BACTERIOLOGICAL QUALITY AND OCCURENCE OF E*SCHERICHIA COLI* AND *STAPHYLOCOCCUS AUREUS* IN ICE CREAM SOLD IN ZARIA METROPOLIS, NIGERIA

BY

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A DISSERTATION SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY, ZARIA, IN PARTIAL FULFILLMENT FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE IN VETERINARY PUBLIC HEALTH AND PREVENTIVE MEDICINE

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

OCTOBER, 2015

DECLARATION

I declare that the work in this thesis entitled **THE BACTERIOLOGICAL QUALITY AND OCCURENCE OF *ESCHERICHIA COLI* AND *STAPHYLOCOCCUS AUREUS* IN ICE CREAM SOLD IN ZARIA METROPOLI, NIGERIA** has been performed by me in the Department of Veterinary Public Health and Preventive Medicine. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at this or any other Institution.

Aisha Usman BAGUDU _____ _____

Name of Student Signature Date

CERTIFICATION

This thesis entitled **"Bacteriological Quality and Prevalence of *Escherichia coli* and *Staphylococcus aureus* in Ice cream Sold in Zaria Metropolis, Nigeria"** meets the regulations governing the award of the degree of Master of Science of Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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ABSTRACT

A cross-sectional study was conducted to determine the bacteriological quality and occurrence of *Escherichia coli* and *Staphylococcus aureus* in ice cream sold to consumers by supermarkets and street vendors in Zaria metropolis. 240 samples of industrially and locally produced ice creams were examined for the total aerobic and coliform counts as well as contamination with *Staphylococcus aureus* and *Escherichia coli* species using standard methods. The isolated *S. aureus* and *E. coli* were further subjected to antimicrobial susceptibility testing by the disk diffusion method. Overall, the mean total counts (TAPC) per ml of industrial and locally produced ice cream were 1.14×10^7 and 2.015×10^7 respectively which were statistically significantly different ($P<0.05$) and the mean counts for coliforms (TCC) per ml for industrial ice cream and local ice cream were 5.69×10^3 and 4.73×10^3 respectively . Of the total of 240 samples, comprising 111 industrially produced and 129 local ice cream samples, 19 (8.0%) were positive for *Staphylococcus aureus* and 5 (2.0%) were positive for *Escherichia coli*; 5(26.3%) of the *Staphylococcus aureus* isolates were from industrially produced ice cream while 14 (73.6%) were from locally made ice cream . The differences were statistically significant ($P<0.05$). Brands of ice cream of industrially produced origin had significantly lower frequency of *S. aureus* and *E. coli* than those of local sources. Antibiotic susceptibility profile for *S. aureus* showed that all the isolates were resistant to multiple antibiotics. Antibiotic susceptibility profile for *E coli* showed that all the isolates were resistant to one or more antibiotics except for nitrofurantoin and ciprofloxacin. Locally produced ice creams sold in Zaria appear to pose a higher health risk to consumers compared to imported brands. It is imperative that bacteriological standards be enforced in order to prevent ice cream borne infections. There

is a need also for investigation and control of antibiotic usage and continuous surveillance of antimicrobial resistance trends in the study area and throughout the country.

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CHAPTER ONE

INTRODUCTION

1.1 Background of Study

Ice cream is a frozen dairy food made by freezing a processed mix. The mix is composed of a combination of milk products (condensed milk, milk powder and cream), sugar, emulsifiers, stabilizers, flavouring and colouring agents (Kocak *et al*.,1998). Ice cream is a palatable, nutritive and healthy food. It is undoubtedly one of the most popular and favourite food products in Nigeria among children and adults especially during hot season. Several brands of ice cream with a variety of flavours are being marketed in Nigeria.

Carbohydrate solutions give the sensation of sweetness and control the frozen and melting characteristics of ice cream. Ice cream mix properties depend primarily on lipid and protein quantities. The small quantities of emulsifier and hydrocolloid act as stabilizers of oil-in-water emulsions. For flavouring, natural flavours may be used such as real fruit, cocoa, coffee, ginger and vanilla. All these allow great variations in ice cream flavour, taste and way of structure formation (Vica *et al.*, 2010).

As food, ice cream is a very nourishing product, with a great energy value due to its rich content in carbohydrates, lipids and proteins. It also contains vitamins (especially vitamin A and B) and mineral salts especially calcium and phosphorus salts.

1.2 The Genera *Staphylococcus* and Species *Escherichia Coli.*

The genus *Staphylococcus* is composed of 32 species and 15 sub-species. The species include; *S. aureus, S. epidermidis, S. capitis, S. caprae, S. saccharolyticus, S. warneri, S. pasteuri, S. hominis, S. lugdunensis, S. schleiferi, S. muscae, S. auricularis, S. saprophyticus, S. cohnii, S. xylosus, S. equorum, S. arlettae, S. gallinarum, S. simulans, S. carnosus, S. pisciferrmentans, S. felis, S. lutrae, S. S. intermedius, S. delphini, S. hyicus, S. chromogenes, S. sciuri, S. lentus, S. vitulus* (Kloos, 1997). *Staphyloccocus* are gram positive cocci and *S. aureus* occurs in clusters appearing as bunch of grapes or in pairs and short chains. They are facultative in their oxygen requirement and ferment lactose, maltose and

mannitol. These organisms are catalase positive and majority produce coagulase which coagulates plasma. *Staphylococcus aureus* species are aetiologic agents of skin and soft tissue infections and also isolated from the anterior nares of infected individuals. *Staphylococci* have the unique property of being able to grow in the prescence of high concentration of salt and this property is utilised in the preparation of media selective for their isolation (Sneath *et al*., 1986). MRSA is an acronym for methicillin resistant *Staphylococcus aureus* that grows in clusters, multiplies very rapidly and can cause many different kinds of infections, ranging from simple skin infections (boils, furuncles) to septicaemia and toxic shock syndrome, and is spreading rapidly in the United States and is worldwide (Carla *et al*., 2009).

The Species *Escherichia coli*

Escherichia coli commonly abbreviated *E. coli*) is a gammaproteobacterium commonly found in the lower intestine of warm-blooded organisms(endoderms). In 1885, Theodor Escherich, a German pediatrician, first discovered this species in the faeces of healthly individuals and called it *Bacterium coli* commune due to the fact it is found in the colon and early classifications of Prokaryotes placed these in a handful of genera based on their shape and motility (Lukjancenko *et al.*, 2010).

Following the revision of Bacteria it was reclassified as *Bacillus coli* by Migula in 1895 (Migula *et al.*,1895) and later reclassified as *Escherichia coli* (Castellani and Chalmers, 1919)

Four of the many *E.coli* strains (K-12, B, C, and W) are thought of as model organism strains. These are classified in Risk Group 1 in biosafety guidelines.

1.3 Statement of Research Problem

Ice cream is a nutritious food for human consumption and also an excellent medium for the growth of many microorganisms some of which may cause diseases in human beings e.g. cholera, typhoid, and bacillary dysentery. Contaminated ice creams have caused several outbreaks of gastrointestinal diseases in a number of countries in Asia, Europe and North America (Dijuretic *et al.,* 1997).

The ingredients of ice cream may be various mixtures of milk, cream, evaporated or condensed milk, dried milk, colouring materials, flavours, fruits, nuts, sweetening agents, eggs, egg products, and stabilizers. Any of these may harbour microorganisms and affect the quality of the product as judged by its bacterial load or its content of various specific species of bacteria (Frazier *et al.,* 1992).

Although ice cream is a frozen product, it has been found to be contaminated by bacterial pathogens such as *Salmonella* spp., *Listeria* spp., *Yersinia* spp., *Staphylococcus aureus*, *Escherichia coli* and *Bacillus* spp., in many countries (Aidara-Kane *et al*., 2000; Cadarno and Rocourt, 2001). Most ice creams become contaminated with microbes during production, transit and preservation.

Factors that contribute to ice cream contamination include poor sanitary practices during processing (Kanbakan *et al*., 2004), use of contaminated ingredients particularly shell eggs, contaminated water (Wilson *et al*., 1997) post-process contamination and improper storage temperatures (Warke *et al*., 2000).

To avoid ice cream-borne gastroenteritis, bacteriological standards have been established for ice cream by several governments. In developing countries, although bacteriological standards may exist, there is always the problem of enforcement (Warke *et al*., 2000).

1.4 Justification

In Nigeria, a tropical country with average temperature ranges from 23^0C (73^0F) to 32^0C (90^0F), ice cream is very widely consumed, and the expanding fast food industry remains a contributing factor to the growth of ice cream sales. In Zaria, it is manufactured on small scale and sold in retail outlets or hawked on bicycles.

Adesiyun (1995) surveyed ice cream samples sold locally in Trinidad and detected *S. aureus* and *E. coli* . Also, Osamwonyi (2011) and Yusuf (2013) evaluated ice creams sold in Benin City and Bauchi respectively and found *E. coli* and *Staphylococcus aureus* among other bacteria. The consumption of ice cream is higher among children of vulnerable age groups. Therefore, there is a need to maintain a high microbiological safe standard

(Champagne *et al*., 1994; Elahi *et al*., 2002). The possibility of acquiring food borne diseases by infants, children, elderly people and immunosuppressed individuals from this product is likely (Champagne *et al*., 1994). A relatively low storage temperature and pasteurization step during its processing are considered sufficient to eliminate most of the pathogenic microorganisms. However, the addition of contaminated ingredients, improper handling and the use of improper storage temperatures are factors that contribute to the potential hazard of the finished products especially during processing after pasteurization processes.

To date, information is scanty on the bacteriological quality of imported and locally processed ice cream in Zaria. This study therefore was conducted to determine the bacteriological quality of ice cream originating from commercial industrial and local sources sold in Zaria, and to understand the differences in the microbiological qualities between locally and industrially produced ice creams.

1.5 Aim of the Study.

To determine the bacteriological quality and the occurence of *E. coli* and *Staphylococcus aureus* in ice cream sold in Zaria metropolis and their potential risk to Public Health.

1.6 Objectives of the Study

i To determine the total aerobic bacterial and coliform counts in ice cream.

ii To isolate, identify and characterize *E. coli* including O157; H7 and *S. aureus* using conventional biochemical tests and confirmation with Microbact 12E and 12S respectively.

iii To determine the antimicrobial susceptibilities of *S. aureus* and *E. coli* to commonly used antimicrobial agents.

1.7 Research Questions

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CHAPTER TWO

LITERATURE REVIEW

2.1 The Genus *Staphylococcus*

Staphylococcus species belong to the kingdom, Bacteria: Phylum, Firmicutes: Class, Bacilli: Order, Bacillales: Family, *Staphylococcaceae*: Genus, *Staphylococcus* (Toder, 2004). Members of the genus *Staphylococcus* are gram positive cocci (0.5 to 1.5 um in diameter) that occur singly and in pairs, tetrads, short chains and irregular grape like clusters. Staphylococci are non-motile, non-spore forming, and usually catalase positive, facultative anaerobes. Except for *S. saccharolyticus* and *S. aureus* sub specie anaerobius, their growth is more rapid and abundant under aerobic conditions. These exceptional organisms are also catalase negative (Kloos, 1997).

2.1.1 Genome characteristics.

Members of the genus *Staphylococcus* have a DNA G+C content of 30 to 39 mol%. The cell wall of *Staphylococcus* species contain peptidoglycan and teichoic acid. The inter-peptide bridge of the peptidoglycan consists of oligoglycan peptides susceptible to the action of lysostaphin. The genus *Staphylococcus* is composed of 32 species and 15 sub-species (Kloos, 1997).

2.1.2 Natural habitats of *Staphylococcus* species

Staphylococci are widespread in nature, although they are mainly found living on the skin, skin glands and mucous membranes of mammals and birds. They are sometimes found in the mouth, blood, mammary glands, intestinal, genitourinary and upper respiratory tracts of these hosts. The prescence of enterotoxigenic strains of staphylococci (most notably, certain

strains of *S. aureus*) in various food products is regarded as a public health harzard because of the ability of these strains to produce intoxication or food poisoning (Kloos, 1997).

2.1.3 Reservoirs of *Staphylococci*.

Staphylococci found in human and other primates include *S. aureus, S.epidermidis, S. capitis, S. caprae, S. saccarolyticus, S. hominis, S. lugdunensis, S. auricularis, S. saprophyticus, S. cohnii, S. xylosus and S. simulans* and are usually only transient on humans and are primarily acquired from domestic animals and their products. Some of the human *23* species are temporarily residents or transients on domestic animals. *S. schleiferi, S. intermedius, S. simulans and S. felis* are commonly found living on carnivores. However, *S. schleiferi* may produce serious infection in humans and *S. intermedius* may produce infections in humans as a result of dog bites. *S. sciuri* and *S. xylosus* are common residents on a variety of rodents (Talan *et al.*,1989).

2.1.4 Pathogenic members of the genus *Staphylococcus*.

The pathogenic members of this genus include S. *aureus, S.epidermidis, S. haemolyticus, S. lugdunensis, S. warneri,* and *S. saprophyticus* which are commonly associated with human infections, while *S. intermedius* and *S. hyicus* are species of special veterinary interest.

2.1.5 *S. aureus* infection in man.

The coagulase- positive *S. aureus* is well documented as a human opportunistic pathogen. Nosocomial infections caused by *S. aureus* have been a major cause of morbidity and mortality. *S. aureus* infections are often acute and pyogenic and if untreated, may spread to surrounding tissue or via bacteraemia to metastatic sites (involving other organs). Some of the most common infections caused by *S. aureus* involve the skin, and they include furuncles or boils, cellulitis, impetigo and post operative wound infections of various sites.

Some of the more serious infections produced by *S. aureus* are bacteraemia, pneumonia, osteomyelitis, acute endocarditis, myocarditis, pericarditis, meningitis, scalded skin syndrome, and abscesses of the muscle, urogenital tract, central nervous system, and various intra-abdominal organs. Food poisoning is often attributed to staphylococcal enterotoxin (Kloos and Bannerman, 1995).

Methicillin resistant *S. aureus* (MRSA) emerged in the 1980s as a major clinical and epidemiologic problem in hospitals. Nasal carriage of *S. aureus* or MRSA has been suggested as risk factor for the development of infections (Kloos and Bannerman, 1995). International convention has now renamed this agent as methicillin resistant *S. aureus*. This is to comply with European law, which requires the use of the recommended international non-proprietary name (r INN) (www.mrsaresources).

MRSA is an acronym for *Methicillin Resistant Staphylococcus aureus* (and is sometimes referred to as *MERSA*). It is an antibiotic resistant superbug bacteria that grows in clusters, multiplies very rapidly and can cause many different kinds of infection, ranging from simple skin infections (boils, furuncles) to septicaemia (infection of the bloodstream) and toxic shock syndrome, and is spreading rapidly in the United States and is worldwide (www.mrsaresources). In the past 30 years, Methicillin Resistant *Staphylococcus aureus* (MRSA) has been the leading cause of nosocomial infections throughout the world. Healthcare-associated MRSA (HA-MRSA) isolates are resistant to multiple antimicrobial drugs. This resistance severely hampers treatment options (Carla *et al.*, 2009).

Studies approximate that 30-50% of the population carry *Staphylococcus aureus* on their bodies all of the time. Those most susceptible to becoming sick from it are children, the

elderly and others with reduced immune system function from diseases such as diabetes and HIV. MRSA used to be a bacterium that was only found in hospital settings (HA-MRSA), but in recent years, MRSA has infected healthy people who have contracted it within their communities (CA-MRSA). MRSA grows very rapidly in warm, moist areas and is often seen in athletes and gyms (www.mrsaresources). Methicillin Resistant *Staphylococcus aureus* (MRSA) colonization has recently been identified in pigs and people that work with pigs, raising concerns about the role of pigs as reservoirs of MRSA for human infection (Khanna *et al.,* 2007).

Farm animal MRSA was first identified in the Netherlands in July 2004. During routine screening, scientists found the bacteria on a six-month-old baby girl admitted for surgery to a hospital in Nijmegen. For several months, the girl remained colonized by MRSA despite repeated attempts to clear the bacteria. Tests revealed that parents were also positive for MRSA. The family lived on a farm and raised pigs. This finding prompted further, more extensive studies (Voss *et al*., 2005). Latest Dutch figures indicate that 40% of pigs , 13% of calves and a high proportion of chickens carry MRSA and 50% of Dutch pig farmers also carry MRSA. All people living on pig and cattle farms are now considered to be at high risk of being MRSA carriers (Voss *et al*., 2005).

2.1.6 Isolation and identification of *S. aureus*.

Several isolation procedures are currently available. Media for isolation include blood agar, nutrient agar, tryptic soy agar, brain heart infusion agar, mannitol salt agar among others. Specimens should be enriched with Brain Heart Infusion broth (BHI) at 37^{o}c for 24 hours and then plated on a selective media such as Baird Parker Agar and incubated at 37^{0}c for 24 hours. Colonies of *S.aureus* are usually large (6 to 8mm in diameter), smooth, slightly raised and transluscent. The colonies of most strains are pigmented, ranging from cream yellow to orange. Gram staining of the colonies reveals gram positive cocci in clusters. The ability to clot plasma continues to be the most widely used and generally accepted criterion of the identification of pathogenic staphylococci often associated with acute infections, such as *S. aureus* in humans and animals and *S. intermedius* and in animals (Kloos and Bannerman, 1995).

2.1.7 Epidemiology of *S. aureus*.

S. aureus may occur as a commensal on human skin; it also occurs in the nose frequently in about a third of the population (Whitt and Salyers, 2002) and throat less commonly. The occurrence of *S. aureus* under these circumstances does not always indicate infection and therefore does not always require treatment. It can survive on domesticated animals such as dogs, cats and horses, and can cause bumble foot in chickens. It can survive for some hours on dry environmental surfaces, but the importance of the environment in the spread of *S. aureus* is currently debated (Whitt and Salyers, 2002).

S.aureus can invade other tissues when normal barriers have been breached (e.g., skin or mucosal lining). This leads to furuncles (boils) and carbuncles (a collection of furuncles). In infants, *S. aureus* can cause a severe disease ; Staphylococcal scalded skin syndrome

(SSSS) (Curran and Al-Salihi,1980). Methicillin Resistant *Staphylococcus aureus* (MRSA) is a critically important human pathogen and attention has been paid recently to the potential role of food animals in human MRSA infection and colonization. This has widely focused on pigs because of widespread reports of MRSA colonization in pigs internationally (Khanna *et al*., 2007; De Neeling *et al*., 2007) and identification of contact with pigs or veal calves as a significant risk factor for MRSA infection or colonization in some regions (Voss *et al*., 2005; Wulf and Voss, 2008). Most of this has involved one MRSA clone, sequence type 398 (ST398). This clone has been termed a "livestock-associated" strain and has predominated in most studies of pigs in Europe and North America (Khanna *et al*., 2007; Denis *et al*., 2009; Kock *et al.,* 2009) and appears to be widely disseminated in the pig population internationally. Identification of MRSA in food animals led to logical concerns about food-borne contamination, and MRSA has been identified in retail meat in Europe, Asia and North America (Kitai et al., 2005; Kwon et al., 2006; Van Loo et al.,2007) however, the role of food borne contamination in human MRSA infection or colonization is currently unclear and much further studies is required to elucidate this potential problem.

MRSA infections that are acquired by persons who have not been recently (within the past year) hospitalized or had a medical procedure (such as dialysis, surgery, catheters) are known as community- acquired MRSA (CA-MRSA) infections, according to the Centers for Disease Control and Prevention (CDC). Community outbreaks have been reported in sports teams, child care attendees, prison inmates and diverse populations where habitation is relatively concentrated.

The CDC defines hospital-acquired MRSA (HA-MRSA) in persons who have had frequent or recent contact with hospitals or healthcare facilities (such as nursing homes or dialysis

centers) within the previous year, have recently undergone an invasive medical procedure, or are immunocompromised.

CA-MRSA strains usually harbour a staphylococcal cassette chromosome (SCC) (type IV or V) that is smaller than the type I-III SCC elements commonly found in HA-MRSA strains (Carla *et al.*, 2009). CA-MRSA have evolved independently of typical hospital MRSA. The dominant CA-MRSA from different regions belong to genetically unrelated lineages and cause unique clinical problems. In the USA, CA-MRSA belong to lineages CC8 (called USA300 which is the dominant clone) and CCI (USA 400) and are responsible for widespread severe skin and soft tissue infection (SSSI) in healthy people, particularly those in close contact with others such as school children, athletes, prisoners, military personnel and men who have sex with men. Infections can be severe enough to require hospitalization and may cause death. CA-MRSA are spreading quickly, resulting in severe pressure on accident and emergency departments, and USA300 is becoming a major cause of hospital-acquired infection (Seybold et al., 2006). Furthermore, Methicillin-sensitive *S. aureus* that are genetically similar to USA300 and PVL positive have also caused a dramatic increase in SSSI in the last few years (Orscheln *et al*., 2009). In Europe, CA-MRSA are rarer, but when they are present in dominant clone is ST80. Rare haemolytic pneumonia in with a high mortality rate is associated with these strains. In Asia, SSSI are increasing due to CA-MRSA CC59, while in the South Pacific, SSSI caused by CA-MRSA CC30 have been prevalent for more than a decade (Tristan et al., 2007).

Some MRSA lineages tend to predominate in specific geographical regions and show host specificities; therefore, they tend to be associated with animals more than with humans and *vice versa* (Sung *et al*., 2008). CC398 is the MRSA lineage most often associated with

asymptomatic carriage in intensively reared food-producing animals, primarily in pigs, but also in cattle and perhaps in poultry (EFSA, 2012). Although this strain is mainly found to colonize animals without causing clinical diseases, in a few isolated cases, it caused clinical infections in animals. Colonization with livestock associated MRSA, especially CC398, has been reported frequently in people who work with such animals, i.e. farmers, veterinarians and their family members (Leonard and Markey, 2008; van Rijen et al., 2008; Cuny et al., 2009). MRSA isolates can be also shared between personnel and animals, including dogs, cats and horses in veterinary hospitals and between companion animals and their owners in households (Cefai *et al.*, 1994; Baptiste *et al*., 2005; Weese *et al*, 2006). Indeed, in a few cases companion animals have been implicated as sources of human infections (Ferreira *et al*., 2011).

2.1.8. Virulence factors of *S.aureus*.

Depending on the strain, *S. aureus* is capable of secreting several toxins which can be categorized into three groups. Many of these toxins are associated with specific diseases.

2.1.8.1 *Pyrogenic toxin superantigens* (*PTSAgs).*

PTSAgs have super antigen activities that induce toxic shock syndrome (TSS). This group includes the toxin TSST-1, which causes TSS. The staphylococcal enterotoxins, which cause a form of food poisoning are included in this group (Carter *et al*., 2000).

2.1.8.2 *Exfoliative toxins* (*EF Toxins*).

EF toxins are implicated in the disease staphylococcal scalded-skin syndrome (SSSS), which occurs most commonly in infants and young children. It also may occur as epidemics

in hospital nurseries. The protease activity of the exfoliative toxins causes peeling of the skin observed with SSSS.

Some strains of *S.aureus* also produce an enterotoxin that is the causative agent of *S. aureus* gastroenteritis. The gastroenteritis is self-limiting with the person recovering within 8-24 hours (Carter *et al*., 2000).

2.1.8.3 *Other toxins*

Staphylococcal toxins that act on cell membranes include alpha-toxin, beta-toxin, delta-toxin, and several bicomponent toxins. The bicomponent toxin Panton-Valentine leucocidin (PVL) is associated with severe necrotizing pneumonia in children. The genes encoding the components of PVL are encoded on a bacteriophage found in community -associated MRSA strains (Carter *et al*., 2000). In many bacteria, expression of toxin genes encoded on such phage is induced under stress, including antibiotics. Wirtz *et al*., (2009) proved that phage induction increases expression of *luk*-PV mRNA dramatically. But surprisingly, they showed that the clone responsible for most CA-MRSA infections in the USA had defective phage and failed to over express the toxin. Other toxins include coagulase, haemotoxin, fibrinolysin, leucocidin, hyaluronidase and dermonecrotoxin.

2.1.9 Prevalence and occurrence of *S. aureus* in food.

Studies demonstrate that MRSA has been isolated from meat and meat products, although the bacterial load was very low. The pathogen is not likely to cause disease, especially if meat is properly prepared before consumption. However, contamination of food products may be a potential threat for the acquisition of MRSA by those who handle food (Inge *et al.*, 2007) .It is well known that pasteurization of milk eliminates *S. aureus.* Raw milk and raw-milk cheese contaminated with MRSA have been incriminated in the transmission of

the pathogen to humans (Normanno *et al*., 2007; Haran *et al*., 2012). Handling or consumption of raw milk and dairy products may lead to the spread of antimicrobial resistance genes of *S. aureus* to humans (McKay, 2008). Significant differences in MRSA prevalence in milk have been recorded among different countries or even regions within the same country. These differences may be due to differing animal production systems among the various countries. Different national antimicrobial policies and regulations may have also contributed to the different prevalence estimates (Grave *et al.*, 2010). Other factors, such as presence of multiple animal species in the same area that facilitate transfer of genetic material between *S. aureus* strains may affect the MRSA prevalence (Spohr *et al*., 2011).

Umaru *et al*. (2013) reported 12.6% and 4.8% *S. aureus* and MRSA in fresh and fermented milk in Zaria. Twenty six percent (26%) of *S. aureus* was reported by Mas'ud (1989) in ice cream in Pakistan. Also, Ojokoh (2006) reported 100% prevalence of *Staphylococcus* species in ice cream sold in Akure, Nigeria. Osamwonyi (2011) , reported a prevalence of 12.5% *S. aureus* in ice creams sold in some locations in Benin city, 60% prevalence was also reported by Yusuf *et al* ., (2013) in ice cream sold in Bauchi, among others. Studies have indicated the prescence of *S. aureus* and MRSA in many countries in Europe, Asia, Africa and America (Pexara, 2013).

2.1.10 Treatment of *S. aureus* infection and antibiotics resistance

The treatment of choice for S. aureus infection is penicillin; but in most countries, penicillin-resistance is extremely common and first line therapy is most commonly penicillinase-resistant penicillin such as oxacillin or flucloxacillin. Combination therapy

with gentamicin may be used to treat serious infections like endocarditis (Korzeniowski and Sande, 1982; Bayer *et al*.,1998), but its use is controversial because of high risk of damage to the kidneys (Cosgrave *et al*.,2009). The duration of treatment depends usually on the infection and on severity.

Researchers have identified a bacteriophage active against *staphylococcus aureus*, including methicillin-resistant strains, in mice and possibly human (Cappareli *et al.*,2007).

2.1.11 Phage therapy

Bacteriophages are viruses that infect bacteria. Bacteriophages have many potential applications in biotechnology that are currently being explored such as being used as delivery vehicles for vaccines and gene therapy, detecting bacterial pathogens, and screening peptides or antibodies. Phage therapy takes advantage of this particular group of viruses in a relatively simple way. It aims to treat bacterial infections through the application of such phages. Though for sometime, the use of bacteriophages were not considered feasible but since the emergence of many antibiotic resistant pathogens, the field is again on the rise (Inal, 2003). Bacteriophages are found in all bacteria so there is at least a theoretical possibility that therapies can be developed for all species of bacteria (Inal, 2003). Because humans are unlikely to come up with a novel antibiotic continuously and bacteria will probably continue to evolve resistances, it is important that new ways of treating infections are discovered. Bacteriophages are specific to their bacterial hosts and therefore do not harm humans or plants. They can grow in numbers as long as the bacteria are present, and they are self limiting in that once they kill all of the target bacteria, they no longer have a place to live and they die off too (Summers 2001; Clark and March, 2006).

2.1.12 Mechanisms of antibiotic resistance by *S. aureus*.

The discovery of antimicrobial agents has been a critical element of the therapeutic Qarmamentarium of modern medicine, but the treatment of infections caused by *S. aureus* is still a challenge for clinicians. Numerous studies have indicated that *S. aureus* is among the most frequently encountered microorganism in microbiology laboratories in Nigeria. There have been reports of isolation of methicillin resistant *S. aureus* in Nigeria. Olayinka and Olayinka (2003) reported a prevalence of 31.4% MRSA from clinical specimens in Zaria, Nigeria, while Chebremedhin *et al.,* (2009) reported a 20.23% prevalence of MRSA from paediatric and surgical cases in Ibadan, Nigeria.

The extreme danger associated with *Staphylococcus aureus* infections is due to the combination of frequent antibiotic resistance, which prevents efficient treatment with extra ordinary virulence which determines the severity of the disease. *S.aureus* is known to exchange antibiotic resistance and virulence determinants between different strains, thereby spreading the capacity to cause serious infections among the organisms. The genetic information for these determinants is usually found on the so-called mobile genetic elements. It has been noted that such exchangeable elements carry genes for either virulence or both. Mobile genetic element-encoded cytolysin connects virulence to methicillin resistance in MRSA (Shu *et al*., 2009).

The term ‘MRSA’ is used to describe strains of *S. aureus* resistant to semi-synthetic, penicillinase resistant, β-lactams such as methicillin, oxacillin or cloxacillin. MRSA strains are resistant to all cephalosporins, cephems and other β-lactams, such as ampicillin-sulbactam, amoxicillin-clavulanic acid, ticarcillin-clavulanic acid, piperacillin-tazobactam

and the carbapenems. This group of organisms is also frequently resistant to most of the commonly used antimicrobial agents, including the aminoglycosides, macrolides, chloramphenicol, tetracycline and fluoroquinolones (Lee, 2003).

β-lactam antibiotics damage bacteria by inactivating penicillin-binding proteins, enzymes that are essential in the assembly of the bacterial cell wall (Pinho *et al*., 2001). These antibiotics inactivate the four native penicillin-binding proteins found in staphylococci. As a result of the weakened cell wall, treated bacteria become osmotically fragile and are easily lysed. The staphylococcal β-lactamase protein, which cleaves the β-lactam ring structure, confers resistance to penicillin, but not to semi-synthetic penicillins.

In MRSA, resistance to all β-lactam antibiotics, including the semi-synthetic penicillins, is conferred by the penicillin-binding protein PBP2 (or PBP2a) that has a very low affinity for β-lactam antibiotics and is thought to aid cell wall assembly when normal penicillin-binding proteins are inactivated (Hartman and Tomasz, 1984). PBP2a is encoded by the *mecA* gene, which is located in the staphylococcal cassette chromosome (SCC*mec*) (Katayama *et al.*, 2000). The confirmation of the presence of the *mecA* gene, has until recently been the 'golden standard' for detection of MRSA worldwide. However, a novel *mecA* homologue (with approximately 70% similarity to the *mecA* gene) that also confers methicillin resistance was identified in *S. aureus* isolates from dairy cattle and humans. This gene, previously denoted as *mecALGA251,* has been re-designated *mecC* (García-Álvarez *et al*., 2011; EFSA, 2012; Laurent *et al*., 2012; Petersen *et al*., 2012).

Additional genes, which are also found in susceptible isolates, can affect the methicillin resistance phenotype in *S. aureus*, resulting in heterogeneity of resistance and making

detection of resistance difficult (Montanari., 1990; de Lencastre and Tomasz 1994). Some strains of *S. aureus* possess an alternative resistance mechanism, attributable to the hyper-production of the *S. aureus* β-lactamase enzyme, which inactivates the antibiotic agents by hydrolysing the β-lactam ring of penicillin and cephalosporin compounds (Brown *et al.*, 2005). Vancomycin is the only antibiotic available for treating MRSA infections (Wenzeel, 1982) However, vancomycin-resistant MRSA strains, including some community acquired-MRSA strains, have increasingly been reported, thereby causing public health concern (Wenzel, 1982; CLSI, 2004).

2.2 Molecular Epidemiology of *Staphylococcus aureus*

The resistance of *S. aureus* to methycillin and other β-lactam antibiotics is caused by the *mec*A gene, which encodes the 78- kDa penicillin binding protein (PBP)2a (or PBP2'). The 2.1-kb *mec*A gene is located on a mobile genetic element, the Staphylococcal Cassette Chromosome *mec* (SCC*mec*) (Ito et al., 2003). Currently, six main types of SCC*mec* (type 1 to v1) are distinguished, ranging in size from 20.9nto 66.9kb. SCC*mec* types 1 (34.3 kb), IV (20.9 TO24.3 kb), V (28kb) and VI (20.9kb) encode for resistance to β- lactam antibiotics only. SCC*mec* types 11 (53.0 kb) and 111 (66.9 kb) determine multi-resistance,as these elements harbour additional drug resistance genes, which are carried on integrated plasmids, i.e. Pub110, p1258 and Pt181, and a transposon (Tn554). Plasmid pUB110 arries the ant (4') gene, encoding for resistance to kanamycin, tobramycin and bleomycin, and p1258 codes for resistance to penicillins and heavy metals such as mercury. Plasmid pT181 codes for tetracycline resistance, while transposon Tn554 carries the ermA gene, which is responsible for inducing macrolide, lincosamide and streptogramin (MLS) resistance (Ito *et al.*, 2003).

S. aureus can also carry resistance genes on plasmids and on other sites of the chromosome. Recently, It has been shown that SCCMEC TYPE III is a composite element, consisting of SCC*mec* type III and SCC*mecury*, harbouring *ccrC*,P1258 and Tn554 (Chongtrakool *et al.*, 2006) .

Furthermore, SCCmec harbours insertion sequences such as IS431, as well as genes responsible for the regulation of mecA transcription,(on SCCmec types I,IV,V,VI), or mec R1 and mecI (On SCCmec type II and III). Both mecI and MecR1 can be truncated by IS431 or IS1272, and this results in a de- repression of mec A. These genes are situated on the so called mec complexes of which five major classes have been distinguished. (Ito *et al*., 2003).

Genes encoding cassette chromosome recombinases (*crr)* are located within the SCC*mec* into the excision of SCC*mec* from the chromosome at a specific site (*attBscc)*; AT THE 3' end of an open reading frame (ORF) of unknown function, named *orfX).* The *ccr* genes are designated *ccr*A1 and *ccr*B1 (in SCC*mec* type1), *ccr*A2 and ccrB2 (in SCC*mec* type VI) and *ccr*C (in SCCmec type V). The J (junkyard) regions (Ito *et al*.,2003).

SCC*mec* elements are divided into three regions. The JI region is located between the chromosome right junctions to the *ccr* genes, while the region from the *ccr* to the *mec* complex is designated as the J2 region. The J3 region ranges from the *mec* complex to the left extremity (*orf*X) of SCC*mec* (Chongtrakool *et al*., 2006).

Several variants of major SCC*mec* elements have been described in *S. aureus* SCC*mec* (Ito *et al*., 2003); type 1 was harboured in the first MRSA strain (NCTC10442), isolated in 1961 in the united kingdom , and this so called Archaic clone disseminated worldwide in the

1960s. SCC*mec* type II was found in a MRSA strain (N315) isolated in Japan, and this New York/ Japan clone spread around the world. In 1985, an MRSA strain (85/2082) harbouring SCC*mec* type IV disseminated worldwide. At the beginning of the 21st century, the first MRSA strain (WIS) With SCC*mec* type V was described in Australia. SCC*mec* type VI, represented by MRSA strain HDE288, has until now only been found in MRSA strains from Portugal (Van Duijkeren *et al*., 2004).

2.3 Diagnosis of MRSA

Diagnostic microbiology laboratories and reference laboratories are keys for identifying outbreaks of MRSA,. Proper MRSA diagnosis may be a potential problem since samples are sent to different diagnostic laboratories that may not be uniform in the testing of bacterial species and their antibiotic sensitivities. MRSA can be typed by both Phenotypic and molecular methods. Phenotypic methods include: colonial characteristics, biochemical reactions, antibiotics susceptibility patterns, and the susceptibility to various phages and toxin production.

Another common laboratory test is a rapid latex agglutination test which detects the PBP2a protein. PBP2a is a variant penicillin binding protein that imparts to S. aureus the ability to be resistant to oxacilllin (Seiken, 2010). If there is a recurrent or persistant case of skin infection in the animal, a small biopsy of either the infected skin or a sample of the exudates from the site may be submitted for laboratory diagnosis. If *S.aureus* is isolated, further tests are needed to determine if it is a MRSA strain and start appropriate antibiotic selection (Lee, 2003).

2.4. Molecular Typing Methods for MRSA

The most prominient molecular typing methods are : pulsed field gel electrophoresis (PFGE), multiloccus sequence typing (MLST), staphylococcal chromosome cassette (SCC typing) and polymerase chain reaction (PCR) [Real-PCR and Quantitative –PCR] (Mackay, 2008; Francois and Sshrenzel, 2008; Denis, 2009).

Rapid diagnosis of MRSA in animals is still in its early stages of development and to date, there is a significant delay between collection and obtaining tests for animals , as compared to humans (Lee,2003) . Rapid tests that have been validated for use in human cases (i.e. real time – PCR) do not necessarily perform adequately in animal cases , so species-specific validation is required (Anderson *et al*., 2007). There is a new typing method that use several variable number tendem repeat (VNTR) sequences for typing animal MRSA isolates, but to date, there is no published data available (Leonard and Markey, 2008).

2.4.1. Treatment of *S. aureus* and MRSA

Antimicrobial therapy should be guided by the susceptibility profile of the organism.. Beta-lactamase–producing strains of methicillin-susceptible *S. aureus* (MSSA) preferably are treated with a semi-synthetic penicillin (e.g., intravenous nafcillin, oxacillin, oral dicloxacillin in patients not allergic to penicillin. First-generation cephalosporins (e.g., oral cephalexin , intravenous cefazolin are an alternative. Vancomycin should only be used for the treatment of methicillin-susceptible *S. aureus* (MSSA) in patients allergic to penicillins because of overuse and development of resistant organisms, and because clearance of bacteremia may be slow **(** Levine *et al*., 1991).

Vancomycin is preferred for treatment in severe MRSA infections and is used only intravenously because the oral formulation is not readily absorbed from the gastrointestinal tract. Vancomycin-intermediate susceptible and vancomycin-resistant strains of *S. aureus* have been reported. Even in patients with vancomycin-susceptible MRSA, there have been reports of treatment failures with vancomycin, which is thought to be because of heterogeneous subpopulations with varying susceptibility to vancomycin (Liu and Chambers, 2003) or associated with the presence of the regulatory gene *agr* group II polymorphism (Moise-Broder *et al.*, 2004).

Linezolid has bacteriostatic activity against *S. aureus* and is approved for treatment of complicated skin and soft-tissue infections and pneumonia in adults and children. It is included in the oxazolidinone class of drugs and has parenteral and oral formulations, with good oral bioavailability. One retrospective analysis (Wunderink *et al*., 2003) of a database from a prospective randomized trial suggested enhanced effectiveness of linezolid compared with vancomycin in MRSA nosocomial pneumonia. The rationale may be related to enhanced concentrations of linezolid in lung epithelial lining fluid. The main adverse event associated with linezolid is bone marrow suppression, especially thrombocytopenia, which is increased with dosage and duration of therapy. Co administration of selective serotonin reuptake inhibitors and adrenergic drugs should be avoided because of central nervous system toxicity (Wunderink *et al*., 2003).

Community-acquired MRSA isolates often are susceptible to fluoroquinolones, trimethoprim/sulfamethoxazole, tetracyclines, and clindamycin. If the infection site is superficial, it may be reasonable to treat it with one of these agents. On the basis of minimal inhibitory concentration testing, community-acquired MRSA may be reported by the

laboratory as susceptible to clindamycin and resistant to erythromycin. In these cases, clindamycin may have inducible resistance that could emerge on therapy, so the laboratory can perform a double-disc diffusion test to check for inducible resistance and determine true susceptibility (Lewis, 2005). For MRSA, mupirocin can potentially eliminate MRSA from mucus membrane colonization. Previous reports identified a new antibiotic called platensimycin that had demonstrated successful use against MRSA (Bayston *et al*., 2007; Wang, 2007). In another separate study, allicin, a compound found in garlic mixed with a small quantity of silver carbonate was found to successively treat MRSA (Klein *et al*., 2007).

There are different strains of MRSA, with different degrees of resistance to the effects of various antibiotics. For resistant strains, a much higher dose over a much longer period, or the use of alternative antibiotic to which the bacteria has less resistance . Therefore, if antibiotic treatment is necessary, it should be guided by the susceptibility of the organism.. Majority of MRSA isolated from hospital and non-hospital sources in Nigeria were found to be sensitive to ciprofloxacin, clindamycin, rifampicin, chloramphenicol and erythromycin (Taiwo *et al*., 2004; Fusi Ngwa *et al.*,2007; Olowe *et al.*,2007).

2.4.2 Prevention and control of MRSA infection.

2.4.2.1 *Control of MRSA infection in humans*

2.4.2.2 Avoidance of MRSA patients or carriers

Avoidance of making direct contact with skin, clothing, and any items that come in contact with either MRSA patients, is not practical because such infected individuals or carriers are

not immediately identified . Rather,individuals can treat and cover any skin breaks and use excellent hygienic practices, e.g hand washing with soap after personal contact with carrirers and use of disposable items when treating MRSA patients (Buckingham, 2008).

2.4.2.3 Screening programs

Patient screening upon hospital admission, with nasal cultures, prevents the cohabitation of MRSA carriers with non-carriers , and exposureto infected surfaces. In some UK hospitals screening for MRSA is performed on every patient (Leonard and Markey, 2008).

2.4.2.4 Surface sanitization

Alcohol has proven to be effective surface sanitizer against MRSA. Quatenary ammonium compounds can be used in conjunction with alcohol to extend the longevity of the sanitizing action. In health care environments, MRSA can survive on surfaces and fabrics, including privacy curtains or garments worn *by* care providers. Complete surface sanitation is necessary to eliminate MRSA in areas where patients are recovering from invasive procedures. Testing patients for MRSA upon admission, isolating MRSA-positive patients, and terminal cleaning of patients roos and all other clinical areas they occupy is the current best practice for nosocomial MRSA (Leonard and Markey,2008).

2.4.2.5 Hand washing

After a successful pilot scheme to tackle MRSA, the UK National Health Service announced its Clean Your Hands campaign. Wards are required to ensure that alcohol-based rubs are placed near all beds so that staff can wash hands more regularly. A more effective strategy also, is to use an anti-microbial cleaner with persistant cleaning action such as chlorhexidine (Buckingham, 2008).

2.4.2.6 Decolonization

After contact with MRSA patients , doctors can shower using chlorhexidine (Hibiclens) or hexachlorophene (phisohex) antiseptic soap from head to toe , and apply mupirocin (Bacroban) 2% ointment into each nostril twice daily for 7 days, uing a cotton –tipped swab. House hold members are expected to follow the same decolonization protocol. Doctors may also use antibiotics such as clindamycin, doxycycline or trimethoprim/sulfamethoxazole. However, there is very little evidence that using more antibiotics actually have the effect of preventing recurrent MRSA skin infections (Buckingham, 2008).

2.4.3 Control and prevention of MRSA infection in animals

2.4.3.1. Precautions to be taken by Veterinarians

As in human medicine, hand hygiene is an integral part of the intervention of the spread of MRSA between animals and humans. Frequent hand washing and proper disinfection of hard surface and equipment between patients is essential. Hand sanitize should be provided in all consulting rooms and kennels to remind staff of the need for frequent hand sanitization. Uniforms, gloves, disposable aprons, and masks should be worn when changing dressing on infected wounds or to prevent potential contact with body fluids and contaminated tissues.

Eye protection is necessary if splashing or aerosols are expected (Leonard and Markey, 2008).

2.4.3.2 The therapeautic animal handlers

There should be good hand hygiene by all who encounter the animals, botyh before and after touching the animal. Licking should be prevented, as well as “shaking paw”.

When placing an animal on a bed, a clean towel or absorbent pad should be placed between the pet and the bed liners. No animals should be allowed to visit any animal in isolation units (Leonard and Markey, 2008).

Contact precautions recommended by the Centers for Disease Control and Prevention for hospitalized patients with MRSA include use of a private room, wearing gloves on entering the room, wearing a gown if contact with the patient or items in the room is anticipated, and handwashing on removal of the gloves. (Boyce et al., 2004) Despite these guidelines, nosocomial MRSA has been increasing in frequency (NNIS, 2002). It is not clear that these guidelines are effective in controlling MRSA (Cooper *et al.,* 2004 ; Cepeda *et al*., 2005) and well-designed randomized controlled studies have not been performed. Adherence to the guidelines has been suboptimal, and hand-washing in particular is inadequate. About 5 percent of hospitalized patients are colonized with MRSA. Advocates have suggested that more active surveillance using preemptive isolation and screening of patients, with stricter adherence to contact precautions and hand washing, may be more successful (Boyce *et al*., 2004).

Topical mupirocin is effective in reducing nasal colonization of *S. aureus*, (Doebbeling *et al.,* 1994) but the use of topical mupirocin to reduce the risk of surgical or nonsurgical infections caused by *S. aureus* has not been reliably successful (Perl *et al.,* 2002) .It also is not clear that topical mupirocin reduces MRSA colonization. Attempts at combining topical mupirocin with antibacterial baths (e.g., in chlorhexidine or systemic agents) deserve further study.

ESCHERICHIA COLI.

2.5.1 *Escherichia coli*

Escherichia coli (E. coli) consists of a diverse group of bacteria and is the head of the large bacterial family, *Enterobacteriaceae*, the enteric bacteria, which are facultatively anaerobic, Gram-negative rods that live in the intestinal tracts of animals (Kenneth, 2012) The *Enterobacteriaceae* are among the most important bacteria medically (Kenneth, 2012). Theodor Escherich, a German Paediatrician first described *E. coli* in 1885, as Bacterium coli commune, which he isolated from the faeces of new-borns, due to the fact it is found in the colon. It was later renamed *Escherichia coli (*Kenneth, 2012) and for many years the bacterium was simply considered to be a commensal organism of the large intestine until 1935 when a strain of *E. coli* was shown to be the cause of an outbreak of diarrhoea among infants.

Escherichia coli is a Gram-negative, facultative anaerobic, rod-shaped bacterium that is commonly found in the lower intestine of warm-blooded organisms also known as endotherms (Eckburg, 2005). *Escherichia coli* typically colonize the gastrointestinal tract of human infants within a few days after birth (James *et al.*, 2004; Kenneth, 2012), as the bacterium is ingested in foods or water or obtained directly from other individuals handling the infant (Kenneth, 2012). Usually, *E. coli* and its mammalian host coexist in good health and with mutual benefit for decades. These commensal *E. coli* strains rarely cause disease except in immune-compromised hosts or where the normal gastrointestinal barriers are breached (James *et al.*, 2004). Cells are typically rod-shaped, and are about 2.0 microns (μm) long and 0.5μm in diameter, with a cell volume of 0.6–0.7μm^3 (Kubitschek, 1990) and can live on a wide variety of substrates. The niche of commensal *E. coli* is the mucous layer of the mammalian colon. The bacterium is a highly successful competitor at this crowded

site, comprising the most abundant facultative anaerobe of the human intestinal microflora (James *et al.*, 2004) by being able to exploit its ability to utilize gluconate in the colon more efficiently than other resident species. This allows it to occupy a highly specific metabolic niche (Sweeney, 1996) with most human beings having a significant concentration of *E. coli* in their gut typically up to 1,000,000 per gram of faeces (Claudia, 2012). *E. coli* can benefit their hosts by producing vitamin K (Bentley and Meganathan, 1982) and by preventing the establishment of pathogenic bacteria within the intestine (Reid *et al.*, 2001).

Escherichia coli is: "A species that occurs normally in the intestines of humans and other vertebrates, is widely distributed in nature, and is a frequent cause of infections of the urogenital tract and of neonatal meningitis and diarrhoea in infants; enteropathogenic strains (serovars) of *E. coli* cause diarrhoea due to enterotoxin, the production of which seems to be associated with a transferable episome; it is the type species of the genus. Medilexicon's Medical Dictionary (2014).

E. coli and related bacteria constitute about 0.1% of gut flora (Eckburg *et al.*, 2005), and faecal–oral transmission is the major route through which pathogenic strains of the bacterium cause disease. Cells are able to survive outside the body for a limited amount of time and can respond to environmental signals such as chemicals, pH, temperature, osmolarity, which makes them ideal indicator organisms to test environmental samples for faecal contamination (Kenneth, 2012).

However, there are several highly adapted *E. coli* clones that have acquired specific virulence attributes, which confer an increased ability to adapt to new niches and allow

them to cause a broad spectrum of disease. These virulence attributes are frequently encoded on genetic elements that can be mobilized into different strains to create novel combinations of virulence factors, or on genetic elements that might once have been mobile, but have now evolved to become 'locked' into the genome. Only the most successful combinations of virulence factors have persisted to become specific pathotypes of *E. coli* that are capable of causing disease in healthy individuals. Three general clinical syndromes can result from infection with one of these pathotypes; enteric/diarrhoeal disease, urinary tract infections (UTIs) and sepsis/meningitis (James *et al.,* 2004). Six pathotypes are associated with diarrohea and collectively are referred to as diarrheagenic *E. coli*. These are: Shiga toxin-producing *E. coli* (STEC)—STEC may also be referred to as verocytotoxin-producing *E. coli* (VTEC) or enterohemorrhagic *E. coli* (EHEC), Enterotoxigenic *E. coli* (ETEC), Enteropathogenic *E. coli* (EPEC), Enteroaggregative *E. coli* (EAEC), Enteroinvasive *E. coli* (EIEC), Diffusely adherent *E. coli* (DAEC) (Nataro, 1998) (CDC, 2001). An additional animal pathotype, known as avian pathogenic *E. coli* (APEC), causes extra intestinal infections; primarily respiratory infections, pericarditis, and septicaemia of poultry (James *et al.,* 2004). The various pathotypes of *E. coli* tend to be clonal groups that are characterized by shared O (lipopolysaccharide, LPS) and H (flagellar) antigens that define serogroups (O antigen only) or serotypes (O and H antigens) (Whittam, 1996).

2.5.2 *Escherichia coli* O157:H7

Escherichia coli O157:H7 is an enterohemorrhagic strain of the bacterium *Escherichia coli* and a cause of illness through food (Karch *et al.,* 2005). *E. coli* serotype O157:H7 is a Gram-negative, rod-shaped bacterium. The "O" in the name refers to the cell

wall somatic antigen number, whereas the "H" refers to the flagella antigen (Dorlands Medical Dictionary, 2012).

Escherichia coli O157:H7 can be distinguished from normal flora by their possession of virulence factors such as exotoxins. Verocytotoxigenic (or verotoxigenic) *E. coli* (VTEC) produce a toxin that is lethal to cultured African green monkey kidney cells (Vero cells) but not to some other cultured cell types (Galal *et al.,* 2013). There are two major families of verocytotoxins, Vt1 and Vt2. A VTEC isolate may produce one or both toxins. Because verocytotoxin is homologous to the shiga toxins of *Shigella dysenteriae*, VTEC are also called shiga toxin-producing *E. coli* (STEC). Serotypes known to contain EHEC include *E. coli* O157:H7, the non-motile organism *E. coli* O157:H-, and members of other serogroups, particularly O26, O103, O111 and O145. *E. coli* O157 is closely related to *E. coli* O157:H7, but it not simply a nonmotile version of this organism; it also has a distinctive combination of phenotypic and virulence features (CFSPH, 2009). It has been estimated that *E. coli* O157:H7 causes about 73,500 cases of illness and 60 deaths annually in the U.S. (Naugle *et al.,* 2005) and fewer than 40 cells of *E. coli* O157:H7 can cause illness in most people as indicated by epidemiological studies (Harakudo *et al.*, 1997). Infection may lead to haemorrhagic diarrhea and to kidney failure (CDC, 2001).

E. coli O157:H7 can grow from around 7-10^{o}C to 50^{o}C, with an optimum temperature of 37^{o}C. Some EHEC can grow in acidic foods, down to a pH of 4.4, and in foods with a minimum water activity of 0.95 (WHO,1996).

2.5.3 History of *Escherichia coli* O157:H7

E. coli O157:H7 was first recognized as a human pathogen following two haemorrhagic colitis outbreaks in 1982 (Riley *et al.,* 1983). These outbreaks were traced to contaminated

undercooked hamburgers, and the illness was similar to other incidents in the United States and Japan. The aetiologic agent of the illness was identified as a rare O157:H7 serotype of *E. coli*. This serotype had only been isolated once before, from a sick patient in 1975 (Riley *et al.,* 1983). Shortly after *E. coli* O157:H7 was identified to be a human pathogen, (Karmali *et al.,*1983), observed that stool samples from children with haemolytic ureamic syndrome (HUS) contained a substance that was toxic to Vero (African green monkey kidney) tissue culture cells. This verocytotoxin was produced by *E. coli* isolates, with O157:H7 the prominent serotype causing infection.

EHEC are loosely defined by a combination of the symptoms they produce and the virulence factors they possess (Neill *et al.,* 1994). The disease-defining symptom of EHEC is haemorrhagic colitis or bloody diarrhoea. Not all EHEC infections, however, produce overt blood in the stools. While *E. coli* O157:H7 infections have a high rate of bloody stools, this may not be the case for other EHEC strains. All EHEC strains produce Shiga toxin 1 (Stx1) and/or Shiga toxin 2 (Stx2), also referred to as verotoxin 1 (VT1) and verotoxin 2 (VT2). The ability to produce Shiga toxin was acquired from a bacteriophage, presumably directly or indirectly from *Shigella*. The toxin is a 70,000 dalton protein composed of a single A subunit (32 kDal) and five B subunits (7.7 kDal). The B subunits provide tissue specificity by binding to globotriaosylceramide (Gb3) receptors on the surface of eucaryotic cells. The A subunit has an N-glycosidase that inactivates the 28S ribosome, thus blocking protein synthesis (Stirpe and Battelli, 2006). Endothelial cells high in Gb3 receptors are the primary target, accounting for the toxin's affinity for the colon and the renal glomeruli, associated with HC and HUS, respectively. The toxin can also indirectly damage cells by releasing cytokines, such as tumor necrosis factor. Toxin alone,

however, is not sufficient to make *E. coli* pathogenic; apparently nonpathogenic, *stx*-positive isolates are isolated frequently from humans. To be fully pathogenic, EHEC require the presence of other virulence markers. The *eae* chromosomal gene, for example, is ubiquitous among EHEC strai ns, encoding for an outer membrane protein associated with attachment. Although its role is unclear, the presence of a plasmid-encoded enterohemolysin is characteristic of EHEC (IFT, 2006).

There is a marked difference between *E. coli* O157:H7 and other pathogenic *E. coli*. In particular, the O157:H7 serotype is negative for invasiveness, adheres through the *E. coli* common pilus (ECP) (Rendón, 2007), and does not produce heat stable or heat labile toxins. In addition, *E. coli* O157:H7 is usually sorbitol negative, whereas 93% of all *E. coli* with human origin ferment sorbitol (Wells *et al.,* 1983). *E. coli* O157:H7 also lacks the ability to hydrolyze 4-methylumbelliferyl-β-D-glucuronide (MUG) (Doyle and Schoeni, 1984) and does not grow at 45 °C in the presence of 0.15% bile salts. Because of the latter characteristic, this serotype cannot be isolated by using standard faecal coliform methods that include incubation at 45 °C (Szabo *et al.*, 1986).

2.5.4 Geographic distribution and outbreak data of *E. coli* O157:H7

EHEC O157:H7 infections occur worldwide; infections have been reported on every continent except Antarctica (CSFPH, 2009). It tends to be reported more often from more developed countries but this may be an artifact caused by the paucity of sophisticated diagnostic laboratories in developing countries (Doyle *et al.,* 2006). The importance of some serotypes varies with the geographic area. Subsequently, a wide variety of food items have been associated with disease, including sausages (Ammon *et al.,* 1999) unpasteurized

milk (McIntyre *et al.,* 2002; Keene *et al.,* 1997) lettuce, (Hilborn *et al.,* 1999) cantaloupe melon, apple juice (Cody *et al.,* 1999) and alfalfa, raddish sprouts (Breuer *et al.,* 2001; Fukushima *et al.,* 1999). Facilitated by the extremely low infectious dose required for infection (estimated to be <100 cells), EHEC has also caused numerous outbreaks associated with recreational and municipal drinking water (Hrudey *et al.,* 2003) person- to-person transmission (Krishnan *et al.,* 1987), petting zoo (Pollett and Warshawsky, 1999) and farm visitations (Shukla *et al.,* 1995). A report indicates potential airborne transmission after exposure to a contaminated building (Varma, 2003). EHEC strains of the O157:H7 serotype is the most important EHEC pathogens in North America, the United Kingdom and Japan and in many countries.

2.5.5 Reservoir and sources of *E. coli* O157:H7 infection

Ruminants have been identified as the major reservoir of *E. coli* O157:H7, with cattle as the most important source of human infections (Doyle *et al.,* 2006). The association of *E. coli* O157:H7 with undercooked ground beef (Willshaw *et al.,* 1994) and raw milk (McIntyre *et al.,* 2002: Keene *et al.,* 1997) contact with cattle or animal products (Wilson *et al.,* 1998) led to investigations of the role of cattle as reservoirs of the pathogen. Other ruminants that can act as a reservoir of the organism include sheep (Barlow *et al.,* 2006; Beutin *et al.,* 1993), goats (Beutin *et al.,* 1995) and deer (Asakura *et al.,* 1998). *E. coli* O157:H7 has been detected in numerous other animals but none of them are considered a significant source of human infection. These include but not limited to horses, rats, swine, rabbits, poultry, etc (Doyle *et al.,* 2006).

Birds are thought to be possible transport hosts for *E. coli* O157:H7. Some wild birds harbour these bacteria (Wallace *et al.,* 1997). Pigeons might spread these bacteria

around a farm environment (Grossmann *et al.,* 2005) Flies and beetles, including houseflies and filth flies of several species (Alam and Zurek, 2004) and dung beetles (Xu *et al.,* 2003), collected on farms with animals shedding *E. coli* O157:H7, contain detectable levels of these bacteria. These insects frequent faecal deposits and may be able to transfer these bacteria to foods, feed and water. Fruit flies collected from a compost pile of decaying apples and peaches contaminated with *E. coli* contained these bacteria both internally and externally and were able to transfer them to wounds in uncontaminated apples. *E. coli* O157:H7 can grow rapidly in "apple wounds". Fruit flies could contribute to widespread contamination of wounded apples that may be processed into cider (Janisiewicz *et al.,* 1999).

E. coli O157:H7 in ruminant faeces may be directly ingested by persons interacting or working with animals. Faecal material may contaminate meat during slaughter, may enter lakes or drinking water sources by action of rain or wind, and may be deposited on fruits and vegetables inadvertently or by use of manure for fertilization. In addition, some animals may transport these bacteria from a faecal source to drinking water or foods (Doyle *et al.,* 2006).

Routes of human infection include: direct contact in cases where *E. coli* O157:H7 shed by infected animals may be spread to many surfaces in enclosures where ruminants are kept including the hides of other animals (Childs *et al.,* 2006). Person-to-person spread of *E. coli* O157:H7 has been the primary mode of infection in many outbreaks in day cares, schools and hospitals, particularly where there have been lapses in hygiene (Belongia *et al.,* 1993; Pennington 2000)

Food, beef, lamb, and mutton can be contaminated during slaughter and processing by exposure to faeces or hides containing *E. coli* O157:H7 (Doyle *et al.*, 2006). Milk from dairy cows, sheep, and goats may be contaminated with *E. coli* and other bacteria from the environment (Doyle *et al*., 2006).

Manure containing *E. coli* O157:H7 may be a source of contamination for vegetables or fruits that are not normally cooked before eating. Foods can also be contaminated with *E. coli* O157:H7 by cross-contamination during food preparation and by infected workers who do not practice good hygiene. Also, water used for drinking or recreation has been reported as the vehicle of infection (Swerdlow *et al.*, 1992).

2.5.6 Symptoms and effects of *E. coli* O157:H7 infection

The symptoms of *E. coli* O157:H7 infections are distinctive, they may be confused at any single phase with other diseases or conditions (Tarr, 1995) and humans can be infected asymptomatically (CFSPH, 2009). The incubation period for disease caused by EHEC O157:H7 ranges from one to 16 days. However, most infections become apparent 3-4 days after eating contaminated food (CFSPH, 2009), though shorter periods 1–2 days have been reported (Robert and Michael, 1997). Symptoms start with mild, non-bloody diarrhoea that may be followed by a period of "crampy" abdominal pain and short-lived fever. The initial diarrhoea increases in intensity during the next 24–48 h to a 4 - 10 days phase of overtly bloody diarrhoea accompanied by severe abdominal pain and moderate dehydration. Several life-threatening complications may occur in patients of which Haemolytic Uraemic Syndrome (HUS) is the most common; occurring in up to 16% of patients with hemorrhagic colitis (CFSPH, 2009).

The onset of HUS is approximately a week after the onset of gastrointestinal symptoms. Characteristic symptoms are pallor, intravascular destruction of red blood cells (microangiopathic haemolytic anemia), depressed platelet counts (thrombocytopenia), lack of urine formation (oligo-anuria), swelling (oedema), and acute renal failure. HUS occurs most often in children under the age of 10. Approximately 65–85% of children recover from HUS without permanent damage; however, long-term renal complications including hypertension, renal insufficiency and end-stage renal failure also occur. Half of HUS patients require dialysis, and the mortality rate is 3–5%. Other HUS-associated complications may include seizures, stroke, colonic perforation, pancreatitis, and hypertension (IFT, 2006). Approximately 15% of cases lead to early development of chronic kidney failure. Insulin-dependent diabetes may also persist in HUS patients (Robert and Michael, 1997). A small number of HUS cases may recur (Siegler *et al.*, 1993).

A second complication associated with *E. coli* O157:H7 is thrombotic thrombocytopenic purpura. This condition resembles HUS except that it generally causes less renal damage; has significant neurological involvement, e.g., central nervous system deterioration, seizures, and strokes; and is restricted primarily to adults (Boyce *et al.*, 1995).

2.5.7 Treatment and cure of infection

Treatment of hemorrhagic colitis is supportive, and may include fluids and a bland diet. Antibiotics are controversial and are usually avoided: they do not seem to reduce symptoms, prevent complications or decrease shedding, and they may increase the risk of HUS (CSFPH, 2009). The use of antimotility (antidiarrheal) agents in hemorrhagic colitis also seems to increase the risk for developing HUS. Patients with complications may require

intensive care including dialysis, transfusion and/or platelet infusion. Patients who develop irreversible kidney failure may need a kidney transplant (Aurora, 2006; CSFPH, 2009).

2.6 Prevention and Control of O157:H7 Infections

The prevention of infection requires control measures at all stages of the food chain, from agricultural production on the farm, to processing, manufacturing, handling and preparation of foods in both commercial establishments and the domestic environment (WHO, 1996).

The Hazard Analysis and Critical Control Point (HACCP) system continues to be the most effective means for systematically developing food safety protocols that can reduce the risk of EHEC infections (NACMCF, 1993). An important component of HACCP application in animal production is reducing the carriage of *E. coli* O157:H7 by animals. Two approaches that have potential are competitive exclusion (Schoeni and Doyle, 1992) and vaccination (Mayr *et al*., 2005).

It is assumed that the ultimate source of *E. coli* O157:H7 on carcasses is faecal contamination during animal production and slaughter operations. Adequate precautions should be undertaken during this operation to prevent contamination (IFT, 1997).

E. coli O157:H7 can be controlled readily through traditional thermal processing techniques; however, the organism's low infectious dose requires that processing be sufficient to assure a low probability of the pathogen surviving. Similarly, pasteurization would be expected to control *E. coli* O157:H7 in fruit juices (IFT, 1997). In addition, adherence to good food handling practices recommended for food service and home preparation represent the last line of defense for assuring prevention of *E. coli* O157:H7 infection.

Recommendations include;

I. Cook ground beef and venison thoroughly (minimum 160°F) before eating.

II. Drink only pasteurized milk and apple juice. Wash fresh fruits and vegetables thoroughly before eating.

III. Wash hands thoroughly after handling animals, particularly cattle, deer, goats, or dogs,

IV. Wash hands thoroughly after changing diapers or after providing care to children or adults suffering from a diarrheal disease,

V. Do not use fresh manure from ruminants to fertilize vegetables or fruits

VI. Avoid swimming in lakes or ponds used by cattle and drinking surface water that has not been properly treated to eliminate pathogens (IFT, 1997; WHO, 1996).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Materials

Apart from standard laboratory apparatus, equipment and reagents, the following were used;

Baird Parker agar and egg yolk tellurite	Microbact 12S Kit system
Mueller Hinton Agar	Microbact 12E Kit system (Oxoid®)
Nutrient Agar	Test tubes
Eosin Methylene Blue Agar	Cotton wool
Mannitol salt Agar (Oxoid ®)	Brain Heart Infusion Broth
DNase Agar	Antibiotic discs (Oxoid ®)
MacConkey Agar	Glass rod

Antibiotics (Oxoid®) Gentamicin (10µg), Oxacillin (5µg), Methicillin (10µg) Tetracycline (30µg), Ampicillin (10µg), Amoxicillin (25µg), Erythromycin (5µg), Chloramphenicol (30µg), Penicillin (10µg), Nitrofurantoin (300µg), Kanamycin ((30µg), Ciprofloxacin (5µg), Streptomycin (10µg) and Sulphamethoxazole/Trimethoprim (25µg).

3.2 Study Site

The study was carried out in Zaria LGA of Kaduna State. Zaria is located between longitude $11^0$04'N and latitude $7^0$42'E with an altitude of 550 – 700 meters above sea level and has a total land area of 300Km^{sq}. Zaria is characterized by a tropical climate, a monthly mean temperature ranging from 13.8 to 36.7^{0}C and an annual rainfall of 1092.8mm

(Agbogu *et al*., 2006). It has a population of 408, 198 and density of 1,360.7 km² (NPC, 2006). The main occupation of the people is agriculture; approximately 40-75% of the population's livelihood is from agriculture (ABU, 2000).

3.3 Study Design

The two local government areas of Zaria were the sampling locations. Local and industrial ice cream samples were collected based on convenience sampling from supermarkets, hawkers and ice cream sales outlets at various locations of Zaria: Zaria city and Tudun Wada in Zaria Local Government Area and Samaru, Kwangila and Sabon Gari in Sabon Gari Local Government Area.

Local ice cream is ice cream that is produced on small scale, usually in large containers and hawked on bicycles or sold at sale outlets by street vendors. The sale is usually limited to a particular region or location. On the other hand, industrial ice cream is ice cream that is branded and produced in large scale, packaged or dispensed in scoops or cones across the counter and usually sold at supermarkets or fast food joints. The sale of industrial ice cream is usually within national or international border.

All the ice cream Samples were collected twice weekly within a period of 3 months (April-July, 2013).

3.4 Sample Size

Sample size was determined using the formula of Thrusfield (1997) at 95% confidence level.

$$N = \frac{Z^2pq}{d^2}$$

N = sample size

Z = standard normal deviation of 95% confidence level. (1.96)

q = 1-p

p = prevalence (18%, Osamwonyi *et al.*, 2011)

d = level of significance (0.05)

Therefore, $N = \frac{1.96^2 \times (0.18 \times 0.82)}{(0.05)^2}$

$= \frac{0.5670}{0.0025} = 226.$

A total of 240 samples were collected for the study.

3.5 Isolation of *Staphylococcus aureus* and *Escherichia Coli* from Ice Cream in Zaria

3.5.1 Sample collection and handling

Ice cream samples from super markets and street vendors were purchased from super markets and sales outlets. At the supermarkets, samples were collected in sealed containers in which they were sold, while ice creams collected from street vendors were wrapped with sterile polythene bags and were appropriately labeled and transported on an ice pack to the Bacterial Zoonoses Laboratory of the Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, where they were bacteriologically analyzed within four hours of collection.

3.5.2 Sample preparation

Prior to taking samples for processing, the ice cream was placed in water bath at 45 degrees Celsius as per recommendations of Harrigan and McCance (1976). After thawing, an aliquot of 15 ml of liquid ice cream was pipetted out and transferred into a sterile glass bottle and covered. 1 ml of ice cream was pipetted out aseptically and transferred into a test tube containing 9 ml of sterile 0.1% peptone water to give a 1:10 dilution.. Further dilutions were prepared according to standard method given by American Public Health Association (1953).

3.5.3 Determination of total aerobic plate count.

For the determination of total aerobic plate count, 0.1ml of suitably diluted sample (10^3 - 10^4) was spread and inoculated on duplicate nutrient agar using a bent glass rod (hockey stick). The plates were kept in an incubator at 37 degrees Celsius for 24 hours. After incubation, all colonies on the plates were counted and expressed as CFU/ ml of ice cream.

3.5.4 Total coliform counts.

For the determination of total coliform counts, 0.1ml of a defined dilution (10^2-10^3) was transferred to MacConkey agar and spread evenly with sterile glass rod (hockey stick). All the agar plates were incubated at 37^0C for 24 hours. The total coliform counts were expressed as the number of coliforms or colony forming units per ml (CFU/ml).

3.5.5 Isolation procedure for *Staphylococcus aureus*.

About 10 ml of each ice cream sample was suspended in 90ml of Brain heart infusion broth supplemented with NaCl (6.5%) and incubated at 37^0C for 24 hours (Lee, 2003). A sterile loopful of the broth inoculum was streaked onto Baird Parker agar (BPA) and incubated at 37^0c for 24 hours. Colonies that appeared black or greyish-black were Gram-stained and subjected to biochemical tests (McFaddin, 2000).

3.5.6 Isolation procedure for *Escherichia coli*.

A sterile loopful of thawed ice cream from each sample was streaked onto Eosin Methylene Blue (EMB) agar plates and incubated overnight at 37^0C to detect the presence of *E. coli*. Colonies with greenish metallic sheen were subjected to biochemical tests for *E. coli*.

3.5.7 Identification of *Staphylococcus* and *E. coli* species

The colonies presumptively identified were subsequently subjected to Gram staining. Gram positive cocci that occurred singly and in pairs, tetrads, short chains and irregular grape-like clusters were suggestive of *Staphylococcus* species, while gram negative rods that occurred

singly or in short chains were suggestive of *Escherichia coli.* Conventional biochemical tests were carried out by the procedure described by Cowan and Steel (1965).

3.5.8 Conventional biochemical test for *Staphylococcus* and *E. coli.*

Conventional biochemical tests carried out for presumptive identification of *Staphylococus* isolates included gram staining, catalase, coagulase, sugar fermentation (xylose , lactose, sucrose and mannitol), haemolysis on 5 % sheep blood agar, pigmentation (Mannitol salt agar) and DNase activity (Barrow and Feltham, 1995).

Conventional biochemical tests carried out for presumptive identification of *E.coli* isolates included IMViC, namely: indole, methyl red, Voges-Proskauer and citrate utilization and Triple sugar Iron (TSI) and Urease tests. Confirmation of isolates was by the use of Microbact 12E (OXOID) which was used according to manufacturer's instructions. For the detection of *E. coli* O157:H7, suspect isolates of *E. coli* were plated on Sorbitol-MacConkey agar. Inoculated plates were incubated at 37^{o}C overnight and sorbitol non-fermenting (SNF) colonies were observed..

3.5.8.1 Catalase test.

Isolates suspected to belong to the genus *Staphylococcu*s were tested for their ability to produce the enzyme catalase. A drop of 3% hydrogen peroxide was placed on a slide and a colony of the suspected isolate was picked with a wire loop and suspended in the hydrogen peroxide. A positive reaction was indicated by the formation of bubbles (Cowan and Steele, 1965).

3.5.8.2 Coagulase Test

Isolates suspected to be *S.aureus* were tested for coagulase activity using rabbit plasma by the tube method. To prepare rabbit plasma, 10ml of rabbit blood was asceptically collected

into 0.3ml 10% EDTA. This was dispensed in sterile tubes and centrifuged at 1000g for 5 mins. The plasma was then collected and used for coagulase test. The test was carried out by adding 0.1 ml of an 18 to 24 hour tryptone soya broth culture of the test isolate to 0.5 ml of 10 fold diluted plasma in saline and incubated at 37^0C. The tubes were examined after 1, 2, 3 and 6 hours for coagulation. Those that were negative after 6 hours were left at room temperature overnight and re-examined. A positive reaction was indicated by definite clot formation.

3.5.8.3 Sugar Fermentation Tests.

These tests were carried out on isolates suspected to be *Staphylococcus*. 1g of each of the sugars and 1.5g of Andrade peptone was dissolved into 100ml of water from which 3 ml each were dispensed into universal bottles and autoclaved at 115^0C for 15 mins. A single isolated colony from Baird Parker agar was then inoculated into the sugar and incubated at 37^0C for 24 to 72 hours. A change in colour to pinkish red was indicative of fermentation. The organisms were subjected to the following sugar tests: xylose, mannitol, lactose and sucrose.

3.5.8.4 Pigmentation test on Mannitol Salt Agar (MSA)

The suspected *S. aureus* isolates were tested for pigment formation on MSA. The media was prepared according to manufacturer's instructions. The suspected organisms were streaked on the agar and incubated at 37^0C for 24 hours. *S. aureus* is characterized by the formation of yellowish colonies on this medium.

3.5.9 Identification of *S. aureus* and *E.coli* isolates based on conventional biochemical tests.

Isolates were identified as *S. aureus* on the basis of colony morphology when streaked onto Baird-Parker agar with smooth, black shiny isolated colonies, Gram positive cocci in clusters, catalase positive, coagulase production in rabbit plasma, beta haemolysis on 5% sheep blood agar, fermentation of mannitol, sucrose and lactose and non fermentation of xylose, DNase production, yellow pigment formation on mannitol salt agar and the use of commercial identification system (Microbact 12S).

Isolates with a greenish metallic sheen appearance on Eosin Methylene Blue Agar were identified by IMViC, namely: indole, methyl red, Voges-Proskauer and citrate utilization, urease and Triple Sugar Iron and Urease tests. Confirmation of isolates was done by the use of Microbact 12E (OXOID).

3.5.10 Micobact testing of *Staphylococcus* and *E.coli* species.

The Microbact 12S and 12E test systems are standardised micro-substrate systems designed to emulate conventional biochemical substrates used for identification of *Staphylococcus* and *E.coli* species respectively. Reactions occurring during the incubation period are demonstrated by easily interpreted colour changes. Organism identification is based on pH change and substrate utilization. Once each reaction was recorded on the report form, each block of three reactions was converted into a numeric value to obtain 4 digit of the microbact code which was fed into a computer aided identification package for interpretation. An isolate with 75% probability for particular species was considered positive while an isolate with less than 75% probability does not meet the criteria for acceptance by the microbact test system and was considered negative. The substrates for Microbact 12S are: maltose, mannitol, mannose, sucrose, trehalose, N-acetyl glucosamine, arginine, urease, beta-glucosidase, alkaline phosphotase, beta-glucuronidase and beta-

galactosidase: while the substrates for Microbact 12E are: Lysine, Ornithine, H_2S, Glucose, Mannitol, xylose, ONPG, indole, urease, Voges-Proskauer, citrate, and TDA.

3.6 Determination of the Susceptibility of *S. aureus* and *E.coli* to Commonly Used Antimicrobial Agents.

The susceptibilities of 19 *S.aureus* and 5 *E.coli* isolates to 13 and 10 antimicrobial agents respectively were determined by the Kirby-Bauer disk diffusion method on Mueller Hinton agar (Bauer *et al*., 1966), and interpreted according to the recommendations of Clinical and Laboratory Standards Institute (CLSI) (2004).

The drug panel of impregnated disks contained gentamicin (10µg), oxacillin (5µg), methicillin (10µg) tetracycline (30µg), ampicillin (10µg), amoxicillin (25µg), erythromycin (5µg), chloramphenicol (30µg), penicillin (10µg), nitrofurantoin (300µg), kanamycin ((30µg), trimethoprim (25µg) and ciprofloxacin (5 µg) for the susceptibility of *S. aureus* .

For the antibiotic susceptibility of *E.coli*, the drug impregnated disks contained ampicillin (10µg), streptomycin (10µg), chloramphenicol (30µg), ciprofloxacin (5µg), gentamicin (10µg), erythromycin (5µg) tetracycline (30µg), nitrofurantoin ((300µg), kanamycin (30µg) and sulphamethoxazole/trimethoprim (25µg).

The disks were placed individually on the surface of inoculated agar plates using a disk dispenser (Oxoid) and incubated overnight.

The zones of inhibition were measured with a ruler and interpreted according to CLSI (2004).

3.6 Data Analysis.

Prevalence of *Staphylococcus* and *E.coli* species in ice cream were processed using the Statistical Package for Social Sciences (SPSS), version 20, and calculated t-test. All statistical tests were performed and interpreted at an alpha of 0.05. Paired Sampled t-test was carried out to compare the total aerobic plate counts in ice cream of various origins.

CHAPTER FOUR
RESULTS

4.1 Results of Bacteriological Quality of Industrial and Locally Produced Ice Creams Sold in Zaria.

4.1.1 Results of total aerobic plate count (TAPC) of industrial and local ice creams

A total of 240 ice creams comprising 111 industrial and 129 locally produced ice creams were collected from different parts of Zaria and analyzed for total aerobic bacteria and total coliform counts. The results showed that the TAPC per ml of ice cream was highest in locally produced ice cream than in industrially produced ice cream.

Using a significance level of 0.05, the paired sampled T-test result gave a *P*- value of 0.034 (Table 4. 1.1), which showed that there was a significant difference between the TAPC of industrial ice cream and the TAPC of local ice cream.

4.1.2 Results of TCC of industrial and local ice creams

Table 4.1.2 shows the mean total coliform counts of industrial ice cream and local ice cream. The mean of the total coliform counts obtained from both industrially produced and locally produced ice cream samples respectively were 5.69×10^3 CFU/ml and 4.73×10^3 CFU/ml. Using a significant level of 0.05, the paired sampled T- test result gave a P- value of 0.311 which showed that there is no significant difference between the total coliform counts of industrial ice cream and locally produced ice cream.

Table 4.1.1 Paired sampled T-test on the difference between total aerobic counts of industrially and locally produced ice cream.

Variables	**Mean(10^7 CFU/ml)**
Industrial ice cream	1.14
Local ice cream	2.015

P- Value = 0.034 ($p < 0.05$)

Table 4.1.2 Paired sampled T-test on the difference between total coliform counts of industrially and locally produced ice cream samples.

Variables	**Mean(10^3 CFU/ml)**
Industrial ice cream	5.69
Local ice cream	4.73

P- Value = 0.311 ($p > 0.05$)

4.2 Frequency and Percentage Occurrence of *S. aureus* and *E. coli in* Ice cream Sold in Zaria.

Table 4.2 shows the frequency of occurrence of *S. aureus* and *E.coli* isolated from both locally produced and industrially produced ice cream samples. In 111 industrially produced ice creams examined, *S.aureus* was present in 5 samples and *E.coli* was present in 2 samples. Whereas in the 129 locally produced samples examined, *S. aureus* occurred in 14 samples and *E.coli* occurred in 3 samples. The percentage of *S. aureus* and *E .coli* in industrially produced ice cream were 4.5% and 1.8% respectively. The percentage occurrence of *S. aureus* and *E .coli* in locally produced ice cream was 10.8% and 2.3% respectively.

Using a significant level of 0.05, the paired sampled T-test result gave a p- value of 0.0000 and 0.374 for *S.aureus* and *E.coli* respectively, which implies that there is a significant difference between the prevalence of *S. aureus* present in industrial and locally made ice cream and there is no significant difference between the frequency of occurrence of *E.coli* present in industrially and locally produced ice creams in Zaria.

A total of nineteen (19) *S. aureus* and five (5) *E. coli* were isolated from all the 240 ice cream samples collected and analyzed in the study. A total 5 (26.3%) of the 19 *S. aureus* isolated were obtained from ice creams produced industrially while 14 (73.6%) of the *S. aureus* were from ice cream produced by local manufacturers. Also, 2 (40%) of the 5 (five) *E. coli* isolated were obtained from ice creams produced industrially while the remaining 3 (60%) were from local ice creams analysed.

Table 4.2 Prevalence of *S. aureus* and *E.coli* in Ice creams Sold in Zaria Metropolis

Ice cream origin	Total no. Sampled	No. positive for *S.aureus* (%)*	No. Positive for *E.coli(%)***
Industrial	111	5(4.5)	2(1.8)
Local	129	14(10.8)	3(2.3)
Total	240	19(7.91)	5(2.08)

*Paired sampled T- test p- value = 0.000, ** Paired sampled T- test p-value = 0.374

4.3 Absence of *E.coli* O157:H7

Suspected isolates of *E. coli* were plated on Sorbitol MacConkey agar and incubated at 37ºC overnight to observe for the prescence of non sorbitol fermenting colonies which were expected to appear colourless. After 24 hours incubation, the growth on plates were observed and all colonies were seen to be sorbitol fermenters (pinkish colonies) which were therefore an indication for the absence of *E.coli* O157:H7 in the ice cream samples.

4.4 Antibiotic Resistance profile of the *S. aureus* and *E. coli* isolated from Ice creams in Zaria

All the 19 coagulase positive *S. aureus* and 5 *E. coli* isolates were subjected to susceptibility testing with a panel of 13 and 10 antimicrobial agents respectively. The antibiotic susceptibility profiles of S. *aureus* shows that all the isolates were resistant to one or multiple antibiotics. However, 100% of the *S. aureus* isolates were resistant to ampicillin, oxacillin and penicillin, 89.4% to tetracycline, 84.2% to amoxicillin and nitrofurantoin, 73.6% to erythromycin , 63.1% to chloramphenicol, ciprofloxacin, and methicillin , 15.8% to kanamycin and 26.3% to sulphamethoxazole/trimethoprim, 5.2% to gentamicin. Gentamicin and kanamycin were the most effective antibiotics whereas penicillin, ampicillin and oxacillin were the least effective in the *in vitro* susceptibility tests (Table 4.4).

The antimicrobial resistance patterns are shown in Table 4.5. The most common patterns for *S. aureus* were: erythromycin – ciprofloxacin – chloramphenicol- methicillin- amoxicillin- ampicillin- penicillin- nitrofurantoin- oxacillin – tetracycline with a frequency of 10.5%, while other patterns occurred once. Pencillin, ampicillin and oxacillin resistance were common among the various patterns observed.

Table 4.4: Susceptibility of 19 *S. aureus* Isolated from Ice creams in Zaria, Nigeria to 13 Antimicrobial agents.

S/N	Antimicrobial agents	No. (%) Susceptibility
1	Amoxicillin	3(15.7)
2	Ampicillin	0
3	Chloramphenicol	4 (21.1)
4	Ciprofloxacin	4 (21.1)
5	Erythromycin	1 (5.3)
6	Gentamicin	18 (94.7)
7	Kanamycin	13 (68.4)
8	Methicillin	5 (26.3)
9	Nitrofurantoin	1 (5.2)
10	Oxacillin	0
11	Penicillin	0
12	Sulphamethoxazole/trimethoprim	7 (36.8)
13	Tetracycline	1 (5.3)

Table 4.5: Antimicrobial Resistance Patterns of 19 *S. aureus* Isolates from Ice creams in Zaria, Nigeria.

S/N	Pattern	Frequency
1	E, CIP ,C, AMC, AMP, P, F, OX, TE	1
2	E,CIP, C, MET, AMC, AMP, P, F, OX, TE	2
3	E, CIP, C, MET, AMC, AMP, K, P, F, OX, TE	1
4	E, C, MET, AMC, AMP, P, F, OX	1
5	CIP, SXT, C, MET, AMC, AMP, P, F, OX,	1
6	E, MET, AMC, AMP, P, F, OX, TE	1
7	E, C, MET, AMC, AMP, K, P, F, OX, TE	1
8	C, MET, AMP, P, F, OX, TE	1
9	E, CIP, AMC, AMP, P,OX, TE	1
10	E,CIP, C, MET, AMP, K, P, F, OX, TE	1
11	E, CIP, SXT, P, OX TE	1
12	CIP, SXT, C, AMC, AMP, P, F, OX, TE	1
13	E, CIP, MET, AMC, AMP, P, F, OX, TE	1
14	CIP, MET, AMC, AMP, P, F, OX, TE	1
15	E, AMC, AMP, P, F, OX, TE	1
16	AMC, AMP, P, F, OX, TE	1
17	E, CIP, SXT, C, AMC, AMP, P, TE	1
18	E, SXT, C, MET, AMC, AMP, P, F, OX, TE	1

4.6 Multiple Antibiotic Resistance Index of *S. aureus* Isolates from Ice creams Isolated in Zaria, Nigeria

MAR indices were calculated for isolates of *S.aureus* from each of the sources by the method of Kaspar *et al*.1990), as follows:

$$\text{MAR index} = \frac{\text{Number of antibiotics to which the isolates are resistant}}{\text{Total number of antibiotics tested.}}$$

The results showed that all isolates had MAR indices ranging from o.46 to 0.84.

4.7 Antimicrobial Resistance Patterns of 5 *E. coli* Isolates from Ice creams in Zaria, Nigeria.

The antimicrobial resistance pattern for 5 *E. coli* isolated from the ice cream samples from Zaria metropolis showed that each of the 5 isolates had a different pattern. Erythromycin and ampicillin were common among the various patterns observed.

Table 4.6 Mar Index Analysis of the *S. aureus* Isolates Obtained from Ice Creams in Zaria, Nigeria (Test against 13 Antibiotics)

S/N	ISOLATE NO	NO. OF ANTIBIOTICS TO WHICH THE ISOLATE WAS RESISTANT	MAR INDEX
1	SL5	9	0.69
2	SS6	10	0.76
3	POT6	10	0.76
4	FK2	11	0.84
5	SL10	8	0.61
6	SSC1	10	0.76
7	FK2	8	0.61
8	POT9	10	0.76
9	SL3	7	0.53
10	SL2	7	0.53
11	SML9	10	0.76
12	STW3	6	0.46
13	FK10	9	0.69
14	CR6	9	0.69
15	SB7	8	0.61
16	FK8	7	0.53
17	FS2	6	0.46
18	SL9	8	0.61
19	SL6	10	0.76

Table 4.7: Susceptibility of 5 *E. coli* Isolated from Ice creams in Zaria, Nigeria to 10 Antimicrobial Agents.

S/N	Antibiotic	No. (%) Susceptibility
1	Ciprofloxacin	5 (100)
2	Nitrofurantoin	5 (100)
3	Kanamycin	4 (80)
4	Sulphamethoxazole/trimethoprim	2 (40)
5	Chloramphenicol	2 (40)
6	Gentamicin	1 (20)
7	Streptomycin	1 (20)
8	Ampicillin	0
9	Tetracycline	0
10	Erythromycin	0

Table 4.8 Mar Index Analysis of the *E. coli* Isolates Obtained from Ice Creams in Zaria. (Tested Against 10 Antibiotics)

S/N	Isolate No	No. Of Antibiotics To Which The Isolate Was Resistant	Mar Index
1	FK8	6	0.6
2	POT1	3	0.3
3	MB2	7	0.7
4	SB6	3	0.3
5	SB5	4	0.4

CHAPTER FIVE

DISCUSSION

All 240 samples of ice cream samples examined, showed positive growth for Total Aerobic Plate Count (TAPC) and 150 (62.5%) samples showed positive growth for total coliform counts (TCC). Results of bacterial counts revealed a mean value of 2.015 x 10^7 CFU/ml (range; 4.4 x 10^5 to 7.84 x 10^7CFU/ml) for locally produced ice cream. The values were higher than those obtained from industrially produced ice cream which had a mean count of 1.14 x 10^7 CFU/ml (range; 2.0 x 10^4 to 5.56 x10^7 CFU/ml). The analysis between industrial and local ice cream with respect to total aerobic counts revealed that there was significant difference ($p< 0.05$) in the ice cream from industrial and locally produced origin. This implied that significantly higher total aerobic counts may be more commonly associated with local ice cream than the industrial ice cream.

According to International Standard Organization (ISO, 1995), total viable counts of ice cream should not exceed 10^5 CFU/g . In the current study, it was observed that the TVC was within the prescribed limit in 69 (62.1%) for industrially produced ice cream; however, none of the locally produced ice cream conformed to the range of 10^0-10^4 observed by Ikenebomeh and Ogaguvia (1993). This is in line with the study of Ambily *et al.* (2012) of the bacteriological quality of ice cream marketed in Thrissur town, Kerala, India which revealed that the TVC was within the prescribed limit for all industrial ice cream brands (1.2 x 10^2 to 8.2 x 10^3 CFU/ml) and none of the street vended samples conformed to the standards. Other investigators elsewhere have similarly reported that local ice cream have significantly higher levels of bacterial contamination than commercial ice cream (El-Sharef *et a*l., 2005).

Yusuf *et al*. (2013) also conducted a study to assess the bacteriological quality of ice cream offered for public consumption in Bauchi, Nigeria showed that the bacterial counts in all the products fell within acceptable limits. Kumar *et al.* (2011) also conducted a study to assess the bacteriological quality of locally made open scoop ice cream sold by street hawkers in different areas of Jalandhar city , Punjab and and found that the samples from all the areas showed heavy contamination of bacteria ranging from 0.1×10^9 to 10.2×10^9 CFU/g. Although the samples analysed in this study had more counts than those obtained by Ambily *et a*l.(2012) and Yusuf *et al*. (2013) in their respective studies, we recorded lesser counts than that of Kumar *et al.* (2011) and were however not within the safe limits. Unsatisfactory detections of TAB have also been reported in many other countries. In the study of Mas'ud (1989) from Pakistan, 72% of the samples had TVC counts over 10^6 CFU/g. From the city of Bangalore in India, Sarada and Begum (1991) found 53% of the samples had counts of 10^5 cfu/g. Warke *et al.(*2000) in India also reported 33% of the samples having excessive counts of 10^6 cfu/ml, which did not comply with the Indian standard.

The results found in those studies and present work indicate that ice creams sold to consumers from large containers, exposed to the open air have high microbial load indicating low hygienic quality of the products in many countries. These high counts may originate from the initial micro flora of raw milk and other ingredients and their quality, the environment, insufficient heat treatment and poor personal hygiene. Also, there are no regulatory controls on the microbial loads of ice cream from these sources and therefore are susceptible to poor sanitary practices during processing and at the sale outlets.

Better microbiological quality of branded ice cream samples may be attributed to pasteurization of the ice cream mix and clear industry guidance on microbiological standards. Furthermore, industrially made ice creams studied were all sold in packages, locally made ice cream were often sold open by vendors in carts. Contamination of the mix, temperature abuse of the mix, and inadequate cleaning of the equipment and premises of sale can lead to TVC counts in excess of 10^6 cells (Harrigan, 1998).

Results for total coliform counts revealed a mean value of 4.73 $x10^3$ CFU/ml for locally produced ice cream (range,1.0 x 10^3 to 3.00 x 10^4 CFU/ml) and mean count of 5.69 x 10^3 CFU/ml for industrially produced ice cream (range;1.0 x 10^3 to 3.5 x 10^4 CFU/ml).

The findings of paired sampled T- test revealed that there was no significant difference between the industrial and local ice cream for total coliform counts and the observed significant level (p-value) was 0.311.

According to Frozen Confections regulations (2014), any frozen confections for sale should not contain more than $5x10^4$ bacteria per gram or more than 100 coliform organisms per gram. The coliform standards for ice cream should not exceed 10CFU/ml (James and Jay, 1978). In the present study, 39.7% and 79.9% of ice creams analysed from industrial and local producers respectively contained coliforms above the prescribed limit. There have been many reports on the occurrence of coliforms in ice cream. In previous studies, various rates of coliforms which do not comply with the TCC standard were reported as 88.4% and 96.4% by Toklu and Yaygin (2000) and Kanbakan *et al.* (2004) respectively. Likewise, in some developing countries such as Pakistan and India, high rates of coliform contamination were also reported at 66% (Mas'ud 1989) and 100% (Warke *et al*., 2000)

respectively. High coliform counts in ice cream samples may indicate insufficient heat treatment, unhygienic or low hygienic materials or tools used the use of contaminated water and failure to adhere to good manufacturing practices. Plant hygiene and personnel hygiene should be suspected when coliform counts of the product are high (Warke *et al*., 2000). Kanbakan *et al*. (2004) reported that inadequate cleaning of the hands, same person selling ice cream and collecting money, open cones and unclean cloth for cleaning the scoops can contribute to high coliform counts.

Coliforms and *E.coli* are indicators for faecal contamination and their presence is particularly worrying given the fact that their level of presence in food has been described as an index of food hygiene (Frazier and Westhoff, 1992; Jay, 1996). *E.coli* was found in 8 (3.38%) out of 240 samples of ice creams. On the contrary, higher incidence rates of *E.coli* than the present study were also reported in previous studies. Yusuf *et al*. (2013) reported a prevalence of 35% *E.coli* in Bauchi; Osamwonyi (2011) reported 30% and Masud (1989) found that 46% of the samples were contaminated with *E.coli* in Pakistan. Anil *et al*. (2012) reported a rate of 13.3% in Trinidad and Tobago. Various incidence rates of 3.33%, 22% and 70% have also been reported by Erol *et al*. (1998), Kivanc *et al*. (1994), and Toklu and Yaygin (2000) in Turkey, in which 60% of the *E. coli* detected were isolated from locally produced ice cream . The levels of coliforms found and the presence of *E.coli* in ice cream samples may indicate a lack of good manufacturing practice during the production, and ice cream produced in domestic or catering premises may be relatively important vehicles for the transmission of gastrointestinal diseases. Hygienic distribution and retail storage practices for ensuring microbiological safety of ice cream sold in open containers should be implemented. Likewise personal hygiene should be emphasized as Kanbakan *et*

al. (2004) stated that coliform contamination of the hands of personnel in ice cream sales department were higher than on the hands of factory workers (Warke *et al*., 2000).

Coliforms and *E.coli* may contaminate ice cream during processing through contaminated water or faecal materials as a result of poor sanitary practices. Pipeborne treated water processed by Nations Water Sewage Authority was reported to have unacceptable coliform levels (Kruy *et al.* 2001; Welch *et al.* 2000). It was demonstrated that containers and tanks used to store water at homes contributed to coliform contamination of water (Welch *et al.* 2000). There is therefore a possibility that the water used during the production and sale of ice cream particularly locally made ice cream may have served as vehicle for *E.coli* and other coliforms to contaminate the ice cream studied. It is relevant to mention that at the sale outlets, particularly when ice cream is sold in open containers which require dispensing of the product for sale, scoop water may serve as vehicle for coliform contamination of ice cream as reported (Wouafo *et al*., 1996; Wilson *et al.*,1997 ; Kanbakan *et al.*, 2004).

E.coli O157 was not detected amongst isolates of *E.coli* studied, which may be an indication that ice cream sold in Zaria is not expected to be an important source of gastroenteritis due to Verocytotoxigenic *E.coli* (VTEC). Previously, Ansay and Kasper (1997), analysed raw cow milk in U.S.A and Heuvelink *et al*. (1998) in Netherlands and found no *E. coli* O157: H7. However, El-Sharef *et al. (*2005) reported that 1.3% of homemade ice cream sold in Libya were contaminated by *E.coli* O157. Also, There are number of studies from different countries concerning the incidence of *E. coli* O157: H7 isolation on a variety of foods. Abdul Raouf *et al*. (1996) reported that 6% of raw cow milk examined in Egypt were contaminated with *E. coli O157*: H7. Milk-borne and ice cream associated gastroenteritis due to VTEC strains are well documented in the literature (

Gray,1995; Karmali, 1989; Kanbakan, 2004). Presence of *E. coli* O157:H7 is usually attributed to the fact that it is usually made from raw milk, in addition to the unhygienic way of processing, handling and selling.

Out of the total of 240 samples of ice cream examined, 19 (8%) were positive for *S. aureus*, with 14(73.6%) of the isolates from locally produced ice cream while 5 (26.6%) were from industrially produced ice cream (p-value= 0.0000). On the other hand, lower or higher incidence rates of *S. aureus* isolation than the results in this study have been reported. In Ankara, Turkey, *S. aureus* was present in 10% of ice cream samples. Warke *et al.* (2000) also reported a detection rate of 100% in India. The presence of *S. aureus* may be due to either insufficient pasteurisation of milk or contamination due to human exposure from the nostrils where it is commonly found, hands, skin and clothing of handlers. Coughing, talking and sneezing produces droplets which could settle on ice cream during transportation, storage and retailing (Warke *et al.*,2000).

There were 18 antimicrobial resistance patterns observed with *S. aureus* isolates which showed multiple resistance. All the *S. aureus* isolates were of resistance to penicillin, ampicillin and oxacillin, which appeared in all the patterns and a high percentage were resistant to tetracycline and erythromycin which appeared in 17 of the 18 patterns. The frequency of resistance to penicillin and ampicillin were similar to the reports of Kwaga and Adesiyun (1984) and Umoh (1990) who reported widespread resistance of *Staphylococcus* to ampicillin and pe nicillin. The resistance of all the isolates to penicillin, oxacillin and ampicillin is in accordance with the known natural resistance of *Staphylococcus* to the β-lactams. In another research in Nigeria, a high level of resistance to tetracycline was

established which was attributed to the excessive use of the drug in Nigeria (Kabir *et al.*, 2005).

The in vitro susceptibility of the isolates to gentamicin (94.7%) is suggestive of the likely efficacy of the drug in treating *S.aureus* infections and may also reflect the fact that they may be less abused in the study environment.

The *S. aureus* isolates also exhibited 100% multiple drug resistance as all had high multiple antibiotic resistance indexes (MAR) which were greater than 0.2. This indicates misuse of the antibiotics in the study area (Kabir *et al.*, 2005).

Antimicrobial susceptibility of *E. coli* to 10 antimicrobial agents showed all the isolates to be susceptible to ciprofloxacin and nitrofurantoin and high percentage (80%) were susceptible to gentamicin and kanamycin. However, all the isolates were resistant to 7 out of 10 antimicrobial agents. This resistance may be due to indiscriminate use of antimicrobial agents.

There were 5 antimicrobial resistance patterns observed for *E.coli*. All the resistance patterns showed multiple resistance with high frequency of resistance to ampicillin, erythromycin and tetracycline.

CHAPTER SIX

CONCLUSION AND RECOMMENDATION

6.1 Conclusion

Overall 198 (82.5%) and 147 (61.3)% of ice cream samples analysed from Zaria metropolis were contaminated with aerobic bacteria and coliforms respectively above limit prescribed by ISO. Also, significantly higher Total Aerobic Counts were more commonly present in local ice creams than industrial ice creams.

Ice cream sold in Zaria metropolis especially from local manufacturers were found to be more commonly contaminated with significant levels *S. aureus* and *E.coli,* some of which displayed resistance to several antimicrobial agents. The findings in this study point to the possible role of ice cream as a vehicle for food borne disease and the transmission of multiple drug resistant *Staphylococcus aureus* and *Escherichia coli.*

6.2 Recommendations

The following recommendations are proffered based on the findings in the current study.

- To improve quality of ice cream, good manufacturing practices are mandatory especially at all post pasteurization steps.
- The use of good quality raw materials and automated machines to minimize handling will be effective in assuring quality.
- Adoption of good sanitation practices and application of HACCP principles in the industry along with education of workers and vendors selling ice cream on personal

hygiene and maintenance of proper temperature of ice cream storage to further improve the quality of the product.

- There is need for continuous surveillance and enforcement of national standard for ice cream by the regulatory authorities and also periodic monitoring of operations to ensure that they maintain proper and hygienic production, storage and distribution of ice cream.

- Rational use of antibiotics in animals is advocated to reduce spread of multi-drug resistant *Staphylococcus aureus* and *E.coli*.

REFERENCES

Abdul-Raouf, U.M., Ammar, M.S. and Beuchat, L.R. (1996). Isolation of *E.coli O157:H7* from some Egyptian foods. *International Journal of Food Microbiology, 29:423-426.*

Abdul-Raouf, U.M., Beuchat, L.R., and Ammar, M.S. (1993). Survival and growth of *Escherichia coli* O157:H7 on salad vegetables. *Applied Environmental Microbiology*, 59**:**19 99–2006.

Agbogu, V.N., Umoh, V.J., Okuofu, C.A., Smith, S.I. and Ameh, J.B. (2006). Study of the bacteriological and physicochemical indicators of the pollution of surface waters in Zaria, Nigeria. *African Journal of Biotechnology,* 5: 732-737.

Ahmadu Bello University (ABU) (2000). Zaria Master Plan, Development of Urban and regional planning, ABU Zaria, Nigeria

Aidara-Kane, A., Ranaivo, A., Spiegel, A., Catteau, M. and Rocourt, J. (2000). Microbiological quality of street-vendor ice cream in Dakar. *Dakar Medical Journal*, 45:20-24

Alam, M.J. and Zurek, L. (2004). Association of *Escherichia coli* O157:H7 with Houseflies on a Cattle Farm. *Applied and Environmental Microbiology,* **70**:7578–7580.

Ambili, R. and Beena, A.K. (2012). Bacteriological quality of ice cream m arketed in Thrissur town, Kerala, India. *Veterinary World*, Vol 5(12) 738-741.

American Public Health Association (1953). *Standard Methods for Examination of Dairy Products*, 10th edition, APHA. Inc., New York. Pp 147-148.

Ammon, A., Petersen, L.R. and Karch, H. (1999). A Large Outbreak of Hemolytic Uremic Syndrome caused by an Unusual Sorbitol-Fermenting Strain of *Escherichia coli* O157:H7. *Journal of Infectious Diseases,* 179:1274–1277..

Anderson, D.J., Sexton, D.J., Kanafani, Z.A., Auten, G. and Kaye, K.S. (2007). Severe surgical site infection in community hospital: Epidemiology, key procedures, and the changing prevalence of methycillin- resistance *Staphylococcus aureus* infection. *Control of Hospital Epidemiology*, 28:1047-1053.

Anil P., Nadira, S., Karla, G. and. Adesiyun, A. A. (2012) .Evaluation of the bacteriological quality of ice cream sold in Trinidad. *WFL* Publisher Science and Technology. *Journal of Food, Agriculture & Environment, Vol.10 (2): 39-45. 2012.*

Ansay, S.E. and Kaspar C.W. (1997). Survey of retail cheeses and dairy processing environments and raw milk for *E.coli* O157*:H7*. *Applied Microbiology,* 25: 131-134.

Aurora, A.S. (2006). *Escherichia coli* 0157:H7 and the spinach scare. *Food Safety and Technology*, 32:341-350

Baptiste, K.E., Williams, K., Willams, N.J., Wattret, A. and Clegg, P.D. (2005). Methicillin resistant *staphylococci* in companion animals. *Emerging Infectious Diseases,* 11: 1942-1944.

Barlow, R.S., Gobius, K.S. and Desmarchelier, P.M. (2006). Shiga toxin-producing *Escherichia coli* in ground beef and lamb cuts: Results of a one-year study. *International Journal of Food Microbiology*, 111(1):1–5.

Barrow, G.I., Feltham, K.A. (1993) Cowan and Steel manual for the identification of Medical Bacteria 3rd edition. Cambridge.

Bauer, A.W., Kirby, W.M. and Sheris, J.K.T. (1996). Antimicrobial susceptibility testing by a standard single disc method. *American Journal of Clinical Pathology*, 45:493-496.

Bayer, A.S., Bolger, A.F. and Taubert, K.A. (1998). Diagnosis and management of an infective endocarditis and its complications. *Circulation,* 98 (25): 2936-2948.

Bayston, R., Ashrat, W. and Smith, T. (2007). Triclosan resistance in MRSA expressed as small colony Variants: a Novel Mode of Invasion of Susceptibility to Antiseptics. *Journal of Antimicrobials and Chemotherapy,* 59: 848-853.

Belongia, E.A., Osterholm, M.T., Soler, J.T., Ammend, D.A., Braun, J.E. and McDonald, K.L. (1993). Transmission of *Escherichiacoli* O157:H7 Infection in Minnesota Child Day-care Facilities. *Journal of American Medical Association,* 269:883–888.

Bentley, R. and Meganathan, R. (1982). Biosynthesis of Vitamin K (menaquinone) in Bacteria. *Microbiology*, 46 (3): 241–80.

Beutin, L., Geier, D., Steinruck, H., Zimmermann, S. and Scheutz, F. (1993). Prevalence and some Properties of Verocytotoxin (Shiga-like Toxin)-Producing *Escherichia coli* in Seven Different Species of Healthy Domestic Animals. *Journal of Clinical Microbiology,* 31:2483–2488.

Boyce, J.M., Havill, N.L., Kohan, C., Dumigan, D.G. and Ligi, C.E. (2004). Do infection control measures work for methicillin-resistant *Staphylococcus aureus*? *Infection Control and Hospital Epidemiology*, 25:395–401.

Breuer, T., Benkel, D. H., Shapiro, R.L., Hall, W.N., Winnett, M.M., Linn, M.J., Neimann, J., Barrett, T. J., Dietrich, S., Downes, F.P., Toney, D.M., Pearson, J. L., Rolka, H., Slutsker, L. and Griffin, P.M. (2001). A Multistate Outbreak of *Escherichia coli* O157:H7 infections linked to Alfalfa sprouts grown from contaminated seeds. *Emerging Infectious Diseases,* 7:977–982.

Brown, D.F.J., Edwards, D.I., Hawkey, P.M., Morrison, D., Ridgway, G.L., Towner, K.J. and Wren, M.W.D. (2005). Guidelines for the laboratory diagnosis and susceptibility testing of methicillin resistant *Staphylococcus aureus* (MRSA*). Journal of Antimicrobial Chemotherapy,* 56:1000–1018.

Buckingham, S.C. (2008). Prevention of recurrent MRSA skin infections: What you need to know. Available from http://www.consultant.com.

Cappareli, R., Parlato, M., Borriello, G., Salvatore, P. and Lanneli, D. (2007). Experimental phage therapy against *Staphylococcus aureus* in mice. *Antimicrobial Agents and Chemotherapy,* 51 (8):2765-2773.

Cardano, A.M. and Rocourt, J. (2001). Occurrence of *Listeria monocytogenes*in food in Chile. *International Journal of Food Microbiology*, 70:175-178

Carla, V., Varaldo, E. and Alessandro, C. (2009). Methycillin-resistant *Staphylococcus aureus* USA400 clone, Italy. *Journal of Emerging Infectious Disease*, 15:1080-6059.

Carter, A.P., Clemons, W.M., Brodersen, D.E., Morgan-Warren, R.J., Wimberly, B.T. and Ramakrishnan, V. (2000).”Functional insights from the structure of the 30S ribosomal subunits and its interactions with antibiotics”. *Nature*, 407(6802):340-348). PMID 11014183.

Castellani, A. and Chalmers, A.J. (1919). *Manual of Tropical Medicine*, Williams Wood and Co., 3rd ed, New York, Pp 415-245.

CDC, Centers for Disease Control and Prevention (1996). Outbreak of Escherichia coli O157:H7 infections associated with drinking unpasteurized commercial apple juice—British Columbia, California, Colorado, and Washington, October 1996. *Morbidity and Mortality Weekly Report, 45***:**975.

CDC, Centers for Disease Control and Prevention (2001). Outbreaks of Escherichia coli O157:H7 infections among children associated with farm visits-Pennsylvania and Washington, 2000. *Morbidity and Mortality Weekly Report*, 50**:**293-*297.*

Cefai, C., Ashurst, S. and Owens, C. (1994). Human carriage of methicillin resistant *Staphylococcus aureus* linked with pet dog. *Lance*t, 44: 539–540

Cepeda, J.A., Whitehouse, T., Cooper, B., Hails, J., Jones, K. and Kwaku, F. (2005). Isolation of patients in single rooms or cohorts to reduce spread of MRSA in intensive-care units: prospective two-centre study. *Lancet,* 365:295–304.

CFSPH, Center for Food Security and Public Health (2009). Enterohaemorrhagic *E. coli* infections. http://www.cfsph.iastate.edu/Factsheets/pdfs/e_coli.pdf. Retrieved 07/08/2014.

Champagne, C.P., Lang, R.R., Roy, D. and Mafu, A.A. (1994). 'Psychotrophs in dairy products, their effects and their controls. *Food Science and Nutrition*, 34: 1-30.

Chebremedhin, B., Olugbosi, M.O., Raji, A.M., Layer, F., Bakare, R.A., Konig, B. and Konig, W. (2009). Emergence of community -associated methycillin resistant *Staphylococcus aureus* with a unique resistance profile in South West Nigeria. *American Society for Microbiology*, 47:2975-2980

Childs, K.D., Simpson, C.A., Warren-Serna, W., Bellenger, G., Centrella, B., Bowling, R.A., Ruby, J., Stefanek, J., Vote, D.J., Choat, T., Scanga, J.A., Sofos, J.N., Smith, G.C. and Belk, K. E. (2006). Molecular characterization of *Escherichia coli* O157:H7 hide contamination routes: Feedlot to harvest. *Journal of Food Protection,* 69:1240–1247.

Chongtrakool, P., Ito, T., Ma, X.X., Kondo, Y., Trakulsomboon, S., Tiensasitorn, C., Jamklang, M., Chavalit, T., Song, J.H. and Hiramatsu, K. (2006). Staphylococcal Cassette Chromosome *Mec* (SCC *mec*) Typing. *Antimicrobial Agents Chemotherapy,* 50(3): 1001-12.

Clark, J.R., and March, J.B. (2006). Bacteriophages and Biotecnology: vaccines, gene therapy and antibacterials". *Trends in Biotechnology*, 24:212-218.

Claudia, S. (2012). Food Safety Zoonoses and Food Borne Illnesses, World Health Organization Report. www.who.int/foodsafety/consumer/5keys/en

Clinical Laboratory Standard Institute (2004). Performance Standards for antimicrobial disk susceptibility testing. CLSI document M100-514. CLSI, Wayne, PA., USA.

Cody, S.H., Glynn, M.K., Farrar, J.A., Cairns, K.L., Griffin, P.M., Kobayashi, J., Fyfe, M., Hoffman, R., King, A.S., Lewis, J. H., Swaminathan, B., Bryant, R.G. and Vugia, D.J. (1999). An Outbreak of *Escherichia coli* O157:H7 Infection from Unpasteurized Commercial Apple Juice. *Annals of Internal Medicine,* 130:202–209.

Cooper, B.S., Stone, S.P., Kibbler, C.C., Cookson, B.D., Roberts, J.A. and Medley, G.F. (2004). Isolation measures in the hospital management of methicillin

resistant *Staphylococcus aureus* (MRSA): systematic review of literature. *Biotechnology and Medical Journal*, 329:533-538

Cosgrave, S.E. (2009). Initial low- dose – gentamicin for *Staphylococcus aureus* bacteremia and endocarditis is nephrotoxic. *Clinical Infectious Diseases.* 48:713-721.

Cowan, S.T., and Steel, K.J. (1965). *Manual for the Identification of Medical Bacteria.* Cambridge University Press, Cambridge. Uk.

Cuny, C., Nathaus ,R., Layer ,F., Strommenger, B., Altmann, D. and Witte, W. (2009). Nasal colonization of humans with methicillin-resistan *Staphylococcus aureus* (MRSA) CC398 with and without exposure to pigs. *PLoS One*, 274:680

Curran, J.P. and Al-Salihi, F.L. (1980). "Neonatal Staphylococcal scalded skin syndrome: Massive outbreak due to an unusual phage type*"* *Paediatrics*, 66(2):290. PMD 6447271. http://www.cirp.org/library/complications/curran1/.

de Lencastre, H. and Tomasz, A. (1994). Reassessment of the number of auxiliary genes essential for expression of high-level methicillin resistance in *Staphylococcus aureus*. *Antimicrobial Agents and Chemotherapy*, 38:2590–2598.

De Neeling, A.J., Van den Broek, M.J., Spalburg, E.C., Van Santen-Verheuvel, M.G., Dam-Deisz, W.D., Boshuizen, H.C.,Van de Giessen, A.W., Van Duijkeren, E. and Huijsdens, X.W. (2007). High prevalence of methicillin resistant *Staphylococcus aureus* in pigs. *Veterinary Microbiology*, 122:366-372.

De Sousa, M.A. and Lencastre, H. (2004). Evolution of sporadic isolates of methycillin resistant *Staphylococcus aureus* (MRSA) and other similarities to isolates of community- acquired MRSA. *Journal Of Clinical Microbiology*, 41:3806-3815.

Denis, O., Suetens, C., Hallin, M., Catry, B., Ramboer, I., Dispas, M., Willems, G., Gordts, B. (2009). Methicillin-resistant *Staphylococcus aureus* ST398 in swine farm personnel, Belgium. *Emerging Infectious Diseases*, 15:1098-1101.

Dijuretic, T., Walls, P., Nichols, G. (1997). General outbreaks of infectious and intestinal diseases associated with milk and dairy products in England and Wales:1992-1996, *Communicable Diseases Report*, REV. CDR Rev.7, R41-R45.

Doebbeling, B.N., Reagan, D.R., Pfaller, M.A., Houston, A.K., Hollis, R.J. and Wenzel, R.P. (1994). Intranasal mupirocin ointment. A prospective cohort study of *Staphylococcus aureus* carriage. *Annals of internal medicine*, Pp 154:1505-1508

Dorland, W.A. (2012). Dorland's illustrated medical dictionary. Philadelphia. 32nd edition.

Doyle, M.E. John, A., Charles, W.K. and Ronald, W. (2006). Human illness caused by *Escherichia coli* 0157:H7 from food and non-food sources. Food Research Institute Briefings.

Doyle, M.P. and Schoeni, J.L. (1984). Survival and Growth Characteristics of *Escherichia coli* Associated with Hemorrhagic Colitis. *Applied and Environmental Microbiology,* 48 (4): 855–6.

Eckburg, P.B., Bik, E.M., Bernstein, C.N., Purdom, E., Dethlefsen, L., Sargent, M., Gill, S.R., Nelson, K.E. and Relman, D.A. (2005). Diversity of the Human Intestinal Microbial Flora. *Science,* 308: 5728.

EFSA (European Food Safety Authority) (2012). Scientific Report of EFSA: Technical specifications on the harmonised monitoring and reporting of antimicrobial resistance in methicillin-resistant *Staphylococcus aureus* in food-producing animals and food. *EFSA Journal*, 10:2897.

Elahi, A.T.M., Habib, S., Rahman, M.M., Rahman, G. I. and Bhuiyan, J. U. (2002). Sanitary Quality of Commercially Produced Ice Cream Sold in the Retail Stores, Pakistan. *Journal of Nutrition*, 1(2): 93-94.

El-Sharef, N., Ghenghesh, K.S., Abognah, Y.S., Gnan, S.O. and Rahouma, A. (2005). Bacteriological Quality of ice cream in Tripoli, Libya. *Food Control*, **17**:637-641

Erol, I., Kuplulu, O., Siriken, B. and Celik, T.H. (1998). Ankara'daki cesitli pastanelere ait dondurmalarin mikrobiyolojik kalitelerin belirlenmesi. *Journal of Veterinary and Animal Science*, 22: Pp 345.

Ferreira, J.P., Anderson, K.L., Correa, M.T., Lyman, R. Ruffin, F., Reller, L.B. and Fowler,V.G.Jr. (2011). Transmission of MRSA between companion animals and infected human patients presenting to outpatient medical care facilities. *PLoS One,* 6:26978.

Francois, P., Harbarth, S., Huygne, N. (2008). Methycillin resistant *Staphylococcus aureus*. Geneva, Switzerland, 1993-2005: *Emerging Infectious Diseases*, 14:304-307.

Frazier, W.C. and Westhof , D.C. (1992). *Food Microbiology,* McGraw-Hill, New York. Pp295.

Frozen Confections Regulation (2014). Bacteriological Standard of Frozen Confections for Sale, Section (6), Cap 132 AC, section56.

Fukushima, H., Hashizume, T., Morita, Y., Tanaka, J., Azuma, K., Mizumoto, Y., Kaneno, M., Matsu-ura, M. O., Konma, K. and Kitani, T. (1999). Clinical experiences in Sakai City Hospital during the massive Outbreak of Enterohaemorrhagic *Escherichia coli* O157 Infections in Sakai City. *Pediatrics International,* 41:213–217.

Fusi Ngwa, C. N., Egri-okwaji, M. T., Odugbemi, T. and hoha, E. (2007). A study on pediatric Methicillin-resistance Staphylococcus aureus in Lagos, Nigeria. *International Journal of Biological and Chemical Sciences,* 1(1):54-60

Galal, H.M., Hakim, A.S. and Sohad, M.D. (2013). Phenotypic and virulence genes screening of *Escherichia coli* strains isolated from different sources in delta Egypt. *Life Science Journal,* 10: 2

Garcia-Alvarez, L., Holden, M.T.G. and Lindsay, H. (2011). Methycillin-resistant *Staphylococcus aureus* with a novel *mec* A homologue in human and bovine populations in the UK and Denmark: a descriptive study. *Lancet Infectious Disease,* 11:595-603.

Grave, K., Torren-Edo, J. and Mackay, D. (2010). Comparison of the sales of veterinary antibacterial agents between 10 European countries. *Journal of Antimicrobial Agents and Chemotherapy*, 65:2037-2040.

Gray, L.D. (1995). *Escherichia, Salmonella, Shigella* and *Yersinia*. In Murray, P. R., Baron, E. J., Pfaller, M. A., Tenover, F. C. and Yolken, R. H. (eds). *Manual of Clinical Microbiology,* ASM Press, Washington. Pp. 450-456.

Grossmann, K., Weniger, B., Baljer, G., Brenig, B. and Wieler, L.H. (2005). Racing Ornamental and City Pigeons carry Shiga Toxin Producing *Escherichia coli* (STEC) with different Shiga Toxin Subtypes, Urging Further Analysis of their Epidemiological Role in the Spread of STEC. *Berlin Munch Veterinary Weekly Journal,* 118:456–463.

Harakudo, Y., Konuma, H., Iwaki, M., Kasuga, F., Sugitakonishi, Y., Ito, Y. and Kumagai, S. (1997). Potential Hazard of Radish Sprouts as a Vehicle of *Escherichia coli* O157:H7. *Journal of Food Protection*, 60:1125–1127.

Haran, K.P., Godden, S.M., Boxrud, D., Jawahir, S., Bender, J.B. and Sreevatsan, S. (2012). Prevalence and Characterization of *Staphylococcus aureus*, including methicillin-resistant *Staphylococcus aureus*, Isolated from bulk tank milk from Minnesota dairy farms. *Journal of Clinical Microbiology,* 50:688-695.

Harrigan, M.F. and McCance, M.E. (1976). *Laboratory Methods of Food and Dairy Microbiology*. Academic press, London, England. Pp 193.

Harrigan, W.F. (1998). Detection and enumeration of pathogenic and toxigenic organism - pathogenic *Escherichia Coli*. In: Harrigan, W.F. *Laboratory methods in food Microbiology*. 3rd Ed, Academic press, London. Pp.186-189.

Heuvelink, A.E., Bleumink, B. Biggelaar, M.C., Giffel, T.E, Beumer, R.R. and Boer, E.D. (1998). Occurence and Survival of Verocytotoxin producing *E.coli* O157:H7 in raw cow's milk in the Netherlands. *Journal of Food protection, 61:1597-1601.*

Hilborn, E. D., Mermin, J. H., Mshar, P. A., Hadler, J. L., Voetsch , A., Wojtkunski, C., Swartz, M., Mshar, R., Lambert-Fair, M. A., Farrar, J. A., Glynn, M.K. and Slutsker, L. (1999). A Multistate Outbreak of *Escherichia coli* O157:H7 Infections Associated with Consumption of Mesclun Lettuce. *Annals of Internal Medicine*, 159:1758–1764.

Hrudey, S.E., Payment, P., Huck, P. M., Gillham, R.W. and Hrudey, E.J. (2003). A Fatal Waterborne Disease Epidemic in Walkerton, Ontario: Comparison with other Waterborne Outbreaks in the Developed World. *Water Science and Technology*, 47:7-12.

IFT, Institute of Food Technology (2006). Foodborne Disease Significance of Escherichia coli O157:H7 and other Enterohemorrhagic *E. coli*. Retrieved 07/08/2014

Ikenebomeh, M.J. and Ogaguvia, R.A. (1993). Microbiology of ice cream. *Nigerian Journal of Microbiology*, 9:40-46.

Inal, J.M. (2003). " Phage Therapy: A reappraisal of bacteriophages as antibiotics" *Achivum Immunologiae et Therapiae Experimentalis,* 51 :237-244.

Inge H.M., Bram M.W.D., Paul H.M.S., Joyce H.C.W., Robert, R., Alex, B., Nicole, I., Carlo, V., Peter, H.J.K. and Jan, A.J.W.K. (2007). Methicillin resistant *Staphylococcus aureus* in meat products, the Netherlands. *Emerging Infectious Diseases Journal,* 13: 1080-6059.

ISO (1995). Recommendation of the meeting of the subcommittee, international organisation for standardization on meat and meat products. ISO/TC-36/SC.6. *The Netherlands,* 10-18.

Ito, T., Ma, X.X., Takeuchi, F., Okuma, K., Yuzawa, H. and Hiramatsu K. (2003). Novel type v Staphylococcal cassette chromosome recombinase (ccrC). *Antimicrobial Agents and Chemotherapy*, 48(7) 2637-51.

James, B.K., James, P.N. and Harry, L.T.M. (2004). *Escherichia coli.* isolated from major food animals and their potential transmission to humans. *Applied and Environmental Microbiology,* 2:123Pp.

James, M. and Jay, J.M. (1978). Indices of food sanitary quality and microbiological standards and criteria; *Modern Food Microbiology*, 2nd edition. P 311.

Janisiewicz, W.J., Conway, W.S., Brown, M.W., Sapers, G.M., Fratamico, P. and Buchanan, R. L. (1999). Fate of *Escherichiacoli* O157:H7 on fresh-cut apple tissue and its potential for transmission by fruit flies. *Applied and Environmental Microbiology,* 65:1–5.

Jay, J.M. (1996). In: *Modern Food Microbiology*, 5th edition. Van Nostrand Reinhold Co. New York. Pp 661.

Kabir, J.A., Olukayode, C.X, Chidi, E.O., Christopher, C.I. and Kehinde, A.F. (2005). Screening of crude extract of six medicinal plants used in Southwest Nigeria on orthodox medicine for anti-methicillin –resistant *Staphylococcus aureus* (MRSA) activity. *Biomedical Central Complementary and Alternative Medicine*, 5:6.

Kanbakan, U., Con, A.H. and Ayar, A. (2004). Determination of microbiological contamination sources during ice cream production in Denizli, Turkey. *Food Control*, **15**:463-470.

Karch, H., Tarr, P. and Bielaszewska, M. (2005). Enterohaemorrhagic*Escherichia coli* in Human Medicine. *International Journal of Medical Microbiology*, 295: 405–18.

Karmali, M. A. (1989). Infection by verocytotoxin-producing *Escherichia coli. Clinical Microbiology Review*, 2:15-38.

Karmali, M.A., Steele, B.T., Petric, M. and Lim, C. (1983). Sporadic Cases of Hemolytic Uremic Syndrome Associated with Faecal Cytotoxin and Cytotoxin-Producing *Escherichia coli* in Stools. *The Lancet*, 1: 619–620.

Katayama, Y., Ito, T. and Hiramatsu, K. (2000). A new class of genetic element, staphylococcus cassette chromosome mec, encodes methicillin resistance in *Staphylococcus aureus*. *Antimicrobial agents and Chemotherapy,* 44:1549-1555.

Keene, W.E., Hedberg, K., Herriott, D.E., Hancock, D.D., McKay, R. W. and Barrett, T. (1997). A Prolonged Outbreak of *Escherichia coli* O157:H7 Infections Caused by Commercially Distributed Raw Milk. *The Journal of Infectious Diseases*, 176: 815-818.

Kenneth T. (2012). *Todar's Online Textbook of Bacteriology*. Retrieved 07/08/2014. http://textbookofbacteriology.net/e.coli.html

Khanna ,T., Friendship, R., Dewey, C. and Weese, J.S. (2007). Methicillin resistant *Staphylococcus aureus* colonization in pigs and pig farmers. *Veterinary Microbiology*, Vol. 128:-303.

Kitai, S., Shimizu, A., Kawano, J., Sato, E., Nakano, C., Uji, T. and Kitagawa, H. (2005). Characterization of methicillin resistant Staphylococcus aureus isolated from retail raw chicken meat in Japan. *Journal of Veterinary Medical Science*, 67:107-110.

Kivanc, M., Yamac, M., Kunduhoghu, B. (1994). The microbiological analysis of ice cream sold in Eskisehir. *City food*, 14: 317-322.

Klein, E., Smith, D.L., Laxminarayan, R. (2007). "Hospitalizations and deaths caused by methicillin-resistant *Staphylococcus aureus*, United States. 1999-2005". *Emerging Infectious Disease*, 13 (12):1840-6.

Kloos, W.E. (1997). Taxonomy and systematic of *staphylococci* indigenous to humans. In: K.B. Crossley and G.L. Archer (ed.). Church hill livingstone, New York. *The Staphylococci in Human DiseaseS*, PP 113-137.

Kloos, W.E. and Bannerman, T.L. (1995*). Staphylococcus* and *Micrococus*. In: P.R Murray, E.J. Barron, M.A. Pfaller, F.C.Tenover, and R.H.Yolken (Editors), *Manual of Clinical Microbiology*, 6th edition. *American society for microbiology*, Washington D.C. Pp 264-282.

Kocak, C., Akan, M. and Yardemci, H. (1998). Bacteriological quality of ice cream marketed in Ankara. Ankara University. *VeterinerFakultesiDergisi*, 45: 131-134.

Kock, R., Harlizius, J., Bressan, N., Laerberg, R., Wieler, L.H., Witte, W., Deurenberg, R.H. and Voss, A. (2009). Prevalence and molecular characteristics of methicillin – resistant *Staphylococcus aureus* (MRSA) among pigs on German farms and import of livestock- related MRSA into hospitals. *European Journal of Clinical Microbiology and Infectious Diseases*, 28:1375-1382.

Korzeniowski, O. and Sande, M.A. (1982). Combination Antimicrobial Therapy for *Staphylococcus aureus* Endocarditis in Patients addicted to Parenteral drugs and in non-addicts: a Prospective Study. *Annals of Internal Medicine*, 97:496-503.

Krishnan, C., Fitzgerald, V.A., Dakin, S.J., and Behme, R.J. (1987). Laboratory Investigation of Outbreak of Hemorrhagic Colitis caused by *Escherichia coli* O157:H7. *Journal of Clinical Microbiology*, 25:1043–1047.

Kruy, S. L., Soares, J.L., Ping, S. and Sainte-Marie, F.F. (2001). Microbiological quality of ice cream sold on the streets of Phnom Penh. April 1996 -April 1997. *Bullettin Society and Pathology of Exotic Diseases*, 94:411- 414.

Kumar, R., Yadav, B.R. and Singh, R.S. (2011). Antibiotic resistance and pathogenicity factors in *Staphylococcus aureus* isolated from mastitic Sahiwal cattle. *Journal of Biological Sciences,* 36:175–188.

Kwaga, J.K.P. and Adesiyun, A.A. (1984). Antibiograms of *Staphylococcus aureus* isolates from some ready -to-eat products. *Journal of food protection*, 47:865-867.

Kwon, N., Park, K., Jung, W., Youn, H., Lee, Y., Kim, S., Bae, W. and Lim, J. (2006). Characteristics of methicillin resistant *Staphylococcus aureus* isolated from chicken meat and hospitalized dogs in Korea and their epidemiological relatedness. *Veterinary Microbiology,* 117:304-312.

Laurent, F., Chardon, H., Haenni, M., Bes, M., Reverdy, M.E., Madec, J.Y., Lagier, E., Vandenesch, F. and Tristan, A. (2012). MRSA harboring mecA variant gene and mecC. France. *Emerging Infectious Diseases* 18:1465-1467.

Lee, J.H. (2003). Methicillin (Oxacillin)-resistant *Staphylococcus aureus* strains isolated from major food animals and their potential transmission to humans. *Applied and Environmental Microbiology,* 69:6489-6494.

Leonard, F.C. and Markey, B.K. (2008). Meticillin-resistant *Staphylococcus aureus* in animals: a review. *Veterinary Journal,* 175(1):27-36.

Levine, D.P., Fromm, B.S. and Reddy, B.R. (1991). Slow response to vancomycin or vancomycin + rifampin in MRSA endocarditis. *Annals of internal medicine*, 115(9):674-80.

Lewis, J.S. (2005). Inducible clindamicin resistance in *Staphylococci*: Should clinicians and microbiologists be concerned? *Clinical Infectious Diseases*, 40:280-285.

Liu, C. and Chambers, H.F. (2003). *Staphylococcus aureus* with heterogenous resistance to vancomycin : Epidemiology, clinical significance and critical assessment of diagnostic methods. *Antimicrobial agents and Chemotherapy*, 47:3040-3045.

Lukjancenko, O., Wassenaar, T.M. and Ussery, D.W. (2010). Comparison of 61 sequenced *Escherichia coli* genomes. *Microbiology of E.coli*, 60 (4): 708-720.

Mas'ud, T. (1989). Microbiological quality and public health significance of ice cream. *Journal of Pakistan Medical Association,* 39:102-104.

Mayr, U.B., Haller, C. and Haidinger W. (2005). Bacterial Ghosts as an Oral Vaccine: a Single Dose of *Escherichia coli* O157:H7. Bacterial Ghosts Protects Mice Against Lethal Challenge. *Infection and Immunity*, 73(8): 4810- 4817.

McFaddin, J.F. (2000). *Biochemical Tests for the Identification of Medical Bacteria*, 3rd ed. Lippincott Williams & Wilkins, Philadelphia, PA. PP 94-95.

McIntyre, L., Fung, J., Paccagnella, A.M., Isaac-Renton, J., Rockwell, F. and Emerson, B. (2002). *Escherichia coli* O157 Outbreak Associated with the Ingestion of Unpasteurized Goat's Milk in British Columbia, 2001. *Canadian Communicable Disease Report*, 28.

McKay, A.M. (2008). Antimicrobial resistance and heat sensitivity of oxacillin-resistant, mecA positive *Staphylococcus spp*. From unpasteurized milk. *Journal of Food Protection,* 71:186-190.

Medilexicon Medical Dictionary (2014). //medical-dictionary.thefreedictionary.com/*E.coli*

Microbact® 2000 Identification Package V 2.03(Windows (tm) MB1244a. Oxoid R, Ltd, Wade road, Basingstoke Hants Rg24 8pw UK.

Moise-Broder, P.A., Sakoulas, G., Eliopoulos, G.M., Schentag, J.J., Forest, A. and Moellering, R.C. (2004). Accessory gene regulator group 11 polymorphism in methicillin- resistant *Staphylococcus aureus* is predictive of failure of vancomycin therapy. *Clinical Infectious Diseases*, 38(1700-5).

Montanari, M.P., Tonin, E., Biavasco, F. and Varaldo, P.E. (1990). Further characterization of borderline methicillin-resistant *Staphylococcus aureus* and analysis of penicillin binding proteins. *Antimicrobial Agents and Chemotherapy*, 34:911–913.

NACMCF (1993). Generic HACCP for Raw Beef.National Advisory Committee on Microbiological Criteria for Foods, U.S. Dept. of Agriculture. *Food Microbiology*, 10: 449–488

Nataro, J.P. and Kaper, J.B. (1998). Diarrheagenic *Escherichia coli:* A comprehensive review of the pathogenesis, epidemiology, diagnosis and clinical aspects of diarrhoeagenic *E. coli. Clinical Microbiology*, 11: 142–201

National Nosocomial Infections Surveillance System. National Nosocomial Infections Surveillance (NNIS) System Report, data summary from January 1992 to June 2002. *American Journal of Infectious Disease Contro*l, 30:458.

National Population Commission (NPC), Abuja, Nigeria (2006). http://www.population.gov.ng.

Naugle, A.L., Holt, K.G., Levine, P., and Eckel, R. (2005). Food Safety and Inspection Service Regulatory Testing Program for *Escherichia coli* O157:H17 in Raw Ground Beef. *Journal of Food Protection,* 68:462–468.

Neill, M.A., Tarr, P.I., Taylor, D.N., and Trofa, A.F. (1994). *Escherichia coli*. In; *Foodborne Disease Handbook, Diseases by Bacteria*, Vol. 1, ed. Y.H. Hui, J.R. Gorham, K.D. Murrell, and D.O. Cliver, Marcel Dekker, Inc., New York. Pp. 169–213.

Normanno, G., Corrente, M., Salandra, G.l., Dambrosio, A., Quaglia, N.C., Parisi, A., Santagada, G., Firinu, A., Crisetti, E. and Celano, G.V. (2007). Methicillin resistant *Staphylococcus aureus* (MRSA) in foods of animal origin product in Italy. *International Journal Food Microbiology,* 117:219-22

Ojokoh, A.O. (2006). Microbiological Examination of ice cream sold in Akure. *Pakistan Journal of Nutrition,* 5(6): 536-538, 2006.

Olayinka, B.O., Olayinka, A.T., Obajuluwa, A.F., Onaolapo, J.A. and Olurinobi, P.F. (2003). Absence of mec A gene in methicillin resistant *Staphylococcus aureus* isolates. *African Journal of Infectious Diseases*, 3(2): 49-56.

Olowe, O.A., Eniola, K.I.T., Olowe, R.A. and Olayemi, A.M. (2007). Antimicrobial susceptibility and betalactamase detection of MRSA in Osogbo, Nigeria. *Nature and Sciences,* 42: 250-254.

Orscheln, R.C., Hunstad, D.A., Fritz, S.A., Loughman, J.A., Mitchell, K., Storch, E.K., Gaudreault, M., Sellenriek, P.L., Armstrong, J.R. and other authors (2009). Contribution of genetically restricted, methycillin –susceptible strains to the ongoing epidemic of community-acquired *Staphylococcus aureus* infections. *Journal of Infectious Diseases,* 49:536-542.

Osamwonyi, O.U., Obayagbona, O.N. and Olisaka, F. (2011). Evaluation of the bacteriological qua lity of ice cream sold in some locations in Benin City. *Continental Journal of Food, Science and Technology*, 5(3):6-11.

Pennington, T.H. (2000). VTEC: Lessons Learned from British Outbreaks. *Journal of Applied Microbiology*, 88:90S–98S.

Perl, T.M., Cullen, J.J. and Wenzel, R.P. (2002). Intranasal mupirocin to prevent post operative *Staphylococcus aureus* infections. *New English Journal of Medicine*, 346:187-197.

Peterson, A.E., Meghan, F., Kathleen, G., Jullian, G.A., Wallace, H., Greene, L.B. and Andrew, W. (2012). Molecular and phenotypic characteristics of health-care and community –asssociated methicillin resistant *Staphylococcus aureus* at a rural hospital. *Plos one*, Pp 10371-383.

Pexara A., Solomakos N. and Govaris A. (2013). Prevalence of methicillin-resistant *Staphylococcus aureus* in milk and dairy products. *Journal of Hellenic Veterinary Medicine Society,* 64(1): 17-34.

Pinho, M.G., De Lencastre, H. and Tomasz, A. (2001). An acquired and a native penicillin-binding protein cooperate in building the cell wall of drug-resistant *staphylococci*. *Proceedings of National Academic Science USA,* 98 (19):10886-10891.

Pollett, G.L. and Warshawsky, B. (1999). *E. coli* O157:H7 Investigation Complete. Middlesex-London Health Unit.

Reid, G., Howard, J. and Gan, B.S. (2001). Can bacterial interference prevent infection? *Trends in Microbiology* 9(9):424-425.

Rendón, M. A. (2007). Commensal and pathogenic *Escherichia coli* use a common pilus adherence factor for epithelial cell colonization. *Proceedings of the National Academy of Sciences*, 104 (25): 10637–10642.

Riley, L.W., Remis, R.S., Helgerson, S.D., McGee, H.B., Wells, J.G., Davis, B.R., Hebert, R.J., Olcott, H.M., Johnson, L.M., Hargrett, N.T., Blake, P.A. and Cohen, M.L. (1983). Hemorrhagic Colitis Associated with a rare *Escherichia coli* Serotype. *The New English Journal of Medicine*, 308: 681–685.

Sarada, M. and Begum, J.M. (1991). The microbiolgical quality of ice cream sold in Bangalore city. *Journal of Food, Science and Technology*, 28, 5,317-318.

Schoeni, J.L. and Doyle, M.P. (1992). Reduction of Campylobacter jejunicolonization of chicks by cecumcolonizing bacteria producing anti-C.jejunimetabolites. *Applied and Environmental Microbiology*, 58: 664–670.

Seiken, Denka. "MRSA latex test for PBP2". Retrieved June 2010 from http/:www.hardydiagnostics.com/catalog2/hugo/MRSAlatexTest.htm.

Seybold, U., Kourbatova, E.V., Johnson, J.G., Halvosa, S.J., Wang, Y.F., King, M. D., Ray, S.M. and Blumberg, H.M. (2006). Emergence of community- associated methicillin – resistant Staphylococcus aureus USA 300 genotype as a major cause of health care – associated blood stream infections. *Journal of Infectious Diseases, 42:647-656.*

Shu, Y.Q., Burhan A.K., Rong W., Thanh-Huy L.B., Dorothee K., Liang C., Barry N.K., Andreas P., Frank R.D., and Michael O. (2009). Mobile Genetic Element-Encoded Cytolysin connects virulence to Methicillin Resistance in MRSA. *PLoS Pathogens*, 5(7).

Shukla, R., Slack, R., George, A., Cheasty, T., Rowe, B. and Scutter, J. (1995). Escherichia *coli* O157 Infection Associated with a Farm Visitor Centre. *Communicable Disease Report Review*, 5:R86– R90.

Siegler, R.L., Griffin, P.M., Barrett, T.J. and Strockbine, N.A. (1993). Recurrent Haemolytic Uremic Syndrome secondary to *Escherichia coli* O157:H7 infection. *Pediatrics*, 91: 666–668.

Sneath, P.H.A., Mair, N.S., Sharpe, M.E. and Holt, J.G. (1986). *Bergey's Manual of Systemic Bacteriology*, Published by waverly Press, Inc. 2: 1015-1034

Spohr, M., Rau, J., Friedrich, A., Klittich, G., Fetsch, A., Guerra, B., Hammerl, J.A. and Tenhagen, B.A. (2011). methicillin-resistant *Staphylococcus aureus* (MRSA) in three dairy herds in southwest Germany. *Zoonoses and Public Health*, 58:252–261.

Stirpe, F. and Battelli, M.G. (2006). Ribosome-inactivating proteins: Progress and problems. *Cellular and Molecular Life Science*, 44:1850–1866.

Summers, W.C. (2001). "Bacteriophage Therapy". *Annual Review of Microbiology*, 55:437-451.

Sung, J.M., Lloyd, DH. and Lindsay, J.A. (2008). *Staphylococcus aureus* host specificity: comparative genomics of human versus animal isolates by multi-strain microarray. *Microbiology*, 154:1949.

Sweeney, N.J. (1996). The *Escherichia coli* K-12 *gnt*P Gene allows *E. coli* F-18 to occupy a Distinct Nutritional Niche in the Streptomycin-Treated Mouse Large Intestine. *Infection and Immunity*, 64: 3497–3503

Swerdlow, D.L., Woodruff, B.A., Brady, R.C., Griffin, P.M., Tippen, S., Donnell, H.D. Jr., Geldreich, E., Payne, B. J., Meyer, A. Jr. and Wells, J.G. (1992). A Waterborne Outbreak in Missouri of *Escherichia coli* O157:H7 Associated with Bloody Diarrhea and Death. *Annals of Internal Medicine*, 117:812–819.

Szabo, R.E., Todd, E.C.D. and Jean, A. (1986). "Method to Isolate *Escherichia coli* O157:H7 from Food". *Journal of Food Protection,* 49 (10): 768–772.

Taiwo, S.S., Onile, B.A. and Akan, D.A. (2004). Methicillin resistant *Staphylococcus aureus* isolates in Ilorin. *African Journal of Clinical and Experimental Microbiology,* 5(2): 189-197.

Talan, D., Staartz, A., Goldstein, E.J., Singer, K., and Overturf, G.D. (1989). Staphylococcus intermedius in canine gingiva and canine-inflicted human wound infections: laboratory characterization of newly recognized zoonotic pathogen. *Journal of Clinical Microbiology,* 27:78-81.

Tarr, P.I. (1995). *Escherichia coli* O157:H7: Clinical, Diagnostic and Epidemiological Aspects of Human Infection. *Clinical Infectious Diseases,* 20: 1–10.

Thrusfield, M. (1997). *Veterinary Epidemiology,* 2nd edition, Oxford Blackwell science, Edinburgh. Pp. 182-186.

Todar, K. (2004). Web Review of Todar's *Online Textbook of Bacteriology*. 304: P. 142

Toklu, G.S., And Yaygin, H. (2000). Antalya piyasasinda satilan dondurmalarin hijyenik kalitesi ve kimyasal ozellikleri. Gida Bilimi ve Teknoloji Derg, 4(1) 38-45.

Tristan, A., Bes, M., Meugnier,H., Lina,G., Bozdogan, B., Courvalin,P., Reverdy,M. E., Enright, M.C., Vandenesch, F. and Etienne, J. (2007). Global distribution of Panton-Valentine leukocidin – positive methicillin –resistant *Staphylococcus aureus*. *Emerging Infectious Diseases,* 13: 594-600.

Umaru, G.A., Kabir, J., Umoh, V.J., Bello, M. and Kwaga, J.K.P. (2013). Methicillin resistant *Staphylococcus aureus* (MRSA) in fresh and fermented milk in Zaria and Kaduna, Nigeria. *International Journal of Drug Research and Technology,* 3(3):67-75.

Umoh, V.J., Adesiyun, A.A. and Gomwalk, N.E. (1990). Antibiogram of *Staphylococcus* strains isolated from milk and milk products. *Journal of Veterinary Medicine*, 37:701-706.

Van Duijkeren, E., Wolfhagen, M.J., Box, A.J., Wannet, W.J and Fluit, A.C. (2004). Human-to-dog transmission of methycillin- resistant *Staphylococcus aureus. Emerging Infectious Diseases,* 10:2235-2317.

Van loo, I.H., Diederen, B.M., Savelkoul, P.H., Woudenberg, J.H., Roosendaal,R., Van Belkum, A., Lemmens-den Toom, N., and Verhulst, C. (2007). Methicillin resistant *Staphylococcus aureus* in meat products in the Netherlands. *Emerging Infectious Diseases, 13:1753-1755.*

Van Rijen, M.M., Van Keulen, P.H. and Kluytmans, J.A. (2008). Increase in a Dutch hospital of methicillin-resistant *Staphylococcus aureus* related to animal farming. *Clinical Infectious Diseases*, 46:261 263.

Varma, J.K. (2003). An Outbreak of *Escherichia coli* O157 Infection Following Exposure to a Contaminated Building. *Journal of the American Medical Association,* 290: 2709–2712.

Vica, M., Glevitzky, M., Dumitrel, G., Popa, M., and Todoran, A. (2010). Microbiological quality of ice cream produced in Alba county, Romania. *Journal of Agroalimentary Processes and Technologies,* 16:19-23.

Voss, A., Loeffen, F., Bakker, J., Klaassen, C., and Wulf, M. (2005). Methicillin resistant *Staphylococcus aureus* in pig farming. *Emerging Infectious Diseases*, 11:1965-1966.

Wallace, J.S., Cheasty, T. and Jones, K. (1997). Isolation of Verocytotoxin-Producing *Escherichia coli* O157 from Wild Birds. *Journal of Applied Microbiology*, 82:399–404.

Wang, R. (2007). Identification of Novel Cytolytic Peptides as key virulence determinants of Community- Associated Methycillin-Resistant *Staphylococcus aureus. Nature and Medicine*, 45:68-72

Warke, R., Kamat, A., Kamat, M. and Thomas, P. (2000). Incidence of pathogenic psychrotrophs in ice creams sold in some retail outlets in Mumbai, India. *Food Control*, 11:77-83.

Weese, J.S., Dick, H., Willey, B.M., McGeer, A., Kreiswirth, B.N., Innis, B. and Low, D.E. (2006). Suspected transmission of methicillin-resistant *Staphylococcus aureus* between domestic pets and humans in veterinary clinics and in the household. *Veterinary Microbiology,* 115:148–155.

Welch, P., David, J., Clarke, W., Trinidad, A., Penner, D., Berstein, S., McDougall, L. and Adesiyun, A.A. (2000). Microbial quality of water in rural communities in Trinidad. *American Journal of Public Health*, 83:172- 179.

Wells, J.G., Davies, B.R. and Waschsmuth, I.K. (1983). Laboratory investigation of haemorrhagic colits outbreaks associated with a rare *Escherichia coli* serotype . *Journal of Clinical Microbiology*, 18:512-20.

Wenzel, R.P. (1982). The emergence of methicillin-resistant *Staphylococcus aureus*. *Annals of Internal Medicine.* 97:440-442.

Whitt, D. and Salyers, A.A. (2002). Bacterial Pathogenesis. A molecular edition, USA. *American Society of Microbiology Press.* P. 9

Whittam, T.S. (1996). *Escherichia coli* and *Salmonella*. ASM Press, Washington DC, USA. Pp 2708–2720

WHO, World Health Organization (1996). Fact Sheet: *Escherichia coli* O157:H7. http://www.who.int/mediacentre/factsheets/fs125/en/

Willshaw, G.A., Thirlwell, J., Jones, A.P., Parry, S., Salmon, R.L. and Hickey, M. (1994). Vero cytotoxin-producing *Escherichiacoli* O157 in Beefburgers Linked to an Outbreak of Diarrhoea, Haemorrhagic Colitis and Haemolytic Uraemic Syndrome in Britain. *Letters in Applied Microbiology,* 19:304–307.

Wilson, I.G., Heaney, J.C. and Weatherup, S.T. (1997). The effect of ice cream-scoop water on hygiene of ice cream. *Epidemiology and Infection*, 119: (1) 35-40.

Wilson, J., Spika, J., Clarke, R., McEwen, S., Johnson, R., Rahn, K., Renwick, S., Karmali, M., Lior, H., Alves, D., Gyles, C. and Sandhu, K. (1998). Verocytotoxigenic *Escherichia coli* Infection in Dairy Farm Families. *Canada Communicable Disease Report*, 24(3).

Wirtz, C., Witte, W., Wolz, C. and Goerke, C. (2009). Transcription of the phage –encoded Panton- Valentine leukocidin of Staphylococcus aureus is dependent on the phage life-cycle and on the host background. *Journal of Microbiology*, 155: 3491-3499.

Wouafo, M.N., Njine, T. and Tailliez, R. (1996). Hygienic and microbiologic quality of ice creams produced in Cameroon: A public health problem. *Bulelletin.of Social. Pathology and Exotics*, 89:358-362.

Wulf, M., and Voss, A. (2008). Cited by Shuaihua, P., Feifei, H., Beilei,G.(2009). In: Isolation and characterization of methycillin – resistant *Staphylococcus aureus* (MRSA) strains from Lousiana retail meats. *Applied and environmental microbiology*, 75: 265-267.

Wunderink, R.G., Rello, J., Cammarata, S.K., Croos-Dabrera, R.V. and Kollef, M.H. (2003). Linezolid vs vancomycin: analysis of two double-blind studies of patients with methicillin-resistant *Staphylococcus aureus* nosocomial pneumonia. *Chest*, 124:1789–97.

www.mrsaresources.com. Methycillin Resistant *Staphylococcus aureus* . retrieved on 6th June, 2009.

Xu, J.G., Liu, Q.Y., Jing, H. Q., Pang, B., Yang, J. C., Zhao, G. F. and Li, H. W. (2003). Isolation of *Escherichia coli* O157:H7 from Dung Beetles *Catharsius molossus*. *Microbial Immunology*, 47:45–49.

Yusuf, M.A., Abdul, T., Abdul Hamid, T. and Yusuf, M.A. (2013). Assessment of Bacteriological quality of ice cream offered for public consumption in Bauchi, Nigeria. *Journal of Pharmacy*, 3:23-30.

APPENDICES

Appendix 1:

Antibiotic Sensitivity profile of *S. aureus* isolates Following the Disk Diffusion Test.

S/N	Sample code.	E	CIP	SXT	C	MET	AMC	AMP	K	P	F	OX	TE	CN
1.	SL5	R	R	I	R	S	R	R	I	R	R	R	R	S
2.	SS6	R	R	S	R	R	R	R	S	R	R	R	R	S
3.	POT6	R	R	I	R	R	R	R	S	R	R	R	R	S
4.	FK2	R	R	I	R	R	R	R	R	R	R	R	R	S
5.	SL10	R	S	S	R	R	R	R	S	R	R	R	S	S
6.	SSC1	I	R	R	R	R	R	R	S	R	R	R	R	S
7.	FK2	R	S	S	S	R	R	R	S	R	R	R	R	S
8.	POT4	R	S	I	R	R	R	R	R	R	R	R	I	S
9.	SL5(3)	I	S	S	R	R	S	I	S	R	R	R	R	S
10.	SL5(2)	R	R	S	S	R	R	I	R	I	R	R	R	S
11.	SM19	R	R	I	R	R	S	R	R	R	I	R	R	S
12.	S^2TW3	R	R	R	S	S	S	R	S	R	S	R	R	S
13.	FK10	I	R	R	R	I	R	R	S	R	R	R	R	S
14.	CR6	R	R	S	I	R	R	R	S	R	R	R	R	S
15.	SB7	I	R	I	S	R	R	R	S	R	R	R	R	S
16.	FK8(1)	R	I	S	I	I	R	R	I	R	R	R	R	S
17.	FS2	S	S	S	S	S	R	R	S	R	R	R	R	S
18.	S19(2)	R	R	R	R	S	R	R	S	R	I	R	R	S
19.	SB4	R	I	R	R	R	R	R	S	R	R	R	R	S

APPENDIX 11:

Antibiotic Sensitivity Profile of *E. coli* Isolates Following the Disk Diffusion Test.

Strain	C	CIP	E	S	AMP	TE	F	K	CN	SXT	R	S
FK8	R	S	R	R	R	R	S	S	S	R	6	4
POT1	R	S	R	S	R	I	S	S	S	I	3	7
MB2	R	S	R	R	R	R	S	I	R	R	7	3
SB6	S	S	R	I	R	R	S	S	S	S	3	7
SB5	S	S	R	R	R	R	S	S	S	S	4	6

APPENDIX 111:

Table of Substrate and Reactions of Microbact 12E (Oxoid, Basingstoke, UK)

			Reaction Colours	
Well No.	Designation	Symbol	Negative	positive
1	Lysine	LYS	Yellow	Blue-green
2	Ornithine	ORN	Yellow-green	Blue
3	H2S	H2S	Straw colour	Black
4	Glucose	GLU	Blue-green	Yellow
6	Xylose	XYL	Blue-green	Yellow
5	Mannitol	MAN	Blue-green	Yellow
7	ONPG	ONP	Colourless	Yellow
8	Indole	IDN	Colourless	Yellow
9	Urease	URE	Straw colour	Pink-red
10	VogesProskauer	VP	Straw colour	Pink-red
11	Citrate	CIT	Green	Blue
12	TDA	TDA	Straw colour	Cherry-red

APPENDIX 1V:

Table of Substrate and Reactions of Microbact 12S (Oxoid, Basingstoke, UK)

		Reaction Colours		
Well No.	Designation	Symbol	Negative	positive
1	Maltose	MAL	Red	Yellow
2	Mannitol	MAN	Red	Yellow
3	Mannose	MNS	Red	Yellow
4	Sucrose	SUC	Red	Yellow
5	Trehalose	TRE	Red	Yellow
6	N-acetyl glucosamine	NAG	Red	Yellow
7	Arginine utilization	ARG	Yellow/olive green	Green/blue
8	Urease test	URE	Straw/ yellow	Pink/red
9	Beta-glucosidase	β – GL	Pale yellow/colourless	Pink-red
10	Alkaline phosphatase	PHO	Pale yellow/ colourless	Bright yellow
11	Beta-glucuronidase	β – GN	Pale yellow /colourless	Bright yellow
12	Beta-galactosidase	β – GD	Pale yellow /colourless	Plum purple

APPENDIX V:

Composition of Media

Eosin Methylene Blue Agar (Oxoid)

Typical formula (g//dl) Ph 6.8 ± 0.2 at 25ºC

Peptone 10.0

Lactose 10.0

Di-potassium hydrogen phosphate 2.0

Eosin Y 0.4

Methylene blue 0.06

Suspend 37.5 g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterelize by autoclaving at 121ºC for 15 minutes. Dry surface of gel before inoculation.

MacConkey agar (Oxoid, Basingstoke, Uk)

Typical formula (g/dl)

Peptone 20.0

Lactose 10.0t

Bile salts 5.0

Sodium chloride 5.0

Neutral red 0.075

Agar 12.0

Suspend 52g in 1 litre of distilled water. Bring to boil to dissolve completely, sterilize by autoclaving at 121º C for 15 minutes

Brain Heart Infusion broth (Oxoid)

Typical formula (g/dl)

Brain Infusion solids 12.5

Beef heart infusion solids 5.0

Proteose peptone 10.0

Glucose 2.0

Sodium chloride 5.0

Di-sodium Phosphate 2.5

Suspend 37g of the agar base into 1000ml of water. Mix well to dissolve and distribute into final containers. Sterelise by autoclaving at 121º C for 15 minutes.

Nutrient agar (Oxoid)

Typical formula (g/dl) pH 7.4 ± 0.2 at 25ºC

Lab –Lemco beef extract 1.0

Yeast extract 2.0

Peptone 5.0

Sodium chloride 5.0

Agar 15.0

Suspend 28g of the agar base into 1000ml of water. Heat gently to boil to dissolve completely and strelize by autoclaving at 120ºC for 15 minutes.

Mueller Hinton agar (Oxoid)

Typical formula (g/l) pH 7.3 ± 0.1 at 25ºC

Beef dehydrated infusion 300.0

Casein Hydrolysale 17.5

Starch 1.5

Agar .0

Suspend 38g of the agar baase into 1000ml of water. Heat gently to boilto dissolve completely and sterilize by autoclaving atb121ºC for 15 minutes.

Modified tryptone soya broth (Oxoid)

Typical formula (g/dl)

Pancreatic digest of casein 17.0

Papaic digest of soy bean meal 3.0

Sodium chloride 5.0

Di-basic potassium hydrogen phosphate 2.5

Glucose 2.5

Bile salts 1.5

Dissolve 16.5g in 500ml of distilled water and distribute into final containers. Sterelize by autoclaving at 121ºC for 15 minutes.

Sorbitol MacConkey agar

Typical formula	(g/dl)
Peptone	20.0
Sorbitol	10.0
Bile salts No.3	1.5
Sodium chloride	5.0
Neutral red	0.03
Crystal violet	0.001
Agar	15.0

Suspend 51.5g in 1 litre of distilled water. Bring to the boil to dissolve completely. Sterilize by autoclaving at 121ºC for 15 minutes.

APPENDIX VI:

Chart Showing the Expected Result of Microbact 12E System after 18-24 Hours Incubation.

Well No	Symbol	*Echerichia coli* (ATCC 259922)
1	LYS	+
2	ORN	+
3	H_2S	-
4	GLU	+
5	MAN	+
6	XYL	+
7	ONP	+
8	IDN	+
9	URE	-
10	VP	-
11	CIT	-
12	TDA	-

APPENDIX VII:

Chart Showing the Expected Result of Microbact 12S System after 18-24 Hours Incubation.

Well No	Symbol	*Staphylococcus aureus* (ATCC 259922)
1	MAL	+
2	MAN	+
3	MNS	+
4	SUC	-
5	TRE	-
6	NAG	-
7	ARG	+
8	URE	+
9	β -GL	+
10	PHO	+
11	β -GN	-
12	β -GD	-

APPENDIX V111: Antimicrobial Inhibition Zone Size Interpretative Chart

			Diameter of zones of inhibition in mm		
Antimicrobial agent	**symbol**	**Disk content(µg)**	**Resistant (mm or less)**	**Intermediate (mm)**	**Sensitive (mm)**
Erythromycin	Cef	5	12	13-14	15
Chloramphenicol	C	30	12	13-17	18
Ciprofloxacin	Cip	5	15	16-20	21
Sulphamethoxazole/ Trimethoprim	Sxt	25	12	13-16	17
Gentamycin	Cn	10	12	13-14	15
Kanamycin	K	30	13	14 -17	18
Oxacillin	Ox	5	13	14-16	17
Streptomycin	S	10	11	12-14	15
Tetracycline	Aug	30	11	12-14	15
Nitrofurantoin	F	300	14	15-16	17

Adapted from Clinical and Laboratory Standard Institute (CLSI) Zone Diameter and Interpretive Standard (2007).

Based on results obtained using Mueller Hinton Agar and *E. coli* (ATCC 25922) as positive control.

APPENDIX IX: Antimicrobial Inhibition Zone Size Interpretative Chart

			Diameter of zones of inhibition in mm		
Antimicrobial agent	**symbol**	**Disk content(µg)**	**Resistant (mm or less)**	**Intermediate (mm)**	**Sensitive (or more)**
Erythromycin	E	15	13	14-22	23
Ciprofloxacin	Cip	5	15	16-20	21
Sulphamethoxazole /Trimethoprim	Sxt	25	10	11-15	16
Chloramphenicol	Chl	30	12	13-17	18
Methicillin	Met	5	9	10-13	14
Amoxicillin	Amc	10	19	-	20
Ampicillin	Amp	10	28	-	29
Kanamycin	K	30	13	14-17	18
Penicillin	P	10	28	-	29
Nitrofurantoin	F	300	14	15-16	17
Oxacillin	Ox	1	10	11-12	13
Tetracycline	Te	30	14	15-18	19
Gentamicin	Cn	10	12	13-14	15

Adapted from Clinical and Laboratory Standard Institute (CLSI) Zone Diameter interpretive Standard (2007).

Based on results obtained using Mueller-Hinton Agar and *Staphylococcus aureus* (ATCC 25923) as positive control