Utilization test usingTriple Sugar Iron agar (TSI)

Triple sugar iron (TSI) agar slants were prepared according to Manufacturer's instructions (Merck, Germany). The Triple sugar iron (TSI) agar slants were inoculated by stabbing the butt and streaking the surface of the slants with sterile straight wire. The organisms were incubated at 37°C for 24 h. After 24 h, the result was read.

Indole Test

Sterile trypton water was prepared according to the Manufacturer's instructions. The presumptive isolate was inoculated in a bijou bottle containing 3ml of sterile trypton water and incubated at 37°C for 48 h. Kovacs' reagent (0.5ml) was added and the culture shaken for 1min. A red colour in the reagent layer indicated the presence of indole (Cheesbrough, 2004)

Motility Test

Motility medium was prepared according to Manufacturer's instructions. The tubes of motility medium were stabbed to a depth of 5 mm to inoculate the isolates and were incubated at 37°C for 24 h. Motile organisms migrated throughout the medium, which becomes turbid; growth of non-motile organisms is confined to the stab inoculum.

Citrate

Citrate medium was prepared according to Manufacturer's instructions (BIOTECH United Kingdom). Citrate slants were inoculated using sterile wire loop to swab the surface and the test organisms were then be incubated at 37°C for 24 h.

Microbact^{TM} 12E Gram-Negative Bacillus (GNB) Rapid Identification System

A 24 h culture of colonies on selective media was obtained and the test was carried out and interpreted as recommended by the Manufacturer (Oxoid, UK). Four digit codes were then obtained which were fed into the computer identification software; which gave the probable identity of the organisms tested in percentage score. The Microbact software recommends a 75% cut-off point for a probable identification. All tests that gave less than 75% were not accepted.

3.5.4.4. Antibiotics Susceptibility Pattern of the Isolates

All the biochemically confirmed isolates were tested for antibiotic susceptibility using Mueller Hilton agar and were tested in vitro for susceptibility to 10 different antibacterial disc as described by Kirby–Bauer method (Bauer *et al.* 1966): Chloramphenicol (30 μg), Gentamycin (10 μg), Nitrofuratoin (100 μg), Ciprofloxacin, (10 μg), Tetracycline (30 μg), Amoxicillin-Clavulanate, (30 μg), Ampicillin (30 μg), Sulfamethoxazole/Trimethoprim (25 μg) , Imipenem (10 μg), and Erythromycin (5 μg) selection of antibiotic was based on (CLSI, 2014) recommendation.

Diffusion Method:

A small part of biochemically confirmedisolates were taken from an overnight culture plate and a colony was touched with a sterile loop and streaked onto Mueller Hilton agar and incubated overnight at 37°C, a loopfull of well isolated colonies were transferred into a tube of sterile physiological saline and vortex thoroughly. The bacterial suspension was compared with 0.5 McFarland standard, the comparison was made by viewing this tube against a sheet of white paper on which black lines were drawn.

A sterile cotton swab was dipped into the bacterial suspension. The excess fluid of swab was removed by pressing firmly against the inside of the tube just above the fluid level, the swab was streaked over the entire surface of Mueller-Hinton agar (Himedia, India) medium three times, rotating the plate approximately 60 degrees after each application to ensure an even distribution of the inoculum.

The antibiotic discs were placed individually using sterile forceps and then gently pressed down onto the agar. The plates were inverted and incubated at 37°C. After incubation the diameter of the zone of complete inhibition (including diameter of the discs) were measured in millimetres with a meter rule.

The zone of growth inhibition was compared with the zone-size interpreted based on standard provided by Clinical and Laboratory Standards Institute (CLSI, 2014). Antibacterial testing results were recorded as resistant, intermediate and susceptible according to zone diameter interpretive standards provided by CLSI (2014).

3.6 Polymerase Chain Reaction (PCR) Analysis of Tetracycline ResistantIsolates

3.6.1 DNA extraction

Lauria Bertani (LB) medium preparation

Aliquots of 1.5g of peptone, 0.5g yeast extract, 0.5g sodium chloride, 1ml of 1N sodium hydroxide, were dissolved in distilled water and made up the volume to 100ml.

Aliquot of 5 ml of the LB medium was dispensed into each of the seven universal sample bottle and autoclaved at 121°C for 15minutes. A colony of the tetracycline resistant isolate from overnight cultured plate was inoculated into the LB medium and incubated at 37°C for 24 h.

3.6.1.1 DNA Extraction Using Zymo Research Kits

One (1ml) of the cultured broth was dispensed to each of the seven 1.5ml eppendorff tubes, the tubes were centrifuged at 10000g for 1min, supernatant was discarded, and pellets were re-suspended in 600µl lysis buffer and incubated at 56°C for 30mins. The mixture was transferred to spin column in a collection tube and centrifuged at 10000g for 1min and the flow through together with the column were discarded.

The spin column thereafter was inserted into a new collection tubes and 500µl of wash buffer I was added and centrifuged at 10000g for 1min. Flow through was discarded, 500µl of buffer II was also added and centrifuged at 10000g for 1min. The flow through were also discarded. The spin column was then centrifuged empty at 10000g for 2mins to remove residual alcohols and salt, the collection tube was discarded and the spin column inserted into a 1.5ml micro-centrifuge eppendorff tube. One hundred (100ml) of elution buffer was added to the spin column and incubated at room temperature for 2mins, and centrifuged at 10000g for 2mins, and then the spin column was discarded.

The resultant DNA in 1.5ml micro-centrifuge tube was stored in -86°C refrigerator for later use in the downstream applications.

3.6.2 The Design of Polymerase Chain Reaction Amplification Primers

Polymerase Chain Reaction primers (Forward GCG CGA TCT GGT TCA CTC G and Reverse AGT CGA CAG YRG CGC CGG C) were used to target the *tet*A gene *(*Aminov *et al*., 2002)**.** An amplicon of 164bp was expected.

3.6.3. Polymerase Chain Reaction Amplification

Polymerase Chain Reactionwas carried out in a total volume of 25µl containing 12.5µl of Master mix containing Taq (*thermosaquaticus*) polymerase, dNTPs and 2 mM $MgCl_2$ was

mixed with 7µl template DNA, 1µl forward, 1µl reverse primer and 3.5µl nuclease free water in a PCR microtube. The cycling parameter involved initial denaturation at 95°C for 5mins and 35 amplification cycles consisting of denaturation at 94°C for 30 seconds, annealing at 58°C for 30 seconds, extension at 72°C for 30 seconds and final extension at 72°C for 7 minutes. The PCR was performed in a DNA thermal cycler according to Manufacturer's instructions.

3.6.4. Electrophoresis

The casting apparatus was assembled with the combs placed in position, 1.5g of Agarose powder was weighed and 100ml of TBE buffer was added, the mixture was microwaved and cooled to 45°C. Five (5µl)ethidium bromide was added and shaken to be uniformly distributed, the preparation was carefully poured into the casting tray and allowed to solidify. The combs were carefully removed and the casting tray was removed and transported to electrophoretic tank containing TBE buffer. The vacuum of the buffer was adjusted to the maximum.

Fifteen (15µl) of PCR product was mixed with 5µl of 6 times loading buffer and loaded into each well, the first well was loaded with 100bp DNA ladder, the tank was covered and connected to electric power pack, which was switched on and ran at 80V for 45min.

The gel tray was taken into the gel documentation unit, the gel was carefully slipped down from the tray to the gel documentation system, and the gel was viewed and documented in a computer system.

Data analysis

The results were analysed using SPSS version 2.0 and Microsoft excel.ANOVA was used for quantitative variable and Duncan multiple range tests were used to analyse values at different significance levels, $P<0.05$ was taken as significant.

CHAPTER FOUR

4.0 RESULTS

4.1 Proximate Composition of Raw beef and "Tsire"

Proximate composition of raw beef and "Tsire" is shown in Fig. 4.1. Moisture content values obtained ranged from 69.00 - 73.38% and 42.83 - 60.05% for raw beef and "Tsire" respectively. The highest percentage moisture of 73.38% was recorded from Pepsi Garden raw beef sample (PR) and the least of 42.83% was recorded from Sculpture Garden "Tsire" sample (ST).

The ash content obtained ranged from 1.05 - 1.20% and 2.45 - 3.95% for raw beef and "Tsire" respectively. The highest percentage ash of 3.95% was recorded from Pepsi Garden "Tsire" sample (PT) and the least of 1.05% was recorded from Faculty of Art raw beef sample (FAR).

The fat content obtained ranged from 8.80 - 17.30% and 23.08 - 30.00% for raw beef and "Tsire" respectively. The highest percentage fat of 30.00% was recorded from Community Market "Tsire" sample (CT) and the least of 8.80% was recorded from Pepsi Garden raw beef sample (PR).

Protein content obtained ranged from 5.25 - 8.75% and 5.25 - 10.50% for raw beef and "Tsire" respectively. The highest percentage protein of 10.50% was recorded from Pepsi Garden "Tsire" sample (PT) and the least of 5.25% was recorded from Sculpture Garden raw beef sample (SR) and Sculpture Garden "Tsire" sample (ST).

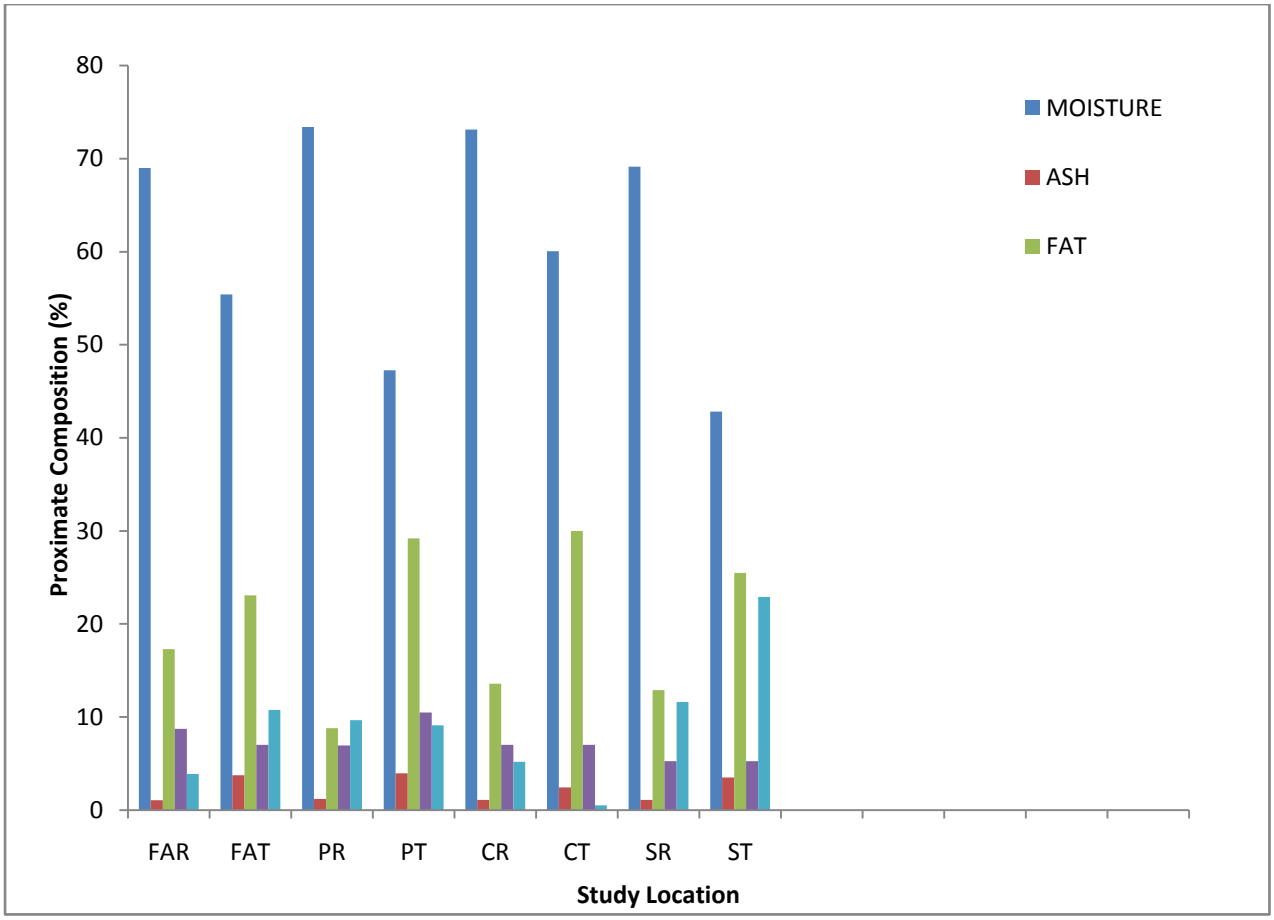


Fig. 4.1: Proximatecomposition of raw beef and "Tsire"

Key

FAR Faculty of Art raw beef Sample

FAT Faculty of Art "Tsire" Sample

PR Pepsi Garden raw beef Sample

PT Pepsi Garden "Tsire" Sample

CR Community Market raw beef Sample

CT Community Market "Tsire" Sample

SR Sculpture Garden raw beef Sample

ST Sculpture Garden "Tsire" Sample

R Raw beef sample

T "Tsire" samples

The carbohydrate (CHO) content obtained ranged from 3.90 - 11.63% and 0.5 - 22.92% for raw beef and "Tsire" respectively. The highest percentage CHO of 22.92% was recorded from Sculpture Garden "Tsire" sample (SS) and the least of 0.50% was recorded from Community Market "Tsire" sample (CS).

4.2 Total Bacterial Counts

Total bacterial counts of all the samples of raw beef and "Tsire" sold in Ahmadu Bello University Main Campus Samaru Zaria is shown in Table 4.1. The total bacterial count ranged from 2.61 - 5.24 x 10^4 cfu/g for raw beef and 1.37 - 1.87 x 10^4cfu/g for "Tsire". The standard deviation (SD) ranged from 4.89 to 9.41 x 10^3 for raw beef and 1.77 to 4.1 x 10^3 for "Tsire". No significant difference in total bacterial count was observed in the raw beef and "Tsire" samples analysed ($P>0.05$).

4.3 Morphological and Biochemical Characteristics of the Bacterial Isolates from Raw Beef Samples.

The morphological and biochemical characteristics of the isolates from raw beef samples are shown in Table 4.2. All of the 16 isolates were Gram negative rod organisms; the probable organisms include *Salmonella*, *Escherichiacoli*, *Klebsiella*, *Enterobacter*, *Hafnia* and *Tatumella* species respectively

Table 4.1: Total Bacterial Counts of Raw Beef and "Tsire" Samples(CFU/g) From the Study Locations

Location	Raw beef	Range of counts of Raw beef	Average of counts of Raw beef	"Tsire"	Range of counts of "Tsire"	Average of counts of "Tsire"
FA	$2.61x10^4$±$4.89x10^{3a}$	$1.0x10^3$-$1.35x10^5$	$2.76x10^4$	$1.87x10^4$±$2.78x10^{3a}$	$1.0x10^3$-$5.5x10^4$	$1.81x10^4$
PG	$4.57x10^4$±$8.3x10^{3ab}$	$3.0x10^3$-$3.03x10^5$	$6.13x10^4$.	$1.74x10^4$±$4.1x\ 10^{3a}$	$2.0x10^3$-$1.09x10^5$	$1.5x10^4$
CM	$3.57x10^4$±$6.89x10^{3ab}$	$1.0x10^3$-$1.58x10^5$	$3.34x10^4$	$1.37x10^4$±$1.77x10^{3a}$	$1.0x10^3$-$3.6x10^4$	$1.29x10^4$
SG	$5.24x10^4$±$9.41x10^{3b}$	$2.0x10^3$-$1.72x10^5$	$5.21x10^4$	$1.59x10^4$±$3.49x10^{3a}$	$2.0x10^3$-$7.7x10^4$	$1.43x10^4$
P-value	0.078			0.716		

MEAN±SD ANOVA, DMRT.

Key
Samples were in duplicate
FA Faculty of Art
PG Pepsi Garden
CM Community Market
SG Sculpture Garden

Table 4.2: Morphological and Biochemical characteristic of the isolates from raw beef samples

Isolates	Gram's reaction	Citrate	Motility	Indole	Urease	Oxidase	TSI	Probable organism
1	Gram –ve rod	-	+	-	-	-	K/AGH_2S^+	*Salmonella spp*
2	Gram –ve rod	-	+	-	-	-	K/AGH_2S^+	*Salmonella spp*
3	Gram –ve rod	-	+	-	-	-	K/AGH_2S^+	*Salmonella spp*
4	Gram –ve rod	-	+	-	-	-	K/AGH_2S^+	*Salmonella spp*
5	Gram –ve rod	-	+	+	-	-	A/AG^+	*Escherichia coli*
6	Gram –ve rod	+	-	+	-	-	A/AG^+	*Klebsiella spp*
7	Gram –ve rod	+	+	-	-	-	A/AG	*Enterobacer spp*
8	Gram –ve rod	-	+	+	-	-	A/AG^+	*Escherichia coli*
9	Gram –ve rod	-	-	-	-	-	A/AG^+	*Tatumella ptyseos*
10	Gram –ve rod	-	+	+	-	-	A/AG^+	*Escherichia coli*
11	Gram –ve rod	-	+	-	-	-	A/AG^+	*Hafnia alvei*
12	Gram –ve rod	-	+	+	-	-	A/AG^+	*Escherichia coli*
13	Gram –ve rod	-	+	+	-	-	A/AG^+	*Escherichia coli*
14	Gram –ve rod	-	+	+	-	-	A/AG^+	*Escherichia coli*
15	Gram –ve rod	-	+	+	-	-	A/AG^+	*Escherichia coli*
16	Gram –ve rod	-	+	+	-	-	A/AG^+	*Escherichia coli*

Key
-ve – Negative
_ _ Negative
+ _ Positive

4.4 Identification of the Isolates Using Microbact™ (12E) Kit

Microbact™ (12E) Gram negative system was used to confirm the isolates. The result is as shown in Table 4.4. It revealed that three of the isolates were *Enterobacter sakazakii,* four of the isolates were *Hafnia alvei,* seven were *Escherichia coli,* one was *Klebsiella oxytoca* and the last one was *Tatumella ptyseos* (Table 4.4).

4.5 Antibiotic Susceptibility Test

Most of the *Escherichia coli* strains were found to be susceptible Nitrofuratoin, Imipenen Chloramphenicol and Ciprofloxacine. It was also noted be resistant to Erythromycin, Amoxicillin Clavulanate, Sulfamethoxazole/Trimethoprim, Ampicillin and Tetracycline as it is shown in Table 4.4.

All of the *Enterobacter sakazakii* strains were susceptible to Amoxicillin Clavulanate, Imipenen, Ampicillin, Tetracycline, Gentamycin, Nitrofuratoin and Choramphenicol. But was found to be resistant to Erythromycin and Sulfamethoxazole/Trimethoprim (Table 4.5).

Table 4.6 reveals that all of the *Hafnia alvei* strainswere susceptible to Imipenen, Nitrofuratoin, Gentamycin and Chloramphenicol. Some of this isolates were resistant to Erythromycin, Amoxicillin Clavulanate, Sulfamethoxazole/Trimethoprim, Tetracycline and Ciprofloxacine (Table 4.6).

As it is shown on Table 4.7 Strain of *Klebsiella oxytoca* was susceptible to Amoxicillin Clavulanate, Imipenen, Ampicillin, Nitrofuratoin, Gentamycin and Chloramphenicol, and it was resistant to Erythromycin, Sulfamethoxazole/Trimethoprim, Tetracycline, (Table 4.7).

Table 4.3: Identification of the isolates using Microbact™ (12E) kit.

Isolates	Four digit code	Percentage probability(%)	Final identity
1	2745	98.91	*Enterobacter sakazakii*
2	6745	83.29	*Hafnia alvei*
3	6767	84.34	*Enterobacter sakazakii*
4	6745	83.29	*Hafnia alvei*
5	6761	96.39	*Escherichia coli*
6	4765	82.51	*Klebsiella oxytoca*
7	2765	97.82	*Enterobacter sakazakii*
8	7765	70.69	*Escherichia coli*
9	0101	98.50	*Tatumella ptyseos*
10	6761	96.39	*Escherichia coli*
11	6741	81.01	*Hafnia alvei*
12	6761	96.39	*Escherichia coli*
13	6761	96.39	*Escherichia coli*
14	4761	76.80	*Escherichia coli*
15	6741	81.01	*Hafnia alvei*
16	6761	96.39	*Escherichia coli*

Table 4.4: Antibiotic susceptibility pattern of *Escherichia coli* Isolated from Raw Beef Samples

Antibiotics (n=7)	Susceptible(%)	Intermediate(%)	Resistance(%)
AMC	1(14.3)	2(28.6)	4(57.1)
E	0(0.0)	1(14.3)	6(85.6)
SXT	2(28.6)	0(0.0)	5(71.4)
IPM	7(100)	0(0.0)	0(0.0)
AMP	3(42.9)	0(0.0)	4(57.1)
TE	2(28.6)	0(0.0)	5(71.4)
CIP	2(28.6)	5(71.4)	0(0.0)
F	7(100)	0(0.0)	0(0.0)
CN	6(85.6)	1(14.3)	0(0.0)
C	7(100)	0(0.0)	0(0.0)

Key
AMC= Amoxicillin Clavulanate, (30 µg)
E= Erythromycin (5 µg)
SXT= Sulfamethoxazole/Trimethoprim (25 µg)
IPM= Imipenem (10 µg)
AMP= Ampicillin, (30 µg)
TE= Tetracycline (30 µg)
CIP= Ciprofloxacin, (10 µg)
F= Nitrofuratoin (100 µg)
CN= Gentamycin, (10 µg)
C= Chloramphenicol, (30 µg)

Table 4.5: Antibiotic susceptibility pattern of *Enterobacter sakazakii* Isolated from Raw Beef Samples

Antibiotics (n=3)	Susceptible(%)	Intermediate(%)	Resistance(%)
AMC	3(100)	0(0.0)	0(0.0)
E	0(0.0)	0(0.0)	3(100)
SXT	2(66.7)	0(0.0)	1(33.3)
IPM	3(100)	0(0.0)	0(0.0)
AMP	3(100)	0(0.0)	0(0.0)
TE	3(100)	0(0.0)	0(0.0)
CIP	2(66.7)	1(33.3)	0(0.0)
F	3(100)	0(0.0)	0(0.0)
CN	2(66.7)	1(33.3)	0(0.0)
C	3(100)	0(0.0)	0(0.0)

Key
AMC= Amoxicillin Clavulanate, (30 µg)
E= Erythromycin (5 µg)
SXT= Sulfamethoxazole/Trimethoprim (25 µg)
IPM= Imipenem (10 µg)
AMP= Ampicillin, (30 µg)
TE= Tetracycline (30 µg)
CIP= Ciprofloxacin, (10 µg)
F= Nitrofuratoin (100 µg)
CN= Gentamycin, (10 µg)
C= Chloramphenicol, (30 µg)

Table 4.6: Antibiotic susceptibility pattern of *Hafnia alvei* Isolated from Raw Beef

Antibiotics (n=4)	Susceptible(%)	Intermediate(%)	Resistance(%)
AMC	1(25)	1(25)	2(50)
E	0(0.0)	1(25)	3(75)
SXT	3(75)	0(0.0)	1(25)
IPM	4(100)	0(0.0)	0(0.0)
AMP	3(75)	0(0.0)	1(25)
TE	3(75)	0(0.0)	1(25)
CIP	2(50)	2(50)	0(0.0)

Samples

F		4(100)	0(0.0)	0(0.0)
CN		4(100)	0(0.0)	0(0.0)
C		4(100)	0(0.0)	0(0.0)
Antibiotics	(n=1)	Susceptible(%)	Intermediate(%)	Resistance(%)

Key
AMC= Amoxicillin Clavulanate, (30 µg)
E= Erythromycin (5 µg)
SXT= Sulfamethoxazole/Trimethoprim (25 µg)
IPM= Imipenem (10 µg)
AMP= Ampicillin, (30 µg)
TE= Tetracycline (30 µg)
CIP= Ciprofloxacin, (10 µg)
F= Nitrofuratoin (100 µg)
CN= Gentamycin, (10 µg)
C= Chloramphenicol, (30 µg)

Table 4.7: Antibiotic susceptibility pattern of *Klebsiella oxytoca* Isolated from Raw Beef Samples

AMC	1(100)	0(0.0)	0(0.0)
E	0(0.0)	0(0.0)	1(100)
SXT	0(0.0)	0(0.0)	1(100)
IPM	1(100)	0(0.0)	0(0.0)
AMP	1(100)	0(0.0)	0(0.0)
TE	0(0.0)	0(0.0)	1(100)
CIP	0(0.0)	1(100)	0(0.0)
F	1(100)	0(0.0)	0(0.0)
CN	0(0.0)	1(100)	0(0.0)
C	1(100)	0(0.0)	0(0.0)

Key
AMC= Amoxicillin Clavulanate, (30 µg)
E= Erythromycin (5 µg)
SXT= Sulfamethoxazole/Trimethoprim (25 µg)
IPM= Imipenem (10 µg)
AMP= Ampicillin, (30 µg)
TE= Tetracycline (30 µg)
CIP= Ciprofloxacin, (10 µg)
F= Nitrofuratoin (100 µg)
CN= Gentamycin, (10 µg)
C= Chloramphenicol, (30 µg)

Table 4.8 reveals that *Tatumella ptyseos* strainwas susceptible to Amoxicillin Clavulanate, Sulfamethoxazole/Trimethoprim, Imipenen, Ampicillin, Tetracycline Nitrofuratoin, Gentamycin and chloramphenicol, and it was resistant to Erythromycin (Table 4.8).

Multi drug resistance indices, and resistance pattern of the isolates is summarised in Table 4.9. Two of the *Hafnia alvei* isolates from Sculpture-Garden "Tsire" spot raw beefsamples were resistant to more than two antibiotics, four of the *Escherichia coli* isolates from Pepsi-Garden and Sculpture-Garden "Tsire" spot raw beefwere resistant to more than four antibiotics, one of the *Enterobacter sakazakii* isolates from Faculty of art "Tsire" spot raw beef was resistant to two antibiotics, and *Klebsiella oxytoca* isolates from Community-Market "Tsire" spot raw beef was resistant to three antibiotics.

4.6 Detection of *tet*A genes by PCR.

In Plate I, Lane M represent molecular weight marker, 100 bp ladder, and lane 1-7 represent tetracycline resistance in raw beef isolates i.e. five isolates of *Escherichia coli* from Community market "Tsire" spot, Pepsi garden "Tsire" spot and Sculpture garden "Tsire" spot, one isolate of *Hafniaalvei* from Sculpture garden "Tsire" spot and one isolate of *Klebsiellaoxytoca* from Community market "Tsire" spot.Lane one to five represents *Escherichia coli,* lane six represents *Klebsiella oxytoca,* and lane seven represents *Hafnia alvei.* Out of seven Tetracycline resistant isolates examined, i.e., five *Escherichia coli,* one *Hafnia alvei and* one *Klebsiella oxytoca tet*A gene was detected in two (*Escherichia coli*), lane six (*Klebsiella oxytoca*) and lane seven (*Hafnia alvei*) as it is shown in plate: I

Table 4.8: Antibiotic susceptibility pattern of *Tatumella ptyseos* Isolated from Raw Beef Samples

Antibiotics (n=1)	Susceptible(%)	Intermediate(%)	Resistance(%)
AMC	1(100)	0(0.0)	0(0.0)
E	0(0.0)	0(0.0)	1(100)
SXT	1(100)	0(0.0)	0(0.0)
IPM	1(100)	0(0.0)	0(0.0)
AMP	1(100)	0(0.0)	0(0.0)
TE	1(100)	0(0.0)	0(0.0)
CIP	0(0.0)	1(100)	0(0.0)
F	1(100)	0(0.0)	0(0.0)
CN	1(100)	0(0.0)	0(0.0)
C	1(100)	0(0.0)	0(0.0)

Key
AMC= Amoxicillin clavulanate, (30 µg)
E= Erythromycin (5 µg)
SXT= Sulfamethoxazole/Trimethoprim (25 µg)
IPM= Imipenem (10 µg)
AMP= Ampicillin, (30 µg)
TE= Tetracycline (30 µg)
CIP= ciprofloxacin, (10 µg)
F= Nitrofuratoin (100 µg)
CN= Gentamycin, (10 µg)
C= Chloramphenicol, (30 µg)

Table 4.9: Multiple Drug Resistance Indices, and Resistance Pattern of the Raw Beef Isolates

Isolates	Source	Location	Number of antibiotic showing resistance	Resistance pattern	MAR Index
Enterobacter sakazakii	Raw beef	PG	1	E	0.1
Hafnia alvei	Raw beef	SG	4	AMC, E, SXT, TE	0.4
Enterobacter sakazakii	Raw beef	SG	1	E	0.1
Hafnia alvei	Raw beef	SG	1	E	0.1
Escherichia coli	Raw beef	CM	1	TE	0.1
Klebsiella oxytoca	Raw beef	CM	3	E, SXT, TE	0.3
Enterobacter sakazakii	Raw beef	FA	2	E, SXT	0.2
Escherichia coli	Raw beef	FA	2	E, SXT	0.2
Tatumella ptyseos	Raw beef	CM	1	E	0.1
Escherichia coli	Raw beef	PG	5	AMC, E, SXT, AMP, TE	0.5
Hafnia alvei	Raw beef	PG	0		0
Escherichia coli	Raw beef	PG	5	AMC, E, SXT, AMP, TE	0.5
Escherichia coli	Raw beef	CM	1	E	0.1
Escherichia coli	Raw beef	CM	5	AMC, E, SXT, AMP, TE	0.5
Hafnia alvei	Raw beef	SG	3	AMC, E, AMP	0.3
Escherichia coli	Raw beef	SG	5	AMC, E, SXT, AMP, TE	0.5

Key
AMC= Amoxicillin clavulanate, (30 µg)
E= Erythromycin (5 µg)
SXT= Sulfamethoxazole/Trimethoprim (25 µg)
AMP= Ampicillin, (30 µg)
TE= Tetracycline (30 µg)
CM= Community Market "Tsire" spot
FA= Faculty of art "Tsire" spot
PG= Pepsi Garden "Tsire" spot
SG= Sculpture Garden "Tsire" spo

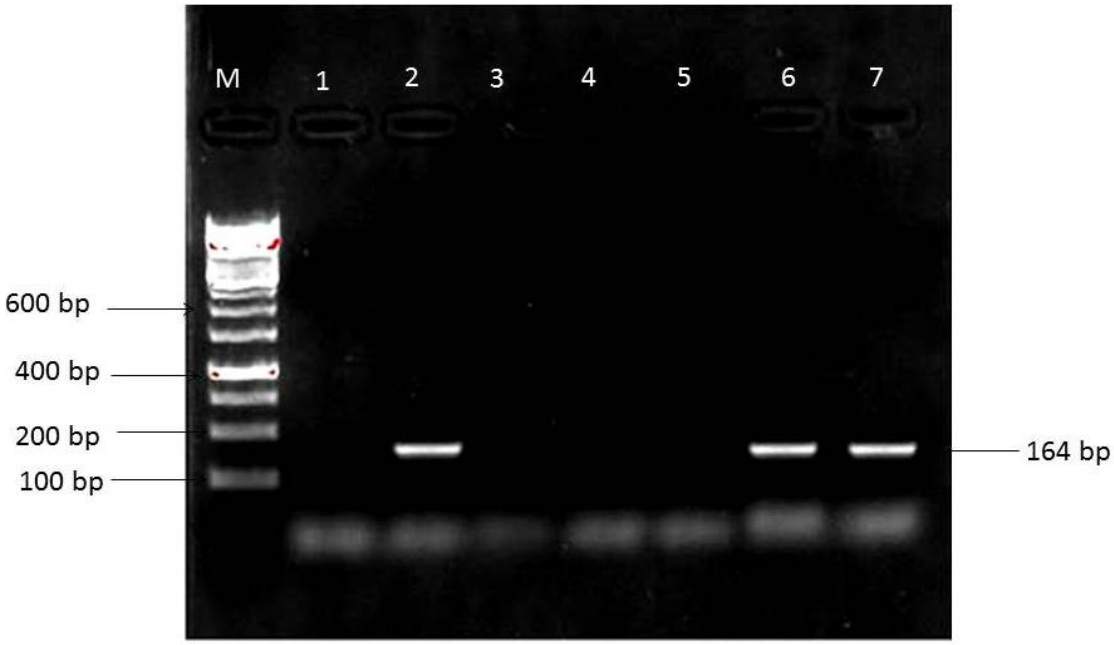


Plate I: Lane 2, 6 And 7 Are Amplicon Of *Teta* Genes Of *Escherichia coli, Klebsiella oxytoca* and *Hafnia alvei* of size 164 bp respectively

CHAPTER FIVE

5.0 DISCUSSION

5.1 Proximate Composition of Raw Beef and "Tsire"

The moisture content of 69.00 - 73.38% was observed for raw beef in this present study. The value is higher than 42.83 - 60.05% obtained for "Tsire". The difference observed might be due to processing of the "Tsire". The high percentage of moisture content of raw beef obtained agrees with the findings of Nikmaram *et al*. (2011), and Bradeeba and Sivakumaar (2012), who reported 73.45 and 73.01% in their respective studies, but is contrary to the findings of Abd El-aal and Suliman (2007), who reported 43.61% when he carried out his research.

The moisture content of "Tsire"obtained was contrary to the findings of Edema *et al.* (2008),and Fakolade and Omojola (2008), who reported 57.17 and 23.29% respectively in their study. The moisture content of both raw beef and "Tsire"varied with study locations. Moisture content of meat could be attributed to the health status of the animals, genetic factors, feeding or method of processing.

High moisture content of food accelerates its spoilage and generally provides a good medium for the growth and proliferation of microorganisms especially bacteria. Raw beef usually tend to have high percentage of moisture which is a major intrinsic factor of food contamination as reported by Yah *et al*. (2009).

Ash content is the measure of total amount of minerals in food. Ash content of 1.05 - 1.20% was obtained from raw beef in this study. This value is lower than 2.45 - 3.95% obtained from "Tsire". According to Abdel-Moneim*et al.* (2012), ash content of meat increases with

addition of additives. The higher ash content of raw beef agrees with the findings of Lijalem *et al.* (2015), who reported 1.2% when he carried out his research on quality of beef.

The higher ash content of "Tsire"obtained also agrees with the findings of Abdullahi *et al.* (2006), and Ogbonna *et al.* (2012), who reported 2.4 and 2.86% respectively when carrying out their respective researches.

Fat content of 8.80 - 17.30% was obtained from raw beef in this study. The value is lower than 23.08 - 30.00% obtained for "Tsire". The increase in fat content of "Tsire" may be due to the addition of oil during "Tsire" preparation as reported by Abdel-Moneim*et al.* (2012). Generally high fat content may be responsible for its juiciness and sweet aroma upon cooking as reported by Adeniyi *et al*. (2011). The highest fat content of raw beef is higher than the findings of Bradeeba and Sivakumaar (2012), and lower than the findings of Venia *et al*. (2006), who reported 2.12% and 10.9 -29.6 % respectively in their respective researches. The fat content of"Tsire"agrees with the findings of Abdullahi *et al. (*2006), and Ogbonna *et al.* (2012), who reported over 23.7 and 20.90 % respectively.

Protein content of 8.80 - 17.30% was observed for raw beef, a value that is less by 23.08 - 30.00% obtained from "Tsire". This agrees with the findings of Omojola (2008). This variation could be due to the fact that "Tsire" preparation involves the addition of other proteineous ingredients (such as groundnut); hence elevating its protein content as reported by Omojola (2008). The highest protein content of raw beef observed is lower than 16.05% reported by Bradeeb and Sivakumaar (2012), the highest protein content of "Tsire"obtained was lower than 31.8 and 28.33% reported by Abdullahi *et al.* (2006), and Ogbonna *et al.* (2012) respectively.

Carbohydrate content of 3.90 - 11.63% was observed for raw beef, a value that is less than 0.5 - 22.92% obtained from "Tsire". The carbohydrate content of both the raw beef and "Tsire"is contrary to the findings of Pushparajan *et al*. (2012).

Generally, the proximate composition of both the raw beef samples and "Tsire" samples affect the growth and survival of microorganisms in the both samples sold in Ahmadu Bello University, Zaria, Nigeria.

5.2 Total Bacteria count of Raw beef and "Tsire"

The total bacterial counts for the raw beef from the four sampling locations varied. This may be due to the level of hygiene during slaughtering and meat processing. The total bacterial counts range of $1.0x10^3$ - $1.72x10^5$cfu/g was observed in the raw beef in this study. The counts were higher than the range of $1.37x\ 10^4$ - $1.87x\ 10^4$ obtained from "Tsire". The analysis of the bacterial counts of the raw beef and "Tsire" samples indicated variations between both samples; bacterial load was lower in "Tsire"samples probably due the effect of heat on the bacteria during the preparation of "Tsire" and lower moisture contents of "Tsire"samples. The results of microbial load of "Tsire"samples also revealed the level of hygiene surrounding the meat and the ingredients used in preparing the "Tsire"as well as that of environment and "Tsire"processors as reported by Uzeh *et al*., (2006). The counts of both the raw beef and "Tsire" were within the acceptable standard specified by International Commission on Microbiological Specification (i.e. < $1.0\ x\ 10^6$ cfu/g) (ICMS, 1986). The highest count of $1.72x10^5$ Cfu/g was from raw beef sample obtained from Sculpture Garden. Though the count was the highest it was below 10^7-10^8 Cfu/g for which spoilage of meat is apparent as reported by Pikkemaat *et al*. (2011). "Tsire" sample counts from this study agreed with the findings of Osho (2004), who reported ranged of 10^4 Cfu/g and is contrary to the findings of Edema *et al*. (2008), and Inyang *et al*. (2005), who reported comparably higher values for total plate counts in "Tsire" samples in the order of 10^5 and 10^6cfu/g respectively

and stated that the values placed the “Tsire” samples consumed in Nigeria within satisfactory limit according to the International Commission of Microbiological Standards for Foods (ICMSF, 1978). However, these values place the “Tsire” samples examined in this work within the acceptable and satisfactory range under the Public Health Laboratory Service guidelines for the bacteriological quality of ready-to-eat foods samples at the point of sale (PHLS, 2000) since no pathogenic organism was isolated from all the “Tsire” samples.

5.3 Gram Negative Organisms Isolated from Raw beef and “Tsire”

Raw beef samples from this study yielded marked growth of bacteria with Community Market and Sculpture Garden “Tsire spot” having the highest number of isolates. The bacterial isolates were identified as *Echerichia coli*, *Enterobacter sakazakii*, *Hafnia alvei*,*Klebsiella oxytoca*, and *Tatumella ptyseos* by comparing their morphologicaland biochemical characteristics with standard reference organisms (Cheeshrough, 2009). *Escherichia coli* and *Enterobacter sakazakii* were isolated from samples obtained from Faculty of Art, *Escherichia coli*, *Escherichia sakazakii* and *Hafnia alvei* were isolated from samples obtained from Pepsi Garden, *Escherichia coli*,*Klebsiella oxytoca* and *Tatumella ptyseos* were isolated from samples from Community Market, *Escherichia coli*, *Enterobacter sakazakii* and *Hafnia alvei* were isolated from samples obtained from Sculpture Garden with an average incidence rate of 12.5, 25, 31.25 and 31.25% respectively. The presence of *Escherichia coli*, *Enterobacter sakazakii*, *Hafnia alvei*, *Klebsiella oxytoca*, and *Tatumella ptyseos* suggest faecal contamination which is the cause of enteric diseases in developing countries and is a cause of morbidity and mortality (Talaro and Talaro 2006).

Tatumella ptyseos have been implicated in tracheobronchial /pulmonary infections such as pneumonitis, asthmatic bronchitis, pharyngitis, Wegener granulomatosis, pneumonia, chronic lung disease, pulmonary edema, pulmonary tuberculosis, and gastrointestinal infection

reported by Berktaş *et al.* (2013). It's have been isolated from other sources such as soil, poultry carcasses, edible microalgae, water supplies, and pineapple in different developing and developed countries as reported by Jimenez *et al.* (2003).

Thesource of *Enterobacter sakazakii* and its vehicles of transmission are not always clear. Although the organism has been detected in multiple food sources as reported by Drudy *et al.* (2006). It is susceptible to some antibiotics, including tetracyclines, aminoglycosides, numerous b-lactams, chloramphenicol, antifolates, and quinolones , Stock and Wiedemann (2002).

Hafnai alvei have been reported in rare cases to cause extra-intestinal diseases. This organism is seldom considered to be pathogenic as reported by Podschun *et al*. (2016). It is commonly isolated from warm blooded animals, animals, contaminated water, sewage, soil and food. It is a natural inhabitant of gastro- intestinal tract in human; they are associated with diarrheal associated with virulent factors similar to toxigenic *Escherichia coli* Podschun *et al*. (2016).

5.4 Antibiotic Resistance

Drug resistance is favoured by the extensive use of antibiotics in animal health or herd health in our communities. The overuse and misuse of these drugs have led to the death of sensitive strains leaving the resistant ones to survive, multiply and infect new hosts (Hug, 1980; Kendal, 1980; Yusha'u *et al.,* 2007). This work has shown some of these organisms to be multidrug resistant with strains of *Escherichia coli* isolates resistant to five antibacterial agents. In a similar vein, strains of *Hafnia alvei* wereresistant to four antibacterial agents while *Klebsiella oxytoca* isolate was resistant to three antibiotics. The medical implication of these is obvious; the public health importance is more glaring in the light of the transferability of these traits among both pathogenic and potentially pathogenic bacteria. The use of antibiotics, whether for prophylaxis or chemotherapy, does not only affect the

pathogenic bacteria but also the commensal bacteria. This maintains a number of resistant bacteria with a pool of resistant genes in the population which further contributes to the general increase and dissemination of bacterial resistance and can be a source of resistant genes for other pathogens (Abatih *et al*., 2009; Blake *et al*., 2003).

5.5 *Tet*A Genes for Tetracycline Resistance in the Isolates Using PCR

Characterization of the antibiotic resistant genes using polymearase chain reaction revealed that five isolates of *Escherichia coli*, one isolate of *Klebsiella oxytoca* and one isolate of *Hafnia alvei* were resistant to tetracycline. *Tet* A gene was detected in three of the tetracycline resistant isolates i.e. *Escherichia coli, Klebsiella oxytoca* and *Hafnia alvei*. Most common genes responsible for tetracycline resistance are *tet*A gene. Skočková *et al*. (2012) reported that in 2010-2011 the most common gene responsible for tetracycline resistance was *tet*A gene.

The fact that 18.75% of the analysed strains possessed *tet*A genes could be due to strong selection pressures provided by environments containing elevated levels of tetracycline as suggested by Bryan *et al*. (2004). High resistance to tetracycline among enterobacterial strains could be caused by the use of antibiotics as animal growth promoters.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 CONCLUSION

In conclusion the proximate composition of raw beef and "Tsire" revealed the various proportions of moisture, protein, fats, carbohydrate and ash. "Tsire" had higher value than raw beef in all the parameter except in moisture.

The total bacterial counts from both the raw beef and "Tsire" were within the acceptable level (<1.0 x 10^6 Cfu/g) (ICMS, 1986) and deemed safe for humanconsumption. The number of cells could increaseto an unacceptable level both raw beef and "Tsire" is notproperly preserved.

Sixteen different bacterial isolates were detected in raw beef but there was no isolate from the "Tsire". The bacteria isolates were identified as *Echerichia coli, Enterobacter sakazakii, Hafnia alvei,Klebsiella oxytoca,* and *Tatumella ptyseos*. The finding from this study is of public health concern, as most of the isolates have been reported to cause various health problems basically foodborne infection and intoxications in humans. There is possibility of transfer of these organisms to the consumers, if the organisms are ingested in uncooked or undercooked meat.

Hundred (100%) of the isolates were susceptible to Imipenen, Nitrofuratoin and Chloramphenicol. Thirteen (13) of the isolates were susceptible to Gentamycin, 69% of the isolates were susceptible to Ampicillin and 31% were resistant to it. Fifty-six percent of the isolates were susceptible to Tetracycline and 44% were resistant to it, 8 of the isolates were susceptible to Sulfamethoxazole/Trimethoprim and 8 were resistant to it. Seven of the isolates were susceptible to Amoxicillin Clavulanate and 6 were resistant to it. Thirty eight

percent of the isolates were susceptible to Ciprofloxacine and 62% were intermediately susceptible to it, 88% of the isolates were resistant to Erythromycin.

The presence of residues of antibiotics in beef as observed in this study has a great public health concern. Human consumption of beef containing antibiotic residue may be associated with the development of antibiotic resistance in human pathogens that are difficult to treat with the commonly available antibiotics.

Polymerase chain reaction revealed the presence of *TetA* gene in three of Tetracycline resistant isolates namely, *Escherichia coli, Hafnia alvei* and *Klebsiella oxytoca.*

6.2 RECOMMENDATIONS:

Thus based on the above conclusions the following recommendations are suggested:

i. meat should not be kept under ambient condition for 48 h so that proximate composition of the meat will not be altered.
ii. Raw meat and "Tsire" sold in A.B.U. main campus had bacterial counts within normal range. However improved conditions of processing is recommended to further enhance the quality.
iii. Good slaughter house practise is recommended to prevent contamination with enteric organisms which are found in meat.
iv. Narrow-spectrum antibiotics should be the first choice when antibiotic therapy is justified.
v. Use of antibiotics as growth promoters should be prohibited.

REFERENCES

Abatih, E. N., Alban, L., Ersboll, A. K., Wong, D. M. (2009).Impact of antimicrobial usage on the transmission dynamics of antimicrobial resistant bacteria among pigs. *Journal of Theory of Biology*,256**:** 561-573.

Abd El-aal, H. A. and Suliman, A. I. A. (2007). Carcass traits and meat quality of lamb fed on ration containing different levels of leucaena hay (*Leucaena leucocephala*) scientific paper, presented at International Congress on Animal Husbandry "New Perspectives and Challenges of Sustainable Livestock Farming", Belgrade, 24(3-4) 77-92.

Abdel Moneim E. S, Mandour, H. A., and El Rakha. B. B. (2012). Some Chemical and Microbiological Characteristics of *Agashi* Meat Product.*Food and Nutrition Sciences*, 3: 851-856

Abdul, U. M., Beuchat, C. R., and Ammar, M. S. (1995). Survival and growth of *Escherichia coli* in ground roast beef as affected by pH, acidulates and temperature. *Journal of Applied and Environmental Microbiology,* 59(8): 2364-2368.

Abdullahi, B., Abdulfatai, K., Wartu, J. R., Mzungu, I., Muhammad, H. I. D. and Abdulsalam, A. O. (2013)Antibiotics susceptibility patterns and characterization of clinical *Salmonella* serotypes in Katsina State, Nigeria. *African Journal of Microbiology Researches.*

Abdullahi, I. O., Umoh, V. J, Ameh, J. B., and Galadima, M. (2004): Hazards associated with kilishi preparation in Zaria, Nigeria. *Nigeria Journal of Microbiol*ogy, 18 (1-2): 338-345.

Abdullahi, I.O., Umoh, V.J., Ameh, J.B. and Galadima, M. (2006). Some hazards associated with the production of a popular roasted meat (tsire) in Zaria. Food Control. 17 (5): 348-352.

Acar. J., and Rostel, B. (2001). Antimicrobial resistance: an overview. *Reviewed Science and Technology*,20:797- 810.

Acar, J. F., and Moulin, G. (2006).Antimicrobial resistance at farm level.*Revue Scientifique et Technique (International Office of Epizootics),* 25(2):775-792.

Adeleke E. O., Omafuvbe B. O. (2011). Antibiotic Resistance of Aerobic Mesophilic Bacteria Isolated from Poultry Faeces. *Research Journal of Microbiology*, 6(4):356-365.

Adeniyi, O. R., Ademosun, A. A., and Alabi, O. M. (2011)Proximate composition and economic values of four common sourcesof animal protein in South-western Nigeria. *Zootecnia Tropica*, 29(2): 231-234.

Agbogu, V. N., Umoh , V. J, Charles, A. O., Smith S. I. and Joseph, B. A. (2006). Study of the bacteriological and physiological indicators of pollution of surface waters in Zaria, Nigeria.*African Biotechnology,* 5: 732-737.

Akoachere, J. F. T. K., Bughe, R. N., Oben, B. O., Ndip, L. M., Ndip, R. N. (2009a). Phenotypic characterization of human pathogenic bacteria in fish from the coastal waters of South West Cameroon: Public health implications. *Review of Environmental Health,* 24:147-155.

Akoachere, J. F. T. K., Tanih, N. F., Ndip L. M., Ndip R. N. (2009b). Phenotypic characterisation of *Salmonella* Typhimurium isolates from food-animals and abattoir drains in Buea, Cameroon. *Journal of Health Population Nutrition,*27:612-618.

Allos, B. M. (2001). *Campylobacter jejuni* infections: update on emerging issues and trends. *Clinical Infectious Diseases,*32**:**1201-1206.

Alonge, D. O., and Hiko, A. A. (1981).Traditional methods of meat preservation in Nigeria. *West Africa Farm Food Procedures,* Pp. 19 – 21.

Altekruse, S. F., Stern, N. J., Fields, P. I., Swerdlow, D. L. (1999). *Campylobacter jejuni* — an emerging foodborne pathogen.*Emerging of Infectious Diseases,*5:28-35.

Aminov, R. I., Chee-Sanford, J.C., Garrigues, N., Teferedegne, B., Krapac, I. J., White, B. A., Mackie, R. I. (2002). Development, Validation, And Application of PCR Primers For Detection of Tetracycline Efflux Genes of Gram-negative bacteria.*Applied Environmental Microbiolicrobiolgy,* 68(4):1786, 1793.

Amstrong, J. L., Shigeno, D. S., Colomiris, J. J., and Ceider, R. J. (1981) Standard Bacterial Count in Water. *Applied and Environmental Microbiology,*42*:277.*

Anderson, E. S. (2000). Drug resistance in *Salmonella* typhimurium and its implications. *British Medical Journal,* 3:333-339.

Anderson, S. R., Saadbye, P., Shukri, N. M., Rosenquist, H., Nielson, N. L., Boel, J. (2006). Antimicrobial resistance among *Campylobacter jejuni* isolated from raw poultry meat at retail level in Denmark. *International Journal of Food Microbiology,* 107:250-255.

Anjum, Q., Omair, A., Ahmed, Y., Shaikh, S., Usman, J., and Qureshi, F. (2004) Frequency of Food Items among Households in a Low Socio-economic Area of Karachi.*Journal of Pakistan Medical Association,*54: 580-582.

ANON (2008). Scientific Opinion of the Panel on Biological Hazards on a request from the Health and Consumer Protection, Directorate General, European Commission on Microbiological Risk Assessment in feeding stuffs for food-producing animals. *The EFSA Journal*, 720, 1–84.

AOAC.Official methods of analyses [15th Ed], (1990).*Association of official Analytical chemist,* Washington D.C. Pp 808, 831-835, 1113.bacterial pathogens of public health

importance in the developing world. World Health Organization. Atlanta Georges U.S.A, Pp. 133 – 284.

Arnon, S. S., Schechter, R., Maslanka, S. E., Jewell, P. N., Hatheway, C. L. (2006). Human botulism immune globulin for the treatment of infant botulism.*New England Journal of Medicine*, 354:462-471.

AVMA (2010).*Issues of Animal Welfare*. Retrieved from http://www.avma.org/issues/animal_welfare/animal_welfare_bronchure.as

Baade, P., Meng, X., Sinclair, C., and Youl, P. (2012).*Quantifying the future burden of cancers preventable by diet and physical activity in Australia.*Retrieved from https://www.mja.com.au/journal/2012/196/5/estimating-future-burden-cancers-preventable-better-diet-and-physical-activity.

Bahrndorff, S., Rangstrup-Christensen, L., Nordentoft.S., and Hald, B. (2013).Foodborne Disease Prevention and Broiler Chickens with Reduced *Campylobacter* Infection. *Emerging Infectious Diseases*, 19(3):425-430.

Bauer, A. W., Kirby W. M. M., Shrris, J. C., and Truck, M. (1966).Antibiotic susceptibility testing by a standardized single disc method.*American Journal of Clinical Pathology*. 45: 493-496.

Bennett, P. N., and Laurence, D. R. (1989) Chemotherapy of Bacterial Infections. *Clinical Pharmacy* (6th Ed.) Churchill Livingstone Med. Div. Longman Group U.K, Pp. 193.

Beriain, M. J., Sánchez, M. and Carr, T. R. (2009). A comparison of consumer sensory acceptance, purchase intention, and willingness to pay for high quality United States and Spanish beef under different information scenarios. *Journal of Animal Science,* 87(10): 3392-3402.

Berktaş, M. , Uzun, K. , Bozkurt, H. , Kurtoglu, M.G. , Guducuoglu, H. , Aydin, S. (2013)Pulmonary infection of Tatumella ptyseos developed on the background of pulmonary tuberculosis. *East Journal of Medicine*,**6**(1):33–4.

Bhandare, S. G., Sherikarv, A. T., Paturkar, A. M., Waskar, V. S., and Zende, R. J. (2007) A comparison of microbial contamination on sheep/goat carcasses in a modern Indian abattoir and traditional meat shops. *Food Control,*18: 854-868.

Bhargava, K., Wang, X., Donabedian, S., Zervos, M., de Rocha L., and Zhang, Y. (2011). Methicillin-resistant *Staphylococcus aureus* in retail meat, Detroit, Michigan, USA. *Emerging Infectious Diseases*, 17 (6): 1135–1137.

Bhaskaran, S., Polonsky, M., Cary, J., and Fernandez, S. (2006). Environmentally sustainable food production and marketing.*British Food Journal*, 108(8): 677-690.

Bichi, A. H., Mukhtar, M. D. and Abdulkarim, B. Z. (2002) Bacteriology of Drinking Water in Kano Nigeria *International Journal of Life and EnvironmentalScience,*4(2): 214 – 220.

Blake, D. P., Humphry, R. W., Scott, K. P., Hillman, K., Fenlon, D. R., and Low, J. C. (2003). Influence of tetracycline exposure on tetracycline resistance and the carriage of tetracycline resistance genes within commensal *Escherichia coli* populations. *Journal of Applied Microbiology*,94: 1087-1097.

Boles, J. A., Rathgether, B. M. and Shand, P. J. (2000).*Staphylococcus* in salted meat product.*Journal of Meat Science* 55**:** 22-231.

Boothe, D. H., and Arnold, J. W.Y (2003). Resistance of bacterial isolates from poultry products to therapeutic veterinary antibiotics. *Journal of Food Protection*,66:94-102.

Bradeeba, K., and Sivakumaar P. K. (2013).Assessment of microbiological quality of beef, mutton and pork and its environment in retail shops in chidambaram, tamil nadu. *International Journal of Plant, Animal and Environmental Sciences,* 3:94.

Brown, E. A. (2005). *Benson's Microbiological Applications Laboratory Manual in General Microbiology*, (9th Ed.) McGraw-Hill Companies, Inc., New York, pp. 230-280.

Bryan, A., Shapir, N., and Sadowsky, M.J., (2004). Frequency and Distribution of Tetracycline Resistance Gene Genetically Diverse, Non selected, and Non clinical *Escherichia coli* Strains Isolated from Diverse Human and Animal Sources. *Applied Environmental Microbiology*, 70 (4):2503, 2507.

Burgos, J. M., Ellington, B. A., and Varela, M. F. (2005).Prevalence of Multi-Drug Resistant Enteric bacteria in Dairy farm Topsoil.*Journal of Dairy Science*,88:1391-1398.

Butzler, J. P. (1984).*Campylobacter infections in Man and Animals.*CRC Press, Boca Raton, FL., USA.

Center for Disease Control (CDC). (2008). National Antimicrobial Resistance Monitoring System (NARMS): Enteric Bacteria; Human Isolates Final Report.

Center for Disease Control and Prevention (2011).*Escherichia coli O157:H7 and other Shiga toxin-producing Escherichia coli (STEC).* Available from: http://www.cdc.gov/nczved/divisions/ dfbmd/diseases/ecoli_o157h7

Centers for Disease Control and Prevention (CDC) (2004). Diagnosis and management of foodborne illnesses: A primer for physicians and other health care professionals, MMWR 53:RR-4.

Chapman, J. S., (1998). Characterizing bacterial resistance to preservatives and disinfectants. *International Journal of Biodeterioration and Biodegradation,*41**:**241-245.

Cheesbrough, M. (1984).*Medical Lab. Manual for Trop. Countries* Vol. 2 the Thetford Press Ltd.

Cheesbrough M. 2004. District laboratory practice in tropical countries.Part 2.p. 62-70 Cambridge University Press, Great Britain.

Cheesbrough, M. (2009**),** Microbial test in: *District Laboratory Practice in Tropical Countries Part 2*. Cambridge University Press, London. Pp 1-197.

Chryssohoidis, G. M., and Krystallis, A. (2005). Organic consumer's personal values research: testing and validating the list of values (LOV) scale and implementing a value based segmentation task. *Food Quality and Preference, 16*(7): 585-599.

Cleveland, J., Montville, T.J., Nes, I. F., and Chikindas, M. L. (2001). Bacteriocins: safe, natural antimicrobials for food preservation. *International Journal of Food Microbiology,* 71:1-20.

Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS) (2014): Performance standards for antimicrobial susceptibility testing. 17th Informational Supplement document M100-S17: 1. Wayne, Pennsyslvania, pp: 32-50.

Codex Alimentarious Commission (1997). Hazard Analysis and Critical Control Point (HACCP) System and Guidelines for Its Application. Codex Alimentarious Commission. Food Hygiene Basic Texts, Secretariat of the Joint FAO/WHO Food Standards Programme. Rome: Food and Agriculture Organization of the United Nations.

Collee, J. G., Stainer, and Duguid, J. P. (1989) *Enterobacteriaceae* In: Practical Med Microbiology (13th Ed.) Churchill Livingstone Med. Div. Longman Group U.K Pp. 110.

Conter, M., Paludi, D., Zanardi, E., Ghidini, S., Vergara, A., and Lanieri, A. (2009). Characterization of antimicrobial resistance of foodborne *Listeria monocytogenes*.*Int. Journal of Food Microbiology* 128**:**497-500.

Courvalin, P. (2005). Antimicrobial drug resistance: prediction is very difficult, especially about the future. *Emerging Infectious Diseases,*11(10).

Curtis, K. R., Cowee, M. W., Lewis, S. R. and Harris, T. R. (2007).*Consumer Preferences for Meat Attributes*. Retrieved from www.ag.unr.edu/uced/reports/technicalreports/fy2006_2007/2006_07_13.pdf.

Davis, C. R., Wingfield, D. L., Peak, K. K., Veguilla, W., Amuso, P. T., Cannons, A. C., and Cattani J. (2007). Molecular characterization of *Vibrio parahaemolyticus* strains associated with foodborne illness in Florida. *Journal of Food Protection,* 70:2396-2401.

Davis, C., Yen, S., and Lin, B. H. (2007). Does Consumer Knowledge Affect Meat Consumption in the US, paper presented at Annual Meeting of Southern Agricultural Economics Association, Alabama, February 4-7.

De Paula C. M. D., Geimba M.P., do Amaral P.H., Tondo E.C. (2010). Antimicrobial resistance and pcr-ribotyping of *Shigella* responsible for foodborne outbreaks occurred in Southern Brazil. *Brazilian Journal of Microbiology,*41**:**966-977.

Dineen, P., Emori, T. E., and Harley, R. N. (1999).Effects of Smoked Meat.*Food Preservation Journal* 69:25.

Deborah, M., Gascoyne, B., Peter, M. H., Neil, T., Lau, J., Lewis, and Cliff, B. (1995). MolecularEpidiemiologyAntibiotic Resistant Enterobacteria.*In Biology Abstract* 100:1995.

Dodds, B., and Gillies, D. (1984).*Bacteriology Illustrated* (5th Ed.) Churchill Livingstone's, New York Pp. 2 – 89.

Dorner, J. W., Cole, R. J. (2002). Effect of application of nontoxigenic strains of *Aspergillus flavus* and *A. parasiticu*s on subsequent aflatoxin contamination of peanuts in storage. *Journal of Stored Produce Research,*38**:**329-339.

Drudy, D. Mullane, N. R. Quinn, T. Wall, P. G. and Fanning S. (2006).*Enterobacter sakazakii:* An Emerging Pathogen in Powdered Infant Formula. *Food Safety,*42:998

Duguid, J. P. (1968) The Genus *Proteus.* In: Jean, F.M. (1976) Ed. *Biochemical Tests for Medical Bacteria.* Williams and Wilkins Co. Baltimore, Pp. 194.

Earnest, J., Joseph. L., and Melnik, E. A. (1987) The Family *Enterobacteriaceae. Review of MedicalMicrobiology.*(17th Ed.)Appleston and Lange Med. Pub.Norwak, Pp. 232 – 45.

Edema, M. O., Osho A. T., and Diala, C. I. (2008). Evaluation of microbial hazard associated with processing of suya (a grilled meat product). *Scientific Research and Essay*, 3:(12) 621-626.

Ekundayo, O.S. (2011). *Bacterial load and sources of Salmonella contamination of cattle carcass and beef from Bodija abattoir, Ibadan.(Unpublished master's thesis).* Ahmadu Bello University, Zaria, Nigeria.

El-Sam, S., Linton, A. H., Bennett, P. M., and Hinton, M .(1993). Effects of low concentrations of ampicillin on the intestinal *Escherichia coli* of chickens.*Journal of Applied Bacteriology,* 75: 108- 112.

Esteban, J. I., Oporto, B., Aduriz, G., Juste, R. A., and Hurtado, A. (2008).A survey of food-borne pathogens in free-range poultry farms.*International Journal of Food Microbiol*ogy, 123(1-2):177- 182.

Exner, and Martins (1995) Infections Disease from the Hygienic View with Special Reference to Infectious Disease from the Environment *Journal of Environmental Microbiology,* 197(1 – 3): 134 – 61.

Fakolade, P. O., and Omojola, A, B. (2008).Proximate composition, pH and microbiological evaluation of "kundi" (dried meat) product from beef and camel meat.Tropeng. University of Hohenheim, October 6-9, 2008. Conference on International Research on Food Security, Natural Resource Management and Rural Development. 2008.

Farzana K, Akhter S, Jabeen F (2009). Prevalence and antibiotic resistance of bacteria in two ethnic milk based products. *Pakistan Journal of Botany,*41: 935-943.

Foley, J., Lynne, A. M. (2008). Food animal associated *Salmonella* challenges: Pathogenicity and antimicrobial resistance. *Journal of Animal Science,* 86**:**173-187.

Food and Agricultural Organization (FAO) (1992).*Meat Quality*.Retrieved from http://www.fao.org/ag/againfo/themes/en/meat/quality_meat.html.

Food and Agricultural Organization (FAO) and World Health Organization (WHO).(1999). *Antimicrobial Resistant Bacteria in Food.*Report of Codex Committee on Food Hygiene.Washington DC, U.S.A.Pp. 1.

Food and Agricultural Organization (FAO) (2013).*Meat Quality*.Retrieved from http://www.fao.org/ag/againfo/themes/en/meat/quality_meat.html.

Food and Agricultural Organization (FAO) (2014).*The role of livestock in climate change*. Retrieved from http://www.fao.org/agriculture/lead/themes0/climate/en/

Fortomaris, P., Arsenos, G., Georgiadis, M., Banos, G., Stamataris, C., and Zygoyiannis, D. (2006).Effect of meat appearance on consumer preferences for pork chops in Greece and Cyprus.*Meat Science*, 72**:**688–696.

Fraiser C.W. Westholff C.D. (2001). Pathogens in meat and meat-borne illnesses.*Food Microbiology.4th Edition* . McCraw Hill Book Company, U.S.A. Pp. 401-411.

Frank, C., Faber, M. S., Askar, M., Bernard, H., Fruth, A., Gilsdorf, A., … Werber D. (2011).*Large and ongoing outbreak of haemolytic uraemic syndrome,* Germany, Euro Surveill. 16(21):pii=19878. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19878.

Franz, C. M., Holzapfel, W. H., Stiles, M .E. (1999). *Enterococci* at the crossroads of food safety *International Journal Food Microbiol*ogy,47:1–24.

Franz, E., van Bruggen, A. H. C. (2008). Ecology of *E. coli* O157:H7 and *Salmonella enterica* in the primary vegetable production chain. *Critical Reviewed of Microbiology,*34:143-161.

Fratamico, P. M., Bhunia, A. K., and Smith, J. L. (2005).*Foodborne pathogens in Microbiology and Molecular Biology*, Caister Academic press, Wymondham Norfolk, UK. Pp. 270-275.

Galanis, E., Lo Fo Wong, Patrick, M. E., Binsztein, N., Cieslik, A., and Chalermchikit T. (2006). Web-based surveillance and global *Salmonella* distribution 2000-2002. *Emerging of Infectious Diseases,* 12:381-388.

Gantois, I., Ducatelle, R., Pasmans, F., Haesebrouck, F., Gast, R., Humphrey, T. J., Van Immerseel F. (2009). Mechanisms of egg contamination by *Salmonella enteritidis*. FEMS *MicrobiologyReview,*33:718-738.

Gashaw, A., Kassu, A., Moges, F., Tiruneh, M., and Huruy K. (2008) Prevalence of bacteria and intestinal parasites among food handlers in Gondar Town, *NorthwestEthiopia Journal of Health Population Nutrition,* 26:451-455.

Gibreel, A., and Taylor, D. E. (2006).Macrolide resistance in *Campylobacter jejuni* and *Campylobacter coli*. *Journal of Antimicrobial Chemotherapy,*58:243-255.

Gilbert, D. N., Moellering, R. C., Eliopoulos, G. M, and Sande, M. A. (2004).*The Sanford Guide to Antimicrobial Therapy*. (34th Ed.) Antimicrobial Therapy Inc., Hyde Park, VT.

Gilbert, P., McBain, A. J. (2003). Potential impact of increased use of biocides in consumer products on prevalence of antibiotic resistance.*Clinical Microbiology Reviewed,* 16(2):189-208.

Gill, C. O. (2005). Sources of microbial contamination a slaughtering plants. In: (J. N. Sofos, Ed.) *Improving the safety of fresh meat,* , Cambridge, UK: *CRC/Woodhead Publishing Limited*, pp. 231–243

Gill, C.O., and Molin, G. (1991). Modified atmospheres and vacuum packaging, In (Russell, N.J., and Gould, G.W., Eds) *Food Preservatives,* Blackie. Glasgow. pp. 172-99

Gracia, A., and de-Magistris, T. (2013). Preferences for lamb meat: A choice experiment for Spanish consumers. *Meat Science,* 95(2): 396–402.

Griggs, D. J., Hall, M. C., Jin, Y. F., and Piddock, L. J. V. (1994).Quinolone resistance in veterinary isolates of *salmonella*.*Journal of Antimicrobial Chemotherapy,* 33: 1173-1189.

Gyles, C. L. (2006). Shiga toxin-producing *Escherichia coli*: an overview. *Journal of Animal Science* 85**:**45-62.

Haman, D.O. (1997). Haemorrhagic cystitis and balantitis associated with verotoxin producing *Escherichiacoli* O157: H7. Lancetii: 150. *Microbiology of Meat Food Technology* 23(6):66-71.

Hart, C. A. and Kariuki, S. (1998).Antimicrobial resistance in developing countries. *Biomedical Journal,* 317:647-650.

Hayes, J. R., Enghish, L. L., Carter, P. J., Proescholt, T., Lee, K. Y., Wagner, D. D. and White, D. G. (2003). Prevalence and antimicrobial resistance of *Enterococcus* Species isolated from retail meats. *Applied and Environmental Microbiology,* pp.7153-7160.

Hedrick, H. B., Aberle, E. D., Fore, Y. C., and Merkel, R. A. (1994).*Principles of meat science*.3rd Kendall Hunt Publishing *D*. Ugugue Iowa.

Hilde – Kruse, and Hening, S. (1994) Bacterial Pathogens Resistant to Antimicrobial Agents in the Environment. *Journal of Applied and Environmental Microbiology,* 60(11): 401.

Hug. (1980) Multiple Antibiotic Resistance in Bangladesh.*Bangladesh Medical Researches Council Bull,* 4:126.

ICMSF, (1996).International Commission on Microbiological Specifications for Foods Microorganisms in Foods 5.Characteristics of Microbial Pathogens. 1st Edn., Aspen Publishers. Chapter 14, pp 218

Igene, J. O., and Ekanem, E. O. (1985).Effect of processing method on the nutritional and sensory characteristics of tsire-type suya meat products.*Nigeria Journal of Applied Science,* 3:1-20.

Igene, J. O., Farouk, M. M., and Akanbi, C. T. (1990).Preliminary studies on the traditional processing of Kilishi.*Journal of Science Food Agriculture,*50: 89-98.

Igene, J.O. and Mohammed, I.D., (1983). Consumers' attitudes towards '*'Suya'* ' meat product.*Tropical Veterinary Medicine Journal,* 1: 85-91.

Indakwo, P. and Abu, G. O. (2004) Distribution and Statistical analysis of bacteria in lake Alau in Northern Nigeria. *Journal of Applied Science and Environ. Management,* 8 (1): 5 – 9.

Ingham, J. (2001). Food Facts for You Http//:Uwex.edu/Ces/flp/specialist/ingham.htlm.

Inistitute of Food Technology (IFT).2002a. Resistance and adaptation to food antimicrobials, sanitizers, and other process controls. A Scientific Status Summary of the Institute of Food Technologists, Chicago, Ill. Davidson PM, Harrison MA, authors. *Food Technology,* 56:69-78.

International Commission on Microbiological Specifications of Foods (ICMSF, 1978). Microorganisms in Foods 1:110-117. University of Toronto Press, Canada.

International Commission on Microbiological Specifications for Foods (ICMSF) (1986). Microorganisms in food sampling for microbiological analysis: Principle and specific application 2nd Edition. Blackwell Scientific Publications Pp139-140; 213-215.

International Organization for Standardization (ISO) (1996). ISO 7218 Microbiology of food and animal feeding stuffs – General rules for microbiological examinations. International Organization for Standardization, 1, rue de Varembé, Case postale 56 CH-1211 Geneva 20, Switzerland.

International Organization For Standardization (ISO) (1999). ISO 6887-1: Microbiology of food and animal feeding stuffs – Preparation of test samples, initial suspension and decimal dilutions for microbiological examination – Part 1: General rules for the preparation of the initial suspension and decimal dilutions. International Organization for Standardization, 1, rue de Varembé, Case postale 56 CH-1211 Geneva 20, Switzerland.

Inyang, C. U., Inyor, M. A., and Uma, E. N. (2005).Bacteriological quality of a smoked meat product ("suya").*Nigerian Food Journal,*23: 230-242.

Jacob, H. M., Herbert, M., Gillies, D. and Luois, A. (1970).*Micro Organisms of Medical Importance.*A Short Textbook of Preventive Medicine for the Tropics.

Jay, J. M. (2002). *'Suya'* in West African Recipes.*Modern food microbiology*. 12**:**15-20.

Jazrawi, and Hindawi, A. K. (1986) Pollution Indicator Bacteria *Journal of Biological Science Researches,* 17**:**31.

Jean, F. M. (1976).*Biochemical Tests for Identification of Medical Bacteria*. The Williams and Wilkins Co. Baltimore, Pp 15 – 31.

Jimenez S.M, Tiburzi M.C, Salsi M.S, Pirovani M.E, Moguilevsky M.A. (2003) The role of visible faecal material as a vehicle for generic Escherichia coli, coliform, and other enterobacteria contaminating poultry carcasses during slaughtering. *Journal of Applied Microbiology*; **95**(3):451–6.

Joint Expert Technical Advisory Committee on Antibiotic Resistance (JETACAR).1999.*The use of antibiotics in food-producing animals.*Report of the Joint Expert Technical Advisory Committee on Antibiotic Resistance (JETACAR). Canberra, Australia. Pp 42.

Judge, D. M., Robert, A. M., and Morris, M. J. (2002).Preparation of suya in Africa.*Journal of African Foods***,** 20:52-55.

Kalalou, I., Faid, M., and Ahami, A. T. (2004).Extending the shelf life of fresh minced camel meat at ambient temperature by *Lactobacillusdelbruekii* subsp. *delbruekii*.*Electronic Journal of Biotechnology.* 7:246-251

Kearney, J. (2010). *Food consumption trends and drivers*. Retrieved from http://rstb.royalsocietypublishing.org/content/365/1554/2793,

Kendal, H. (1980) Resistance of *Yersinia enterocolitica* to Antibiotics.*Journal of TropicalMedicine and Hygiene,*161(1994).

Klerks, M. M., Franz E., van Gent-Pelzer, M., Zijlstra, C., and van Bruggen, A. H. C. (2007). Differential interaction of *Salmonella enterica* serovars with lettuce cultivars and plant-microbe factors influencing the colonization efficiency. *International Society of Microbiology and Ecology Journal,*1:620-631.

Koenraad, P. M., Rombouts, F. M., and Notermans, S. H. W. (1997). Epidemiological aspects of thermophilic *Campylobacter* in water-related environments: a review. *Water Environment Researches,*69:52

Kokkinakis, E. N., and Fragkiadakis, G. A. (2006). HACCP effect on microbiological quality of minimally processed vegetables: a survey in six mass-catering establishments. *International Journal Food science technology,* 42:18-23.

Koutsoumanis, K. P., and Sofos, J. N. (2004) Microbial contamination of carcasses and cuts. *In Encyclopedia of Meat Sci.* (Jensens, W.K., Ed.). Amsterdam: Elsevier Academic Press. pp. 727–737

Kramiliah, W. E., Pearson, A. M., and Tauber, F. (1973).*Processed meat*. AV Pul. Co Inc. West Pork. pp. 331-341

KwaZulu-Natal Department of Health (2001).*Nutrition and ageing*.Retrieved from http://www.kznhealth.gov.za/nutrition/ageing.htm.

Lambert, A. D., Smith, J. P., and Dodds, K. L. (1991).Shelf life extension and microbiological safety of fresh meat - A review.*Food Microbiology,* 8: 267-297

Latvala, T., Niva, M., Mäkelä, J., Pouta, E., Heikkilä, J., Kotro, J. & Forsman-Hugg, S. (2012). Diversifying meat consumption patterns: consumers' self-reported past behaviour and intentions for change. *Meat Science*, 92(1):71-77.

Lawries, R. A. (2001).*Microbiology Growth in Meat*.Meat Science 6th Edition. Pergoman Publishing Competition, Switzerland. Pp.43-49.

Lee, J. H. (2006). Occurrence of methicillin-resistant *Staphylococcus aureus* strains from cattle and chicken, and analyses of their mecA, mecR1 and mecI genes. *Veterinary Microbiol*ogy,113:137-141.

Levin, B. R. (2007). The population genetics of antibiotic resistance.*Clinical Infectious Diseases*,**2**4 :(Suppl. 1), S9–S16.

Lijalem, T., Beyan, M., and Banerjee, S. (2015).Quality of beef, chevon and mutton at Hawassa, Southern Ethiopia.*African Journal of Food Science*.9(5):301-306

Linscott, A. J. (2011). Foodborne illnesses.*Clinical Microbiology,Newsletter* 33(6):41-46.

Liu, F., Kariyawasam, S., Jayarao, B. M, Barrangou, R., Gerner-Smidt, P., Ribot, E.M., ... Dudley E.G. (2011). Subtyping *Salmonella enterica* Serovar Enteritidis isolates from different sources by using sequence typing based on virulence genes and clustered regularly interspaced short palindromic repeats (CRISPRs). *Applied and Environmental Microbiology* 77:4520-4526.

Luois, O. (1987) *Gastroenteristis Medical Dictionary*. Butter worth Publishers London.

Magnus, C. A., Ingledew, W. M., Mccurdy, A. R. (2008). Thermal resistance of *Streptococci* isolated from pasteurized ham. *Canada Institute of Food Science Technology Journal,* 19:62–67.

Maimuna, A., and Arzai, A. H. (1996).Effects of Some Disinfectants on Bacteria.Deptment of Biological Sciences.Bayero University, Kano, Nigeria.

Mare, I. J. (1998). Incidence of R factors amongst Gram-negative bacteria in drug free human and animal communities. *Nature,* 22:1046-1047.

Marie, C. N., and Fernando, B. C. (1997) Molecular Biology of Antibiotic Resistance in Enterobacteria. *Biology Essay,* 19(8): 736.

Martin, B., Garriga, M., Hugas, M., and Aymerich, T. (2005). Genetic diversity and safety aspects of *Enterococci* from slightly fermented sausages. *Journal of Applied Microbiology,*98:1177-1190.

Martinez, S., Hanagriff, R., Lau, M. and Harris, M. (2007).*Factors affecting demand for branded beef*. Paper presented at the 39th Annual Meetings Program Southern Agricultural Economics Association Mobile, United States.

May, R. D., Margesin, R., Klingbichel, E., Harhugen, E. D., Yenewe, D., Schiner, F., and Mark, I. T. (2003). Rapid detection of meat spoilage by measuring volatile organic compounds by using proto transfer reaction mass spectrophotometry.*Applied Environmental Microbiol*ogy, 69:4697-4705.

McEntire, J. (2004). Bacteria associated with foodborne diseases, scientific status review, IFT (Institute of Food Technologists), 34(6): 388-392

Mead, P. S., Slutsker, L., Dietz, V., McCaig, L. F., Bresee, J. S., Shapiro C., and Tauxe R.V. (1999).Food-related illness and death in the United States.*Emerging of Infectious Diseases*,5:607-625.

Mesa, R. J., Blanc, V., Blanch, A. R., Cortés, P., González, J. J., Lavilla, S. and Tórtola M.T. (2006). Extended-spectrum β-lactamase-producing *Enterobacteriaceae* in different environments (humans, food, animal farms and sewage).*Journal of Antimicrobial Chemotherapy*, 58:211-215.

Michino, H., Araki, K., Minami, S., Takaya, S., Sakai, N., Miyazaki, M. and Yanagawa H. (1999). Massive outbreak of *Escherichia coli* O157:H7 infection in school children in Sakai City Japan associated with consumption of white radish sprouts. *Antimicrobial Journal of Epidemiology*,150: 787-796.

Ministry of Economic Development (1996). Kaduna State Statistical year book, Kaduna Nigeria Ministry of Economic Development, Statistics Division

Mølbak, K. (2005). Human health consequences of antimicrobial drug resistant *Salmonella* and other foodborne pathogens. *Clinical Infectious Diseases*,41:1613-1620.

Moloney, A. P. Teagasc, and Dunsany. (2002). *The fat content of meat and meat products*. Retrieved from http://www.enq.ufsc.br/disci/eqa5217/material_didatico/MEAT_PROCESSING/1539_ch07.pdf

Momoh, O. M. and Ochaba, A. O. (2002). Herd Structure of small holder goat production in Okukpo L.G.A of Borno state, Nigeria. *Tropical Journal of Animal Science*, 5: 53-57.

Morvan, A., Moubareck, M., Leclercq, A., Herve'-Bazin, M., Bremont, S., Lecuilt, M. and Le Monnier A. (2010). Antimicrobial resistance of *Listeria monocytogenes* strains isolated from humans in France. *Antimicrobial Agents of Chemotherapy*,54: 2728-2731.

Mukhtar, M. D. and Oyeyi, T. I. (2005) Drug Resistant Enetrobacteria in Drinking Water sources of some local government areas in Kano Nigeria. *African Scientist*,4(3): 115 – 120.

Mukhtar, M. D., Oyeyi, T.I. and Kawo, A.H. (2001) Enterobacteria infections in *Journal of Kano State.*The Nigerian Society of Experimental Biology NISEB, 1(1): 61 – 70.

Nachamkin, I. (2003). *Campylobacter* and *Arcobacter*. In: *Manual of Clinical Microbiology*, 8th ed. ASM Press, Washington, DC. pp. 902-914.

Nachamkin, I., Engberg, J., Aarestrup, F. M. (2000). Diagnosis and antimicrobial susceptibility of *Campylobacter* spp. In: Nachamkin I. Blaser MJ, eds. *Campylobacter*, 2nd edn. Washington. *American Society of Microbiology*, pp. 45- 66.

Nakai, S., Modler, and W. H. (2000).*Food protein*, 1st ed. Wiley-VCH, Inc., New York, U.S.A. pp. 128-133.

Neal, P. (1995) Enterobacteria Subvert the Biology of Their Host Cells. *Elsevier Scientific Publishers Ireland.*

Nester, E. W., Aderson, D. G., Roberts, C. E., Pearsall, N. N. and Nester, M. T. (2001). Microbiology; A Human Perspective. Third Edition. McGraw Hill Company, U.S.A: Pp. 822- 809.

Nigeria Primary Healthcare (PHC) Standing Order (1995).Fedederal Mininistry of Health Office of the Minister. Kano State Ministry of Health (1996) Statistics on Enteric Infections and the Control Strategies (1995Reports), Pp 1 – 40.

Nikmaram, P., Said, Y. M., and Emamjomeh, Z. (2011). Effect of cooking methods on chemical composition, Quality and cook loss of camel muscle (Longissimus dorsi) in comparison with veal.*African Journal of Biotechnology*,10(51):10478-10483.

Nordman, P., Poirel, L., and Dortet, L. (2012).Rapid Detection of Carbapenemase-producing *Enterobacteriaceae*.*Emerging of Infectious Diseases,*18(9):1503-1507.

Nychas, G. E., Skandamis, P. N., Tassou, C. C., and Koutsoumanis, K. P. (2008). Meat spoilage during distribution.*Meat Science,* 78: 77–89

Nychas, G-J.E., Marshall, D., and Sofos, J. (2007).Meat poultry and seafood. In: (Doyle, M.P., Beuchat, L.R, and Montville, T.J., eds.). *Food Microbiology Fundamentals and Frontiers*. ASM press, USA

Nyenje, M. E., Odjadjare, C. O., Tanih, N. F., Green, E., and Ndip, R. N. (2012b). Foodborne pathogens recovered from ready-to-eat foods from roadside cafeterias and retail outlets in Alice, Eastern Cape Province, South Africa: Public health implications. *International Journal of Environmental Research andPublic Health* **9:**2608-2619.

Nyenje, M. E., Tanih, N. F., Green, E., and Ndip, R. N. (2012a). Current status on antibiogram of *Listeria ivanovii* and *Enterobacter cloacae* isolated from ready-to-eat foods in Alice, South Africa: a cause for concern. *International Journal of Environmental Research andPublic Health* 9:3101-3114.

Odusole, K. A, and Akinyanju, O. O. (2003).Red "suya" syndrome – acute intravascular IeAdminstration and control.*Consumer safety bulletin*. 2(2): 20-24.

Ogbeide, O. A. (2013). *Consumer willingness to pay premiums for the benefits of organic wine and the expert service of wine retailers*, PhD thesis, The University of Adelaide. http://digital.library.adelaide.edu.au/dspace/handle/2440/83363.

Ogbeide, O. A., Ford, C. and Stringer, R. (2014).*The Environmental Benefits of Organic Wine: Exploring Consumer Willingness-to-Pay Premiums*? Retrieved from http://dx.doi.org/10.1080/10454446.2013.856054.

Ogbeide, O. A., Stringer, R. and Ford, C. (2014). Are Australian wine consumers willing to pay for the expert service of wine retailers? Retrieved from http://dx.doi.org/10.1080/09571264.2014.917617.

Ogbonna, I. O., Danladi, M. S., Akinmusire, O., and Odu, C. E. (2012).Microbiological Safety and Proximate Composition of Suya Stored at Ambient Temperature for Six Hours from Maiduguri, Northern Nigeria. *Internet Journal of Food Safety*, 14: 2012, p.11-16

Okunlola, O. O. (2012). Meat Preference and Meat Consumption Pattern of South-western Nigeria: *Journal of Agriculture, Forestry and the Social Sciences*, 10(1): 23-31.

Olaitan, J. O., Shittu, O. B, and Akinliba, A. A. (2011). Antibiotic resistance of enteric bacteria isolated from duck droppings. *Journal of Applied Biological sciences* 45:3008-3018.

Olaoye, O. A., and Idowu, O. A. (2010). Features and functional properties of lactic acid bacteria used as biological preservatives of meat processing: A review article. *Journal of Agricultural Technology,* 6: 449-460.

Olsen, S. J., MacKinnon, L. C., and Goulding, J. S. (2001).*Surveillance for foodborne disease outbreaks—United States, 1993-1997*. MMWR 49:1-11.

Omojola, A. B. (2008). Yield and Organoleptic Characteristics of Suya (an intermediate moisture meat) prepared from three different muscles of a matured bull. *African Journal of Biotechnology*, 7(13):2254-2257.

Oporto, B., Esteban, J. I., Aduriz, G., Juste, R. A., and Hurtado, A. (2007). Prevalence and strain diversity of thermophilic campylobacters in cattle, sheep and swine farms," *Journal of Applied Microbiology,*103(4):977-984.

Oporto, B., Ramon, A. J., and Hurtado, A. (2009). Phenotypic and genotypic antimicrobial resistance profile of *Campylobacter jejuni* isolated from Cattle, Sheep, and Free-Range Poultry faeces. *International Journal of Microbiology*, 456573.

Osho, A.T. (2004): *Evaluation of bacterial contamination of grilled meat (Suya) processed in Abeokuta.*Undergraduate research project.Development of Nutrition and Dietetics. University of Agriculture Abeokuta. Pp. 49

Paige, J.C., Chaudry, M. H., and Pell, F. M. (1999).Federal surveillance of veterinary drugs and chemical residues (with recent data).*Veterinary Clinical North American Food Animal Practices,*15:45-61.

Paulsen, P., Smulders, F. J. M. (2003). Combining natural antimicrobial systems with other preservation techniques: the case of meat. In: *Food preservation techniques* (Zeuthen, P., and Bùgh-Sùrensen, L., eds.). Woodhead Publishing Ltd, Cambridge, England and CRC Press Boca Raton, New York, Washington DC. pp 71-85

Pearson, D. (1976). *The chemical analysis of foods* (7th ed.). Churchill Livingstone, London. Pp. 6-25.

Pelczar, M. J., Jr Chan, E. C., and Nee, R. (1993) *Microbiology International Edition.* Machraw Hilling. New York. Pp. 806 – 833.

Permentier, L., Maenhout, D., Broekman, K., Deley, W., Van de Perre1, V., Verbeke, G. and Geers, G. (2013).Comparison of Growth Performance, Body Composition, Body Conformation and Meat Quality between Three Genetic Pig Lines.*The Open Agriculture Journal*, ***7*:** pp. 96-106.

Pezzotti, G., Serafin, A., Luzzi, I., Mioni, R., Milan, M., Perin R. (2003).Occurrence and resistance to antibiotics of *Campylobacter jejuni* and *Campylobacter coli* in animals and meat in northeastern Italy.*International Journal of Food Microbiology,* 82:281-287.

Phillips, I., Casewell, M., Cox T., De Groot, B., Friis, C., Jones, R. and Waddell, J. (2004). Does the ruse of antibiotics in food animals pose a risk to human health? A critical review of published data.*Journal of Antimicrobial Chemotherapy*, 53:28-52.

Pikkemaat, M. G., Rapallini M. L. B. A., Zuidema, T., Elferink, J. W. A., Oostra-van Dijk, S., Driessen-van Lankveld, W. D. M. (2011). Screening methods for detection of antibiotic residues in slaughter animals: comparison of the EU-four plate method, the Nouws Antibiotic Test and the Premi®Test (applied to muscle and kidney). *Journal of Food Additives and Contaminants*; DOI:10.1080/19440049.2010.535027.

Podpečan B, Pengov A, and Vadnjal S (2007).The source of contamination of ground meat for production of meat products with bacteria *Staphylococcus aureus*.*Veterinary Research* 44: 25-30.

Podschun, R. Fisher, A. and Ullmann, U. (2016). Characterization of *Hafnia alvei* isolates from human clinical extra-intestinal specimens: Haemagglutinins, serum resistance and siderophore synthesis. *Journal of Medical Microbiology*, 50:208-2014

Public Health Laboratory Service (2000): *Communicable Diseases and Public Health* 3: 3.

Pushparajan, N., Soundarapandian, P., and Varadharajan, D. (2012) Proximate Composition of Fresh and Prepared Meats Stored in Tin Free Steel Cans. *Journal of Marine Science Research and Development.* 2:113pp 4

Russell, A. D., (1991). Mechanisms of bacterial resistance to non-antibiotics: food additives and food and pharmaceutical preservatives. *Journal of Applied Bacteriology,*71:191-201.

Russell, A. D. (1997). Plasmids and bacterial resistance to biocides. *Journal of Applied Microbiology,* 83(2):155- 165.

Russell, A. D. (2001). Mechanisms of bacterial insusceptibility to biocides.*American Journal of InfectiousControl* 29:259-61.

Safdar, A., and Armstrong, D. (2003). Antimicrobial activities against 84 *Listeria monocytogenes* isolates from patients with systemic listeriosis at a Comprehensive cancer centre (1955-1997). *Journal of Clinical Microbiology,*41:483-485.

Saghaian, S. H., Maynard, L. J., and Reed, M. R. (2006). Contributed paper presented at the International Association of Agricultural Economists Conference, Gold Coast, Australia, August 12-18.

Sameer, F. (1988).*Pollution – Indicator Bacteria in Drinking Water.Water, Air and Soil Pollution* 39:1988 Khumer Publishers.

Samuel, L., Marian, M. M, Apun K., Lesley, M. B, and Son, R. (2011). Characterization of *Escherichia coli* isolated from cultured catfish by antibiotic resistance and RAPD analyses. *International Food Reseaches Journal,*18(3):971-976.

Sartz, L. B., Jong, B., Hjertqvist, M., Forshell, P. L., Alsterlund R., Fdahl S. L. and Karpman, D. (2008). An outbreak of *Escherichia coli* O157:H7 infection in southern Sweden associated with consumption of fermented sausage; aspects of sausage production that increase the risk of contamination. *Epidemiology of Infections* 136: 370-380.

Savadkoohi, R. B., and Kacho A. M. (2007). Prevalence of *Shigella* species and their antimicrobial resistance patterns at Amirkola Children's Hospital, North of Iran. *Iran Journal of Paediatrics,* 17:118-122.

Schelin, J., Wallin-Carlquist, N., Cohn, M. T., Lindqvist, R., Barker G. C., and Rådström, P. (2011). The formation of *Staphylococcus aureus* enterotoxin in food environments and advances in risk assessment. *Virulence* 2:580-592.

Schmidt, R. H., Goodrich, R. M., Archer, D. L., and Schneider, K. R. (2009).*General overview of the causative agents of foodborne illness.*Food Science and Human Nutrition Department, Florida Cooperative Extension Service, IFAS, University of Florida. Publication: FSHN033 pp. 1-5.

Settepani, J. A. (2004). The hazard of using chloramphenicol in food animals.*Journal of the American Veterinary Medical Association* 184: 30- 39.

Skočková, A., Cupáková, Š., and Karpíšková, R. J. (2012). Detection of tereacycline resistance genes in *Escherichia coli* from raw caw's milk.*Food Science Biotechnology*, 1:777, 784

Skov, M. N., Andersen, J. S., Aobo, S., Ethelberg, S., Aarestrup, F. M., Sorensen, A. H., … Baggesen D.L. (2007). Antimicrobial drug resistance of *Salmonella* isolates from meat and humans, Denmark.*Emerging of Infectious Diseases,*13:638-641.

Sleigh, J. D. and Duguid, J. P. (1989).*Enterobacteriaceae*In Mackie and MacCartney (1989) *Practical Medical Microbiology.* 13th Rd. Longman.

Smith, D. L., Dushoff, J., and Morris, J. G. (2005).Agricultural antibiotics and human health. *PloS Medicine* 2(8):Pp232.

Smith, H. W. (2005). The effect of the use of antibacterial drugs, particularly as food additives, on the emergence of drug resistant strains of bacteria in animals.*New Zealand Veterinary Journal,*15: 153- 166.

Smith, D. H. (2002).R factor infection of *Escherichia coli* lyophilised in (1946).*Journal of Bacteriology* 94: 2071- 2072.

Smith, H. W. and Crabb, W. E. (2008). The sensitivity to chemotherapeutic agents of a further series of strains of *Bacterium coli* from cases of white scours: the relationship between sensitivity tests and response to treatment. *Veterinary Record* 68: 274-276.

Sofos, J. N. (2008). Challenges to meat safety in the 21st century.*Meat Science,* 78**:**3-13.

Sokori, T. J., and Anozie, S. O. (1999).Meat spoilage.*Journal of Food Production* 53 (12):1069-1072.

Stock I, and Wiedemann B. (2002). Natural antibiotic susceptibility of *Enterobacter amnigenus, Enterobacter cancerogenus, Enterobacter gergoviae* and *Enterobacter sakazakii* strains. Clinical Microbiology of Infections; 8:564–78.

Stokes, J. E., and Ridgeway, G. I. (1980).*FamilyEnterobacteriaceae.Clinical Bacteriology* 5th Ed. Edward Arnold.

Sunde, M., Fossum, K., Sloberg, A., and Sorum, H. (1998).Antibiotic resistance in *Escherichia coli* of the normal intestinal flora of swine.*Microbial Drug Resistance* 4: 289- 299.

Swann, M. M. (2009). Use of Antibiotics in Animal Husbandry and Veterinary Medicine. UK Joint Committee Report London: H.M. Stationery Office.

Tafida S.Y., Kabir J., Kwaga J.K.P., Bello M., Umoh V.J., Yakubu S.E, Nok A.J., Hendriksen R. (2012) Occurrence of *Salmonella* in retail beef and related meat products in Zaria, Nigeria. *International Journal of Food Microbiology* p120-121

Talaro K. F., and Talaro, A. E. (2006)**,** *Foundation in Microbiology*. W. M. C. Brown Publisher, Dubuque. Pp.781- 783

Tansuphasiri U., Khaminthakul, D., and Pandii, W. (2006). Antibiotic resistance of *Enterococci* isolated from frozen foods and environmental water. *Southeast Asian Journal of Tropical Medicine Public Health*, 37(1):162-170.

Tarr, P. I., Gordon, C. A., and Chandler, W. L. (2005).Shiga-toxin-producing *Escherichia coli* and haemolytic uraemic syndrome. Lancet365:1073-1086.

Teplitski, M., Wrigh, A.C., and Lorca, G. (2009).Biological approaches for controlling shellfish-associated pathogens. *Current Opinion of Biotechnology*,20:185-190.

Thrum, V. and Gericke.(1994) Nosocomial Outbreak of *CitrobacterFreundii*.*Journal of Applied Bacteriology* 1996:80.

Tsigarida, E., Nychas, G. J. E. (2001). Ecophysiological attributes of a *Lactobacillus* sp. and a *Pseudomonas* sp. on sterile beef fillets in relation to storage temperature and film permeability. *Journal of Applied Microbiology*, 90: 696–705

U. S. Department of Health and Human Services Food and Drug Administration. Center for Food Safety and Applied Nutrition (FDA/CFSAN, 2003).

Umoh, J. U. (2001): *International Conference on Food and Security*. Conference Centre Ibadan, Nigeria. Pp. 109 – 115.

Uzeh, R. E., Ohenhen, R. E., and Adeniji, O. O. (2006).Bacterial Contamination of Tsire-*Suya* a Nigerian Meat Product.*Pakistan Journal of Nutrition, 5*(5): 458-460. http://dx.doi.org/10.3923/pjn.2006.458.460

Vaillant V., De Valk H., Baron E., Ancelle T., Colin P., Delmas M.C. and Desenclos J.C. (2005). *Foodborne pathogens and disease.* 2:221-232.

Van den Bogaard AE & Stobberingh E.E .(1999). Antibiotic usage in animals.*Drugs* 58: 589-607.

Van Looveren M., Daube G., De Zutter L., Dumont J. M., Lammens C., Wijdooghe M., Goosens H. (2001). Antimicrobial susceptibilities of *Campylobacter* strains isolated from food animals in Belgium. *Journal of Antimicrobiology Chemotherapy,* 48: 235-240.

Venia F., Joana B., Sandra V., Ana, M., Fatima S., Maria J. M. and Paula T., (2006). Chemical and microbiological characterization of *alheira*: Atypical Portuguese fermented sausage with particular reference to factor relating to food safety. *Meat Science Journal,73* :(4) .570-575.

Verma, D. P. S., and Gupta, S. (2004). Does Higher Price Signal Better Quality? *The Journal for Decision Makers,* 29(2): 67-77.

Vugai D.J., samuel M., Farley M.M., Marcus R., Shiferaw B., Shallow S. (2004). Invansive *Samonella* infections in the United States, Food-net, 1996-1999: incidence, serotype distribution, and outcome. *Clinical Infectious Diseases*,38:S149-156.

Wagacha J. M., and Muthomi J. W. (2008). Mycotoxin problem in Africa: current status, implications to food safety and health and possible management strategies. *International Journal of Food Microbiology*,10:124:1-12.

Wan Muhamad, A. W. A., Wan AbdulAziz, W. M. A., Nor Azlida, A. and Norizan, M. (2012).Some Practical Guidelines for Effective Sample-Size Determination in

Observational studies.*Aceh International Journal of Science and Technology*, 1(2): 51-53

Wang, H., McEntire, J. C., Zhang, L., Li, X., and Doyle, M. (2012). The transfer of antibiotic resistance from food to humans: facts, implications and future directions. Rev *science technology Office of international Epizootics*,31 (1): 249-260.

WHO (2000).*"Cholera"*, Fact sheetno.107. http://www.who.int/mediacentre/factsheets/fs107/en/Accessed on 9/10/2012

WHO (2007).*Food safety and foodborne illnesses.* Fact sheet no. 237. http://www.who.int/mediacentre/factsheets/fs237/en/ Accessed on 9/10/2012.

WHO (2011).Enterohaemorrhagic *Escherichia coli* (EHEC).Fact sheet no.125 http://www.who.int/mediacentre/factsheets/fs125/en/.Accessed on 9/10/2012.

WHO.*International Health Regulation* (2005) WHO, Geneva (2006). http://whqlibdoc.who.int/publications/2006/9241580380_eng.pdf Accessed on 2/10/2012.

Willey, J. M., Sherwood, L. M., and Woolverton, C. J. (2007).*Prescott, Harley, and Klein's Microbiology* (7th Ed.) McGraw-Hill Companies Inc. New York. pp. 53-854.

Wood, J. D. Enser, M., Fisher, A.V., Nute, G. R., Sheard, P. R. Richardson, R. I., … Whittington, F.M. (2008). Fat deposition, fatty acid composition and meat quality: A review. *Meat Science* 78(4): 343–358.

Woodward, K. N. (1991). Hypersensitivity in humans and exposure to veterinary drugs. *Veterinary and Human Toxicology* 33: 168-172.

World Health Organization (WHO).(1987). World Health Organization Scientific Working Group on Infections Due to *Enterobacteriaceae*.WHO/CD/1987.

World Health Organization (WHO).(1991). Bacteriology of Drinking Water.The International Standard for Drinking Waters, Geneva.

World Health Organization (WHO). (1994). Guidelines on Detection and Monitoring of *Salmonella* Infected Poultry Flocks with Particular Reference to *Salmonella enteritidis*, Wray C. & Davies R.H., eds. WHO, Geneva, Switzerland, ZOON/94.173.

World Health Organization (WHO).(2000). WHO Global Principles for the Containment of Antimicrobial Resistance in Animals Intended for Food. Report of a WHO Consultation, WHO Department of Communicable Disease Surveillance and Response, Geneva, 1-7.

World Health Organization (WHO). (1997). The Medical Impact of Antimicrobial Use in Food Animals. Report of a WHO Meeting. Berlin, Germany. Pp 1-7.

World Health Organization (WHO). (2003). Joint *FAO/OIE/WHO* Expert Workshop on non-Human Antimicrobial Usage and Antimicrobial Resistance: Scientific assessment.

World Health Organization (WHO). (2011). Tackling antibiotic resistance from a food safety perspective in Europe. WHO Library Cataloguing in Publication Data. Copenhagen, Denmark. Pp 8-9.

World Health Organization(WHO). (2000). WHO global principles for the containment of antimicrobial resistance in animals intended for food.Report of a WHO consultation, Geneva, Switzerland.

Wray, C., and Wray, A., EDS (2000).*Salmonella* in Domestic Animals. CAB International, Wallingford, Oxon, UK. *Salmonellosis* Chapter 2.9.9 Book p3

Wu, C. J., Hsueh, P. R., Ko W. C. (2011). A new health threat in Europe: Shiga toxin producing *Escherichiacoli* O104:H4 infections. *Journal of Microbiology and Immunology of Infections*, 44:390-393.

Yah, S. C., Nwinyi, C. O., and Chinedu, N. S. (2009).Assessment of bacteriological quality of ready to eat food (Meat pie) in Benin City metropolis Nigeria.*African Journal of Microbiology Reseaches*,3(6):390-395.

Youl, P., Baade, P. & Meng, X. (2012).Impact of prevention on future cancer incidence in Australia.*Cancer Forum*, *36*(1), 37-41.

Yucel, N., Citak, S., and Onder, M. (2005).Prevalence and antibiotic resistance of *Listeria* species in meat products in Ankara, Turkey.*Food Microbiology*. 22: 241-245.

Yusha'u, M., Aliyu, B. S. and Olonitola, O. S. (2008) Predliminary for Inhibitory Activity against extended spectrum Beta lactamase producing *Enterobacteriacea.* www.Ijpas.com *International Journal of Pure andApplied Sciences*, 2 (2): 1 – 5.

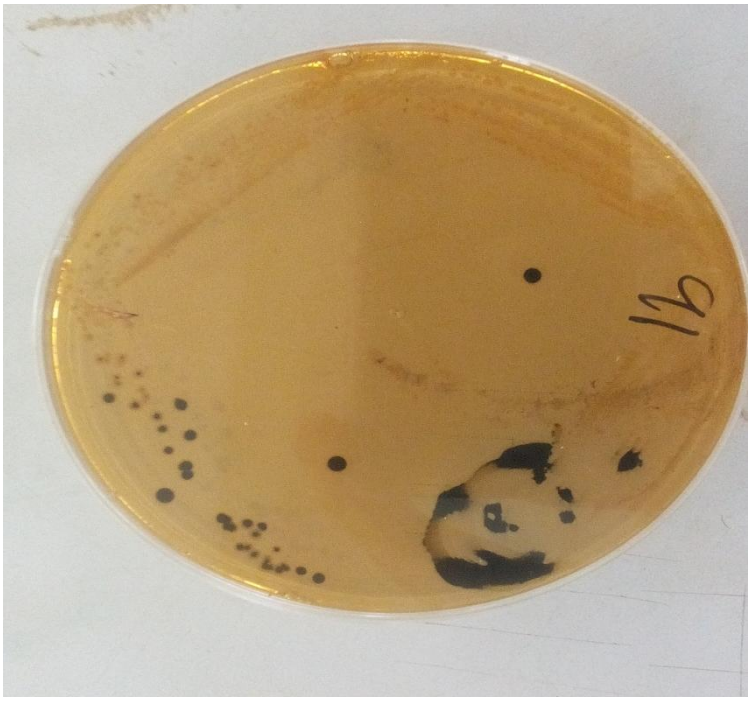
Yusha'u, M., Olonitola, S. O. and Aliyu B. S. (2007) Prevalence of extended spectrum β – lactamases (EBLS) among Clinical isolates of *Enterobacteriaceae* in Kano *Applied sciences*, 1 (3): 42 – 48

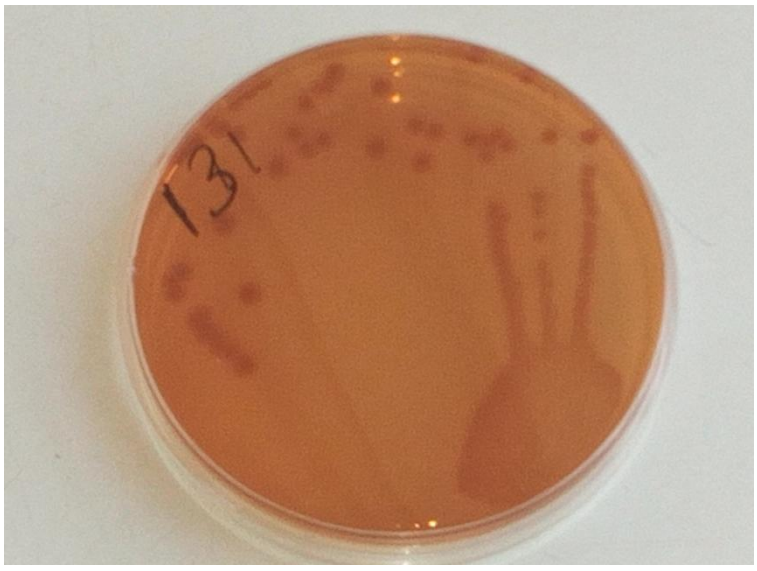
Zhang, G., Ross, C.R., and Blecha, F. (2000). Porcine antimicrobial peptides: new prospects for ancient molecules of host defence. *Veterinary Research*, 31:277-296.

Zhao, S., White, D. G., McDermott P. F., Friedman, S., English L., Ayers S. and Walker R.D. (2001). *Escherichia coli* and *Salmonella* isolates from food animals and ground meat. *Antimicrobial Agents Chemotherapy*. 45:3647-3650.

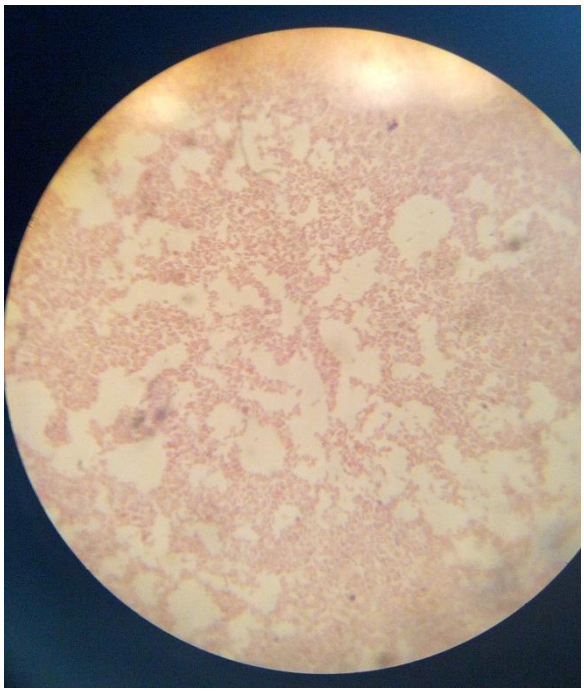
Zweifer, C., Fischer, R., and Stephan, R. (2008).Microbiological contamination of pig and cattle carcasses in different small-scale Swiss abattoir.*Meat. Science*, 78: 225-231.

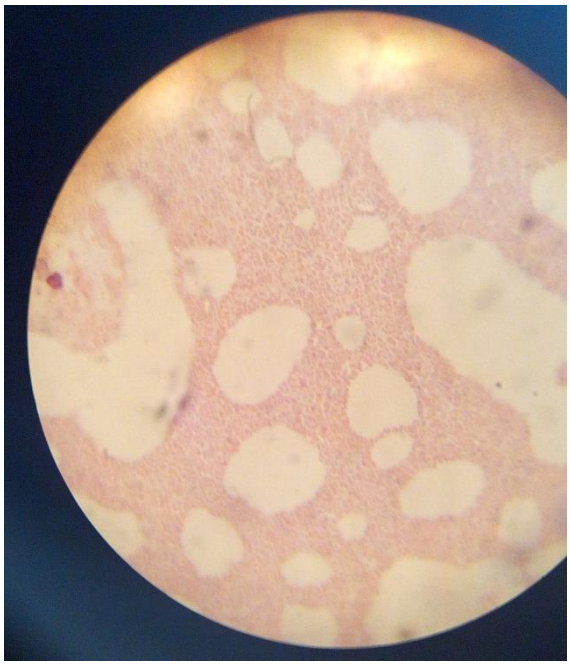
APPENDIX I: Isolates on SSA Plate





APPENDIX II: Microscopy Result



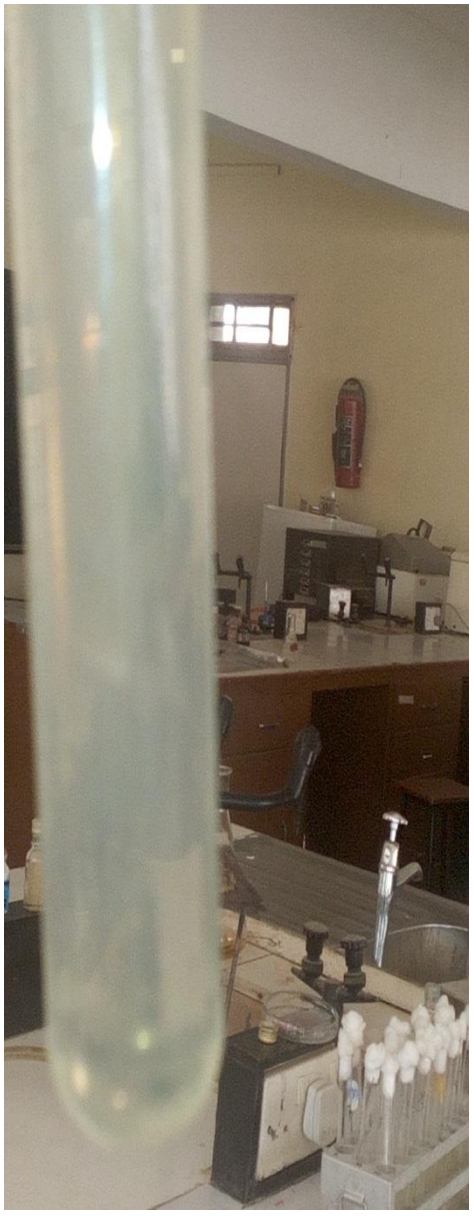


APPENDIX III: Biochemical Test Result

TSI

Urease test

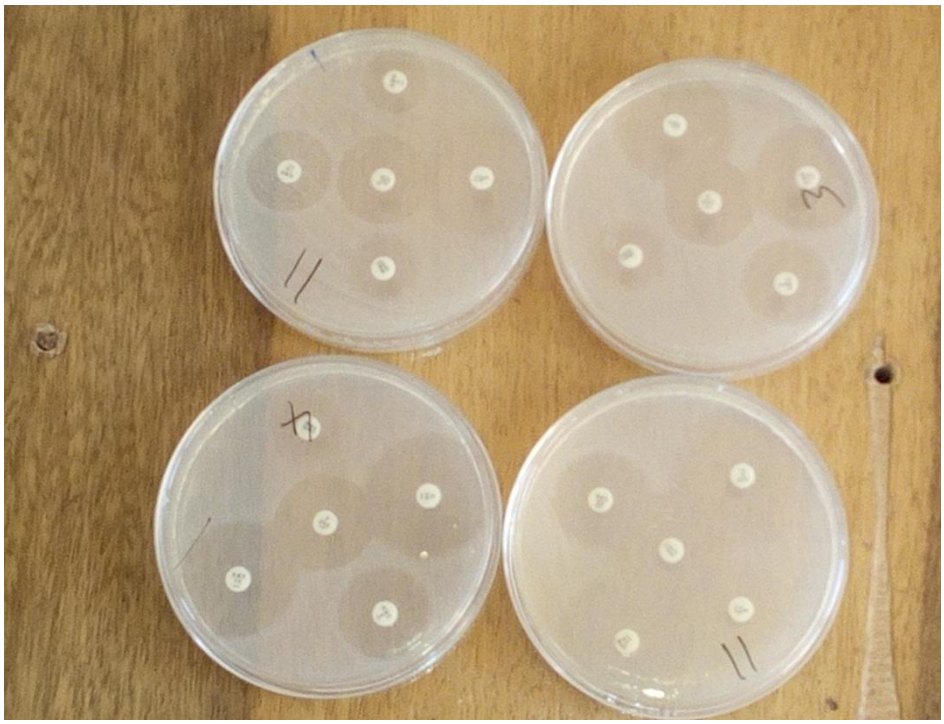
Citrate test



Motility

APPENDIX IV: Microbact 12E Gram-Negative Bacillus (GNB) Rapid Identification System Result

APPENDIX V: Antibiotic Subscebtibility Test Result



APPENDIX VI: Proximate Composition (%) of Raw Beef And Tsire Samples

	SAMPLE Codes	MOISTURE	ASH	FAT	PROTEIN	FIBER	CHO
1	FAR	69.00	1.05	17.30	8.75	-	3.90
2	FAT	55.42	3.75	23.08	7.00	-	10.75
3	PR	73.38	1.20	8.80	6.96	-	9.66
4	PT	47.25	3.95	29.20	10.50	-	9.10
5	CR	73.11	1.10	13.60	7.00	-	5.19
6	CT	60.05	2.45	30.00	7.00	-	0.50
7	SR	69.12	1.10	12.90	5.25	-	11.63
8	ST	42.83	3.50	25.50	5.25	-	22.92

Key

FAR	Faculty of Art raw beef Sample
FAT	Faculty of Art “Tsire” Sample
PR	Pepsi Garden raw beef Sample
PT	Pepsi Garden “Tsire” Sample
CR	Community Market raw beef Sample
CT	Community Market “Tsire” Sample
SR	Sculpture Garden raw beef Sample
ST	Sculpture Garden “Tsire” Sample
R	Raw beef sample
T	“Tsire” samples
-	Not detected