OCCURRENCE AND ANTIBIOGRAM OF *SALMONELLA* AND *SHIGELLA* SPECIES FROM RAW AND FERMENTED COW MILK ("NONO") IN ZARIA AND ENVIRONS, NIGERIA

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

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FEBBRUARY, 2018

Declaration

I declare that the work in this dissertation entitled **"Occurrence and Antibiogram of *Salmonella* and *Shigella* Species from Raw and Fermented Cow Milk ("Nono") In Zaria and Environs, Nigeria.**" has been carried out by me in the Department of Veterinary Public Health and Preventive Medicine under the supervision of Professor M. Bello and Professor C.A. Kudi. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree at this or any other institution.

Elizabeth ONIOSHUN ______________________ ______________

Signature Date

Certification

This dissertation entitled "**Occurrence and Antibiogram of *Salmonella* and *Shigella* Species From Raw and Fermented Cow Milk ("Nono") In Zaria and Environs, Nigeria**" by Elizabeth ONIOSHUN meets the regulations governing the award of the degree of Master of Science in Veterinary Public Health and Preventive Medicine of the Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

Professor M. Bello (Chairman, Supervisory Committee)	________________ Signature	____________ Date
Professor C.A. Kudi (Member, Supervisory Committee)	________________ Signature	____________ Date
Prof. E.C. Okolocha Head of Department Veterinary Public Health and Preventive Medicine	________________ Signature	____________ Date
Prof. S.Z. Abubakar (Dean, School of Postgraduate Studies)	________________ Signature	____________ Date

Dedication

This dissertation is dedicated to the Almighty God; the Father of all creation.

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My sincere appreciation goes to the ALMIGHTY GOD by whose hand I have been able to complete this dissertation despite the terrible health challenge that almost took my life away. I also thank Him for blessing me with wonderful family members, supervisors, friends and colleagues who encouraged me and stood by me all through the course work and research.

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Abstract

The daily demand for the consumption of dairy products necessitates the need for investigating the level of bacterial contamination and the presence of pathogenic organisms like *Salmonella* and *Shigella* in milk and its products. Also, the indiscriminate use of antibiotics in treatment of infections, in the agricultural industry, as household disinfectants and as growth promoters has led to increasing antibiotic resistance in different parts of the world, hence, the need for continuous monitoring of resistance of pathogenic organisms to commonly used antibiotics. One hundred and twenty eight (128) raw milk samples and one hundred and fourty (140) fermented milk samples were collected from dairy herds in 13 villages and 10 market places respectively from Zaria and its environs. The Total Aerobic Count (TAC) of the milk samples was determined and the presence of *Salmonella* and *Shigella* species was investigated following the ISO 6579 2003 standard. *Shigella* species was not isolated from the study. The overall mean log CFU/ml ± standard deviations for raw milk and fermented milk were 7.1±0.5 and 9.0±0.6 respectively. These were above the acceptable level recommended by the European Commission (mean log 5.0) indicating that milking was likely done in an unhygienic manner. Only 7/268 (2.6%) isolates conformed with typical *Salmonella* reactions from biochemical testing. Of the 7 isolates, only 3 were identified as *Salmonella* species using the microbact^{TM} kit. The 3 isolates also agglutinated with the *Salmonella* polyvalent A-S antiserum. None of the 3 isolates harboured the *inv*A virulence gene. The isolates were tested against nine antimicrobial classes and were sensitive to only one class (Folate inhibitor pathway). All the isolates exhibited multidrug resistance to the other 8 antimicrobial classes with each isolate showing resistance to 8 and the highest to 9 antimicrobials within the class of fluoroquinolones, aminoglycosides,

cephem, nitrofurantoin, beta-lactamase inhibitor, tetracyclines, macrolide and phenicols. It is important to combat the indiscriminate use of antibiotics owing to increase in resistance.

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Nomenclature

Abbreviations

TACs	-	Total Aerobic Counts
CFU	-	Colony Forming Units
SPI	-	*Salmonella* Pathogenicity Island
SSA	-	Salmonella-Shigella Agar
XLD	-	Xylose Lysine Deoxycholate Agar
TDA	-	Tryptophan Deaminase

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Milk is a fresh, clean, whole undigested and normal mammary secretion that is obtained by draining of the udder of healthy dairy animals that are properly fed and kept (Frank and Mahony, 1988; Ajogi *et al*., 2005). Milk is made up of fat, protein, total solid, lactose and ash as the main constituents (Armstrong, 1995). Other minor constituents found in milk include milk fat, vitamins, flavor compounds and metal ions. These constituents influence the nutritional, technological and sensory properties of milk and dairy products (Armstrong, 1995). Milk can be obtained from various animals such as cows, goats and sheep for consumption by humans, although, cow is known as the most relevant for commercial purposes (Adams and Moses, 1995) In Nigeria, White Fulani (Bunaji) is known as the major milk producer (Adeneye, 1989). However, in some cultures, milk from animals like goats, camels, horses and donkeys serve as sources of food (Rampling, 1990). Both rural and urban dwellers consume milk and its products (Makut *et al*., 2014).

Raw milk is unfermented and unpasteurized milk that is produced by collection of milk directly from dairy animals (Makut *et al*., 2014). Microorganisms including pathogenic organisms that are capable of causing food-borne illnesses can gain access into milk and proliferate (Rosenthal, 1991). Hence, they could become threatening to the health of the consumers (Rosenthal, 1991). Sources of milk contamination include commensal or pathogenic flora of the udder or teat canal, the animal's skin, faecal soiling of the udder, contaminated milking equipment, use of contaminated water to clean the milking equipment, and storing milk in contaminated containers (Rampling, 1990). Also, milk may be contaminated by commensal or pathogenic organisms from milkers, insects, rodents, birds, and other animals (Rampling, 1990).

In Nigeria, the Fulani tribe locally process cow milk in its raw or boiled form in to products like Nono, Kindrimo and Manshanu (Eka and Ohaba, 1977). Nono is fermented milk. It is produced by boiling cow milk for about 20 minutes; it is then collected in a calabash and allowed to ferment naturally for 24 hours (Eka and Ohaba, 1977; Olasupo *et al.*, 1996). Some people allow the milk to ferment naturally without boiling (Omotosho *et al*., 2013). Even before fermentation, the precipitated milk butter is filtered out for cheese processing (Ogbonna *et al*., 2012). The most common product often mixed with nono is called "Fura" (a dumpling made of millet) to make a preparation called "fura da nono".

The most common method of reducing milk contamination by pathogenic microorganisms is Pasteurization. It kills most potential pathogens but does not render milk sterile. Post-pasteurization contamination can occur since milk is usually placed in containers for sale after pasteurization (Rampling, 1990). There have been reports on the outbreak of milk-borne diseases despite pasteurization; this could be due to improper pasteurization or contamination of milk and milk products (Hartman, 1997; Altekruse *et al*., 1998; Da Silva *et al*., 1998; Nebedum and Obiakor, 2007).

Several researchers have reported milk to be a highly favourable medium for the growth of many kinds of microorganisms (Ekici *et al*., 2004; Chatterje *et al*., 2006; Lingathurai *et al.*, 2009; Muhammad *et al*., 2009; Lingathurai and Vellathurai, 2010; Mubarack *et al*., 2010; Ali and Abdelgadir, 2011; Anshumala and Niranjan, 2012). Microorganisms such as *Shigella* species, *Streptococcus* species, *Listeria monocytogenes*, *Salmonella* species, *Staphylococcus* *aureus*, *Campylobacter jejuni*, and *Mycobacterium tuberculosis* have been isolated from milk and milk products (Flowers *et al*., 1993; Jayarao *et al*., 2006; Egwaikhide *et al*., 2014; Olufunke *et al*., 2014; Usman *et al*., 2016).

Shigella belongs to the bacterial family *Enterobacteriaceae*, and is almost genetically identical to *Escherichia coli.* They are also closely related to *Salmonella* and *Citrobacter* species (Lampel, 2001; Anon, 2010). Based on biotyping the genus *Shigella* is divided into four serogroups: *S. dysenteriae* (Serogroup A; 15 serotypes), *S. flexneri* (serogroup B; 8 serotypes divided into 11 subserotypes), *S. boydii* (serogroup C; 20 serotypes), and *S. sonnei* (serogroup D; 1 serotype) (Ingersoll *et al*., 2002). The four serogroups differ in epidemiology (Ingersoll *et al*., 2002). *Shigella* species are Gram-negative, facultatively anaerobic nonsporeforming rods.

Shigella causes shigellosis which is an infectious disease that causes diarrhoea, vomiting, stomach cramps, and high fever. The infection lasts five to seven days, but children, the elderly, and individuals who are immunosuppressed are more prone to severe cases of shigellosis. If left untreated, the diarrhoea can cause death, and seizures can result due to high fever (Anon, 2010). Species of *Shigella* such as *S. dysenteriae* produce enterotoxins such as Shiga toxin, which causes haemolytic uraemic syndrome (Anon, 2010). CDC in 2015 reported 243 cases of Shigellosis in 32 states of the United States. The infection was traced to international travelers who had multi-drug resistant shigellosis and had spread it to others who had not travelled (CDC, 2015a).

The genus *Salmonella* also belongs to the bacterial family *Enterobacteriaceae* and consists of two species: *S. enterica* which is divided into six subspecies (*S. enterica* subsp. *enterica*, *S. enterica* subsp. *salamae*, *S. enterica* subsp. *arizonae*, *S. enterica* subsp. *diarizonae*, *S. enterica* subsp. *houtenae* and *S. enterica* subsp. *indica*) and *S. bongori* (formerly called *S. enterica* subsp. *bongori*). They are motile with peritrichous flagellae with the exception of *Salmonella* Pullorum-Gallinarum (Perilla, 2003).

Salmonella is one of the most common causes of bacterial foodborne illnesses worldwide, second only to *Campylobacter* (Allos, 2002; WHO, 2005). All species, including humans, may be infected by *Salmonella*, which live in the intestine and may be shed in faeces. However, *Salmonella* can survive and multiply very well outside the intestinal tract, which makes eradication almost impossible (EUFIC, 2006). The most common serotypes involved in human foodborne illness are *S.* Enteritidis and *S.* Typhimurium, but they often cause only mild, if any, disease in livestock. Salmonellosis has four disease patterns which include: enteric fever (Typhoid fever), gastroenteritis, bacteraemia and chronic carrier state (Gray and Fedorka-Cray 2002; Pui *et al*., 2011). Symptoms of salmonellosis usually include: nausea vomiting, diarrheoa, and fever which may vary but lasts between 2 to 7 days (Giannella, 1996). There have been reports on the outbreaks of salmonellosis owing to consumption of milk and its products.

The invasion locus (*inv*) of *Salmonella* consists of at least 12 different genes required for adherence and/or invasion. The *inv*A gene is essential for invasion of epithelial cells by *Salmonella* (Galan and Curtiss, 1991).

1.2 Statement of the Research Problem

Poor hygienic practices by handlers of milk and its products can lead to introduction of pathogenic microorganisms like *Salmonella* and *Shigella* into milk, hence, posing a great risk to the health of the consumers, if the products do not undergo further processing before consumption (Adeyemi and Umar, 1994). Sometimes, not all fermented milk (nono) sent to the market by the sellers is sold the same day and in a country like Nigeria, such unsold products may be returned to the market without proper means of preservation (Egwakhide *et al*., 2014). Thus, pathogenic organisms that might have gained access into the product would have had enough time to multiply or produce harmful metabolites (Egwakhide *et al*., 2014).

Shigellosis is more common in places with low hygienic and sanitary conditions (Kurjak and Chervenak, 2006). Globally, the estimated cases of shigellosis are 165 million per year where 163 million of the cases are from developing countries (Kotloff *et al*., 1999). *Shigella* infection accounted, in 1973, for up to 393,121 cases with up to 29 deaths in Nigeria as reported by the World Health Organization (WHO, 1987). There have been reports on the cases of shigellosis in some parts of the world, but several of the mild cases are not reported (DuPont, 2000).

Salmonella has been recognized as one of the principal pathogens of concern associated with milk and processed milk products (ICMSF, 2006). Okonkwo (2011) isolated *Salmonella* and *Shigella* from fermented milk (nono) samples from markets in Maiduguri metropolis with a prevalence of 24% and 8% respectively. Ogbonna *et al.* (2012) also reported mean counts of *Shigella (*0.15, 0.40 and 0.24 $\log_{10}$ cfu mL^{-1}) and *Salmonella* (1.44, 1.83 and 1.23 $\log_{10}$ cfu mL^{-1}) in raw milk, nono and Kindrimo respectively from different markets in Maiduguri, Nigeria.

Hayes *et al.* (2001) reported that bacteria in milk can occur through colonization of the teat canal or an infected udder (clinical or subclinical mastitis) or get contaminated at various stages which could be from the animal, the milker (manual as well as automated), extraneous dirt or unclean water. Shehu and Adesiyun, (1990) reported that the female Fulani hawkers prior to sales often do engage in the act of adding stream water and milky white supernatant of water obtained from soaked baobab tree seeds in order to increase the volume and improve colour of milk and its products. This act could further lead to the contamination and spoilage of the cow milk and its products. Tamba *et al*. (2016) reported poor sanitary conditions in Sabon Gari Local Government area of Zaria which include: failure of the milk handlers to wash their hands, presence of flies in the environment where milking was done and where milk products were sold, addition of river or stream water and ice block to the milk to increase its quantity. The isolation of pathogenic microorganisms from milk and its products and reported observations by researchers on the unhygienic practices by milk handlers raises a public health concern as the presence of pathogenic microorganisms and bacterial counts exceeding acceptable levels by regulatory bodies could pose a threat to the health of the consumers.

For more than five decades, there has been reports on the progressive resistance of *Shigella* to commonly used antibiotics (Ashkenazi *et al*., 2003; Talukder *et al*., 2007), which has made it difficult to identify efficient drugs for the treatment of shigellosis (Abdu *et al*., 2014). More than 80% of food poisoning bacteria such as *Salmonella* are reported as antibiotic resistant to at least one type of antimicrobial and more than 50% as resistant to two or more (Dabassa and Bacha, 2012).

1.3 Justification of the Study

Dairy products are consumed by millions of people on daily basis worldwide and as such the potential for food-borne illness is a major concern to producers, regulators and consumers (Bryne, 2004).

In Nigeria, most dairy herds that produce fresh milk and milk products for human consumption are not subjected to quality control to ensure the safety of the milk for public consumption (Bertu *et al*., 2010) owing to the fact that greater percentage of the dairy products are locally processed. The methods implored (e.g. use of firewood without observing the adequate pasteurization temperature) by these producers in boiling milk before consumption or fermentation may not adequately decontaminate milk. Hence, pathogenic microorganisms may survive and multiply in the milk. Moreso, lack of proper attention to hygiene may encourage contamination of milk and its products.

These unhygienic milking practices necessitate the investigation of bacterial loads and the presence of pathogenic microorganisms like *Salmonella* and *Shigella* in both raw and fermented milk in Zaria and environs.

The increasing reports on antimicrobial resistance by *Salmonella* and *Shigella* species necessitates the need for continuous antimicrobial susceptibility testing for identification of the right antibiotics for effective treatment.

1.4 Aim and Objectives

1.4.1 Aim of the study

To determine the microbiological quality of raw and fermented cow milk and to isolate and determine the antibiogram of *Shigella* and *Salmonella* species from raw and fermented cow milk in Zaria and environs.

1.4.2 Objectives of the study

The objectives were to:

1. Enumerate the total aerobic counts (TACs) in raw and fermented cow milk from dairy herds in Zaria and environs.
2. Isolate *Salmonella* and *Shigella* species present in raw and fermented cow milk from dairy herds in Zaria and environs and their antibiotic resistant profiles.
3. Confirm the presence of *inv*A virulence gene in the *Salmonella* isolates.

1.5 Research Questions

1. What is the level of bacterial contamination in raw and fermented cow milk from dairy herds in Zaria and environs?

2. Are *Shigella* and *Salmonella* species present in raw and fermented cow milk from dairy herds in Zaria and environs and what are their antibiotic resistant profiles?

3. Is *inv*A virulence gene present in the *Salmonella* isolates from dairy herds in Zaria and environs?

CHAPTER TWO

LITERATURE REVIEW

2.1 Milk Composition

Milk is a heterogeneous mixture which consists of carbohydrates, proteins, vitamins, minerals and other nutrients found in water, which is the main constituent (Harding, 1999; Mehta, 2015). Milk is termed as most complete food of nature owing to the fact that its rich protein value contains more essential amino acid compared with other natural foods (Foley *et al*., 1972). Milk from animals can play a significant function in children's diet in populations where there is not much access to other foods from animals (FAO, 2015). Milk quality and composition are of great significance to the dairy industry and the wellbeing of humans as the composition of milk largely depends on the possibility of processing it into different products (Ozrenk and Selcuk, 2008). Moreso, the composition of milk is not stable as it depends on factors such as breed of the animal, absorption of feed, management practices, lactation stage and season (Walstra and Jenness, 1984; Walstra *et al*., 1999; Lindmark, 2003). The main constituents of cow milk are shown on table 2.1.

Table 2.1. Average Composition of Cows' Milk

Main constituent	Range (%)	Mean (%)
Water	85.5-89.5	87.0
Total solids	10.5-14.5	13.0
Fat	2.5-6.0	4.0
Proteins	2.9-5.0	3.4
Lactose	3.6-5.5	4.8
Minerals	0.6-0.9	0.8

Source: Frank (1988)

2.1.1 Water

The major component of milk is water (FAO, 2015). Eighty-seven (87%) percent of milk is water. The amount of lactose that is being synthesized by the secretory cells of the mammary gland determines the availability of water (Guetouache *et al*., 2014). The level and composition of milk in dairy cattle is determined by water (Barney and VanHorn, 2003).

2.1.2 Milk proteins

Milk is known to have a high value for life's health as it is a good source of essential amino acid (Korhonen *et al*., 1998; Clare and Swaisgood, 2000). Milk protein improves the uptake of nutrients and acts as growth factors, antibodies, hormones, enzymes and immune stimulants (Korhonen *et al*., 1998; Clare and Swaisgood, 2000). Casein makes up 80% of milk protein while whey consists of about 20% of milk protein (Jensen, 1995; Miller *et al*., 2007; Monika *et al*., 2012).

2.1.2.1 Casein

The four major components of casein include: alpha, beta, gamma and kappa-casein. These combine to make up a structure known as the casein micelle (Davies and Law, 1980; Miller *et* al., 2007). Casein can help absorb minerals like calcium and phosphorus (Holt *et al*., 2013). It also contains a high amount of Glutamine which is needed by the body from an outside source of food when under physiological stress as with endurance exercise (Dunford and Doyle, 2008).

2.1.2.2 Whey protein

Whey protein is composed predominantly of beta-lactoglobulin and alpha lactalbumin (Strickland *et al.*, 2001; Miller *et al*., 2007). Other whey proteins include serum albumin, immunoglobulins (IgA, IgG, IgM), protease peptones, lactoferrin and transferrin (Miller *et al*., 2007; Borkova and Snaselova, 2005). Whey can easily be broken down and its nutrient absorbed relatively quickly by the body (Dunford, 2008). Whey maintains good health as it keeps the blood pressure low and improves the body condition during stress (Markus *et* al., 2000; Pal and Ellis, 2010).

2.1.3 Milk carbohydrate

Lactose is the major carbohydrate component in milk (Frank, 1988). It is a disaccharide consisting of two sugars, namely glucose and galactose (Robbins, 2001). It has been considered to be the least cariogenic of the common dietary sugars and helps to prevent dental caries (Banoczy *et al*., 2009).

2.1.4 Milk fat

The appearance, texture, flavor and satiability of milk are characteristics contributed by milk fat (Miller *et al*., 2007). Milk fat has the most complex fatty acid composition of edible fats as it contains over 400 individual fatty acids (Jensen and Newburg, 1995; Jensen, 2002; Parodi, 2004). Milk fat consists mainly of triglycerides, which is approximately 98%. Other milk lipids include diacylglycerol, cholesterol, phospholipids and free fatty acids (Jensen, 1995).

2.1.6 Minerals

Milk contains minerals like Calcium, iodine, phosphorus, potassium, magnesium, zinc and selenium. Calcium is an essential mineral in the body that helps to develop strong and healthy bones and teeth (Insel *et al.,* 2004). Calcium plays numerous role in the body as it helps to regulate the division and differentiation of cells, facilitates weight control, energy metabolism, normal coagulation of blood, reduces the risk at which kidney stones is developed and prevents high blood pressure (Insel *et al*., 2004; EFSA, 2009a; EFSA, 2010c). Iodine is essential for the normal functioning of the thyroid gland and its hormones. It also helps in maintaining the skin (EFSA, 2009b; EFSA, 2010d). The thyroid hormones regulate the rate of metabolism in the body. They also regulate body temperature and aid in growth and reproduction (Haug *et al*., 2007). Iodine content of milk varies with season (FSA, 2002; Haug *et al*., 2007). Phosphorus is the most abundant mineral in the body second to calcium (TDC, 2015). It is needed for metabolism of calcium and protein, healthy bones and teeth, regulation of pH levels in the body (EFSA, 2009c; TDC, 2015). Potassium aids in the maintenance of normal blood pressure and helps to maintain the functions of the muscles and nerves of the body (TDC, 2015). Milk is a good source of magnesium. Magnesium helps in preventing oxidative stress and is effective in treating acute cases of asthma (Hans *et al*., 2002; Cheuk *et al*., 2005). Zinc is more available in milk than in vegetable foods (Insel *et al*., 2004). Zinc is involved in immune function as it helps to fight infections, repairs DNA, involved in growth and replication of cells and metabolism of proteins and lipids (Insel *et al*., 2004; TDC, 2015). Selenium is involved in the synthesis and repair of DNA and acts as an antioxidant (Dodig and Cepelak, 2004). Defficiency in selenium has been associated with worsened asthma symptoms and adverse mood states (Rayman, 2002; Allam and Lucane, 2004). It has also been found to be protective against some types of cancer (Insel *et al*., 2004).

2.3. Microbial Quality of Raw Milk

The high moisture content, a close to neutral pH and the diversity in nutrient content of milk makes it a good medium for the growth of microorganisms which can lead to its deterioration (Acuri *et al*., 2006; Alves, 2006; Lorenzetti, 2006; Barros *et al*., 2011). Milk collected aseptically from clean and healthy cows generally has a low microbial load of less than

1,000cfu/ml; storing the milk for sometime at normal temperature may bring about increase in the microbial load of the milk (Richter *et al*., 1992).Temperature is a significant factor that facilitates the prevalence and multiplication of specific organisms in milk as such, organisms that cannot survive refrigeration temperatures cannot multiply under such conditions (Jay, 1996; Frank, 1997). It is essential to keep milk at a refrigerated temperature in order to have a low bacterial count (Silva *et al*., 2010). Despite cold milk storage, psychrotrophic bacteria can still survive in the milk. These psychrotophic species include: *Pseudomonas, Achromobacter, Aeromonas, Serratia, Alcaligens, Chromobacterium, Flavobacterium* and *Enterobacter* (Sørhaug *et al*., 1997; Ryser, 1999; Martins *et al*., 2006; Mcphee and Griffiths, 2011)). Having a higher number of bacteria could be as a result of factors like: poor hygiene during milking, unclean udder of the individual animal, condition in which the milk is stored, use of unclean utensils for milking and means through which the milk is transported (Spreer, 1998; Murphy and Boor, 2000; Gandiya, 2001).

2.3.1. Standard plate count

Standard plate counts (SPC) indicates the quality of the milk and hygiene as it gives an estimate of the number of colonies of microorganisms in the milk (Hayes *et al*., 2001; Aggad *et al*., 2010). SPC gives the aerobic counts of bacteria in a milk sample (Guide to Udder Health, 2014).Milk is plated on a solid agar and incubated for 48hours at 32^{o}C. After incubation, the plates are observed and the grown bacteria are counted (Extension, 2010). The number of colonies on the plate equates the number of bacteria that can grow in the presence of oxygen. Arithmetical calculation is then carried out to obtain the number of bacteria per ml/milk (Guide to Udder Health, 2014). The regulations regarding bacterial count limits vary within jurisdictions although in the pasteurized milk ordinace it has been mandated that the

SPC of milk should not exceed 100, 000 cfu/ml. However, most parts of the dairy industry recommend a lower count of ≤10,000 cfu/ml as it would improve the milk quality (Extension, 2010). SPC is limiting in that it does not indicate the type of bacteria present. It only gives the count of bacteria that can grow at lower temperatures (Extension, 2010; Perennia, 2013). However, having high bacteria counts in raw milk could be as a result of other bacteria accessing the milk from various sources (Extension, 2010).

The suitable colony counting range for aerobic organisms is 25-250 colonies to avoid false indications of the actual number of bacteria present in the sample; counts higher than 250 colonies but close to 250 colonies can be reported as estimated aerobic plate counts. However, colonies far exceeding 250 should be reported as too numerous to count (TNTC) (IDF, 1987; AOAC, 1990; APHA, 1993).

2.4 Sources of Microbial Contamination of Raw Milk

2.4.1. Microbial contamination from within the udder

The bacterial count in raw milk is normally as low as <1000 colony-forming units per milliliter (cfu/ml) when the milk is separated from the udder of healthy cows (Kurweil, 1973; Wallace, 2008). The normal flora of the cow's teat cistern, teat canal and teat skin does not influence the standard plate count of the milk (Wallace, 2008). Bovine mastitis affects a large percentage of cows in dairy herds worldwide (Bradley, 2002). Mastitis can be caused by pathogens that are termed contagious. These contagious pathogens tend to live on the cow's udder and teat skin, they transfer from affected cow (or quarter) to unaffected cow (or quarter) during milking. These pathogens adhere easily to the skin; they colonize the teat end and grow into the teat canal where infection occurs. High levels of contagious mastitis often have high Somatic Cell

Counts (SCCs). Environmental mastitis pathogens can be present in the herds and can transfer during milking or between milkings, when the cow is eating or lying down. The main pathogens that cause mastitis include: *Escherichia coli*, *Streptococcus uberis* and *Staphylococcus aureus*. They are regarded as those commonly associated with clinical mastitis in dairy cattle.

Milk from mastitic cows can bring about larger number of microorganisms in the bulk milk (Bramley and McKinnon, 1990; Wallace, 2008). Factors which determine the influence of mastitis on the number of bacteria in the milk include the strain of the organism causing the infection, the stage of the infection and the number of the herd infected (Bramley and McKinnon, 1990; Wallace, 2008). *Streptococci* particularly *Streptococci agalactiae* and *Streptococci uberis* most often influence the bacterial counts in the bulk milk (Bramley *et al*., 1984; Wallace, 2008).

2.4.2. Microbial contamination from the udder's exterior

Cows housed in a dirty environment can get their teats soiled with mud and other dirt present in such an environment. Teats soiled with manure can also bring about increase in the number of microorganisms in the milk if the teat surface of the cows is not properly washed prior to milking (Extension, 2010). The extent to which the teat surface is soiled determines if the soiled teat can contribute to the total bacterial count in the milk on the condition that the teat is not properly washed before milking (Wallace, 2008). It is therefore essential to sanitize the teat thoroughly after which a clean napkin can be used to dry it up (Wallace, 2008; Extension, 2010). There is also effect of ascending infection from environment into the teat leading to mastitis.

2.4.3. Microbial contamination from cleaning and sanitization of equipment

It is essential to keep all equipments and materials used in milking clean before milking and after milking the cows. This is because, leaving milk residue on the surfaces of the equipment, can facilitate the growth of various microorganisms (Wallace, 2008). Use of unclean water in milking can also serve as a source of microbial contamination (Bramley and McKinnon, 1990). There is therefore the need to use clean and safe water in milking processes and to ensure that milking machines are clean.

2.5. Overview of Milk Production and Safety Regulation in Nigeria

The major milk production systems in Nigeria include the Traditional system (Pastoral system), the agro-pastoral system, semi- intensive systems and intensive systems (Ndambi *et al*., 2007; Yahuza, 2001). The pastoral system is practiced mainly by the fulanis who are in care of not less than 95% of the cattle population (Yahuza, 2001).Some of these fulanis migrate from one location to another in search of green pasture for their cattle while the sedentary ones live permanently in homes (Ndambi *et al*., 2007). These pastorialists graze their cattle away from their homes during the dry season. The agro-pastoral system is an improved form of pastoral system in which the cattle owners get involved in crop production for the purpose of risk reduction and diversity in production (Ndambi *et al*., 2007). The cattle also graze around and feed on crop residues. The Semi- intensive systems is a peri- urban dairy production system in which the animals graze and also feed on concentrates (Ndambi *et al*., 2007). Crossbreeding is done between exotic bulls and local cows or through artificial insemination (AI) in order to obtain a better milk yield and to retain the ability of the animals to adapt to changing environmental conditions (Bayemi *et al*., 2005). In the intensive system, the animals are totally confined with proper attention to feeding and breeding management for an upgraded and better production (Diop and Mazouz, 1995; Julius, 2012). It involves the use of hired labour as milk is being supplied to large towns (Ndambi *et al*., 2007).

2.6. Bacterial Pathogens Associated with Raw Milk Consumption

Raw milk can get contaminated with pathogenic bacteria that are capable of causing illnesses which can lead to hospitalization or even death (CDC, 2015b). Such bacteria include: *Brucella*, *Campylobacter*, *Listeria*, *Mycobacterium bovis*, *Salmonella*, Shiga toxin-producing *E. coli*, *Shigella*, *Streptococcus pyogenes*, and *Yersinia enterocolitica* (CDC, 2015b). There have been reports on outbreaks of illnesses (from 1998 through 2011) owing to the consumption of raw milk that was contaminated with these pathogens. Most of the illnesses were found to be caused by *Escherichia coli*, *Campylobacter*, *Salmonella, or Listeria* (CDC, 2015b; FDA, 2015).

2.7. *Shigella*

2.7.1. Structure, classification and antigenic types of *Shigella* species

Shigella species is a pathogenic bacteria in the Enterobacteriaceae family which are gram-negative and rod-shaped (Schroeder and Hilbi, 2008). *Shigella* species are non-motile facultatively anaerobic non-lactose fermenters (Thomas and Gerald, 1996). They have been classified into four serogroups based on their biochemical characteristics and specific polysaccharide antigens which can be detected by polyvalent antisera (Thomas and Gerald, 1996; Downes and Ito, 2001; Kweon, 2008). The four serogroups of *Shigella* include *S. dysenteriae*, *S.sonnei, S. flexneri* and *S. boydii* (Ingersoll, 2002; Lampel and Maurelli, 2003). The most virulent species is *S. dysenteriae* which produces shiga toxin (Thomas and Gerald, 1996; Erqou *et al.,* 2007). Shiga toxin is a very potent cytotoxin which inhibits the synthesis of proteins in mammalian cells that are susceptible and it causes heamolytic-uraemic syndrome (Thomas and Gerald, 1996). The mildest form of shigellosis which occurs as diarrhoea is caused by *S.sonnei* (FDA, 2012).

2.7.2. Epidemiology of Shigellosis

Shigella species has a worldwide distribution. The species of *Shigella* that has been isolated most in the developed country is *S.sonnei* while *S. flexneri* is the predominant species in developing countries (Kurjak and Chervenak, 2006; FDA, 2012). Globally, the estimated cases of shigellosis are 165 million per year where 163 million of the cases are from developing countries (Kotloff *et al*., 1999).

Shigellosis is more common in places with low hygienic and sanitary conditions (Thomas and Gerald, 1996; Kurjak and Chervenak, 2006). There have been several reports on the cases of shigellosis in some parts of the world, but several of the mild cases are not reported (DuPont, 2000). In Australia, there was a reported incidence (2.4 cases per 100,000 population which makes 549 cases) of shigellosis in 2012 which were food borne and non-food borne cases (NNDSS, 2013). There was an outbreak of *S. sonnei* in Vilnius, Lithuania between 25th September and 11th October 2004. Forty one cases of shigellosis were reported by the Vilnius public health centre and 36 laboratory confirmed cases were found to be due to the consumption of unpasteurized milk curds prepared in a dairy. Epidemiological investigation confirmed that the patients had bought the curd from the same stalls at one of the two markets in Vilnius and that the *S.sonnei* isolates from the patients (consumers), dairy staff and market staff were of the same type and resistant to the same antibiotics (Zagrebneviene *et al*., 2005). Control and Preventive measures were taken as infected patients were hospitalized and staffs from who *S.sonnei* was isolated were asked to halt working until they had recovered (Zagrebneviene *et al*., 2005).

Shigellosis has been reported to account for 450,000 illnesses in the United States (Haley *et al*., 2010). Shigellosis can be spread through consumption of faecally contaminated foods and water and person-to-person transmission is also possible as outbreaks have been reported in daycare centers, institutional settings and nursing homes (DuPont *et al*., 1989; Ryan *et al*., 1997; Downes and Ito, 2001; Weir, 2002).

2.7.3. Clinical presentation

The two basic clinical presentations of Shigellosis include: watery diarrheoa with vomiting and mild to moderate dehydration and dysentery which is characterized by a small volume of bloody, mucoid stool and abdominal pain (Thomas and Gerald, 1996; Downes and Ito, 2001). Complicated cases of shigellosis is caused by *S. flexneri* which causes reiter's syndrome (reactive arthritis) which is characterized by several symptoms such as severe dehydration, intestinal perforation, toxic mega colon, bacteremia, toxaemia, septicaemia, seizures, toxic encephalopathy with headache and alterations of consciousness, septic shock and convulsion in very rare cases (Ovsyshcher *et al*., 1987; Erqou *et al*., 2007). Ingesting as little as 10-200 cells of *Shigella* can result to infection (Thomas and Gerald, 1996; Kurjak and Chervenak, 2006).

2.7.4. Antibiotic resistance of *Shigella*

Studies have also shown that *Shigella species* are endemic in Nigeria. Efuntoye and Adenuga (2011) isolated species of *Shigella* among nursery and primary school children with diarrheoa in Ago-Iwoye and Ijebu-Igbo, South-western Nigeria. The *S. dysenteriae* strains were resistant to tetracycline, trimethoprim-sulphamethoxazole and amoxicillin while the *S. flexneri* strains were resistant to ampicillin, trimethoprim-sulphamethoxazole, chloramphenicol, tetracycline, streptomycin and amoxicillin. All the 21 isolates were found to be susceptible to norfloxacin, pefloxacin and ofloxacin. Similarly, Abdulrasheed *et al.* (2014) isolated *Shigella* species with multiple drug resistance from children with diarrheoa cases in Ile-Ife, Southwest Nigeria. A total of 102 *Shigella* isolates: *S. flexneri* (45 isolates), *S. dysenteriae* (26 isolates), *S. boydii, (*19 isolates) and *S. sonnei* (6 isolates). Six of the isolates could not be typed under the test conditions. The isolates exhibited 100% resistance to Tetracycline and Ampicilin and >50 % resistance to Trimethoprim (80.4%), Nalidixic acid (64.7%), Cephalothin (64.7%), Chloramphenicol (59.8%), Sulphonamide (56.9%), and Cefotaxime (52.0%). The frequency of resistance of *Shigella* species to the antibiotics indicates the extensive use of antibiotics which necessitates continuous monitoring of antibiotic use.

2.8. *Salmonella*

2.8.1. Structure, classification and antigenic types of *Salmonella* species

Salmonella species belong to the family Enterobacteriaceae. They are gram negative facultatively anaerobes which are motile (Giannella, 1996; Kim *et al*., 2004). There are two (2) species of the genus *Salmonella,* they include *Salmonella enterica* and *Salmonella bongori* (Reeves *et al.,* 1989; Kauffmann, 1996; Popoff and Le Minor, 1997). *Salmonella* species have three major antigens namely; flagellar (H) antigen, somatic (O) antigen and the Vi antigen which is possessed by only a few serovars (Giannella, 1996). The flagellar (H) antigen is susceptible to destruction by heat as all flagella detaches by heating at $100^{o}C$ for 30 minutes (Slack and Synder, 1978). It is responsible for the motility characteristic of the bacteria (Slack and Synder, 1978). The O antigen which is part of the cell wall lipolysaccharide, is a heat stable polysaccharide, it is alcohol stable and hydrophilic (Slack and Synder, 1978; Eliana *et al*., 2012). The Vi antigen is an envelope antigen which enhances the virulence of the bacteria (Slack and Synder, 1978) *Salmonella enterica* has six (6) subspecies namely: (*S.enterica* subsp.*enterica* (I), *S. enterica* subsp.*salamae* (II)*; S. enterica* subsp.*arizonae* (IIIa); *S. enterica* subsp.*diarizonae* (IIIb); *S. enterica* subsp.*houtenae* (IV)*;* and *S. enterica* subsp.*indica* (VI) (Popoff and LeMinor, 1997; Brenner and McWhorter, 1998). There are over 2,500 serovars of *Salmonella enterica* subspecies *enterica* (Grassl and Finlay, 2008; Daniela *et al*., 2014). *Salmonella* possess an important characteristic of being able being able to grow and multiply outside their hosts, which gives them greater chances of survival (Gray and Fedorka-Cray, 2002).

2.8.2. Host preferences of *Salmonella* serovars

Some serovars of *Salmonella enterica* are host-restricted, others can be found in a range of hosts (Grassl and Finlay, 2008). The serovars have been divided into three groups based on their infectivity in relation to their hosts (Singh, 2013). The first group of serovars have a broad host range, hence, are not restricted as they can infect virtually all animals. They cause mild infections which may persist in the host without any serious clinical manifestation (Clarke and Gyles, 1993; Mrutyunjay *et al*., 2006). Serovars of *S.enterica* that cause infections with broad host range include: *Salmonella* typhimurium and *Salmonella* enteritidis (Mrutyunjay *et al*., 2006; Singh, 2013). The disease is more severe in young hosts but milder in adults due to a more developed immune response in adult hosts (Baumler *et al*.,1998).

Serovars like Dublin and Choleraesuis belong to another group as they exhibit host-preference for specific hosts although they are not completely restricted to such hosts (Kingsley and Baumler, 2000; Singh, 2013). Serovar Dublin causes systemic infection and enteric disease in

Cattle, pigs and humans but less frequently in humans (Clarke and Gyles, 1993; Kingsley and Baumler, 2000). The host restricted serovars include serovars Typhi, Paratyphi A, Gallinarum, Pullorum and Abortus equi (Mrutyunjay *et al*., 2006; Singh, 2013). They cause systemic infections in the various hosts they are restricted to. For example, serovar Paratyphi A causes a systemic disease (paratyphoid) in humans, while serovar Pullorum causes the systemic pullorum disease in poultry (Hornick *et al*., 1970; Buxton and Frase, 1977; Uzzau *et al*., 2000).

2.8.3. Epidemiology of Salmonellosis

Salmonellosis is common in developing countries and even in the United States (Cianflone, 2008). Infections caused by *Salmonella* species can be acquired through consumption of contaminated food and water which gives the bacteria access into the small intestine hereby invading the intestinal walls and spreading to other organs in the body (Tierrez and Garcı´a-del , 2005; Paulin *et al*., 2007; Buzby and Roberts, 2009). The most common reservoirs of *Salmonella* include animals like cows, chickens, reptiles like lizards, turtles and snakes; turkeys, pigs, etc (Giannella, 1996; LAH, 2013). Typhoid fever involves person-to-person transmission or consumption of contaminated food that has been prepared by a human carrier of *Salmonella typhi* (Giannella, 1996) Cases of Typhoid fever are low in developed countries, but cases of non typhoidal salmonellosis is increasing worldwide (Pui *et al*., 2011). The control of Salmonellosis tends to be difficult as it is a reservoir exists in various animals and human and animal carrier states exist (Giannella, 1996).

2.8.4. Occurrence and reported outbreaks of *Salmonella* infections

Karshima *et al.* (2013) isolated *Salmonella* species from raw milk in Kanam, Plateau State with a prevalence of 6.4% (38/600). There were reported cases of human *Salmonella Dublin* infection in California in 1981, 1982 and 1983 with 46, 70 and 123 cases respectively (CDC, 1984). These cases resulted from consumption of raw milk of which 24% of the patients reported that the milk was certified.

2.8.5. Pathogenesis of *Salmonella*

The pathogenesis of *Salmonella* involves the mechanism of the disease origin, how it develops in the body and whether it has an acute, chronic or recurrent manifestation (LAH, 2013). Some specific *Salmonella* serovars produce particular syndrome (Giannella, 1996). For example, *Salmonella* Typhi and Paratyphi-A produce enteric fever, *Salmonella* Enteritidis and *Salmonella* Typhimurium produce gastroenteritis while *Salmonella* Choleraesuis produces septicaemia (Giannella, 1996). For most non-typhoidal *Salmonellae*, the organism is able to gain entrance into the body on ingestion of food or water contaminated with *Salmonella*, there is also person-to-person spread (Giannella, 1996). The bacteria enter the gastrointestinal tract but some are killed in the stomach; the ones that survive pass through the acidic environments of the small intestine; they attach and invade the distal ileum and proximal colon, they invade the intestinal epithelium and proliferate within the epithelium (Giannella, 1996; Serman, 2001; Cianflone, 2008; LAH, 2013). Most of the serovars die in the extra intestinal sites, while in the case of gastroenteritis, the bacteria remains confined to the intestine (Giannella, 1996).Following epithelium invasion, there is intracellular multiplication and spread to the mesenteric lymph nodes and to every part of the body; they are then taken up by the reticuloendothelial cells which confines and regulates the spread of the bacteria (Giannella, 1996). Infection of the liver, spleen, kidneys, bones and other organs depends on the host defenses and the serotype (Giannella, 1996; Cianflone, 2008; LAH, 2013). Following invasion of the intestine, there is an induction of acute inflammatory response by most *Salmonella* serovars; this could result to ulceration. This invasion mediates the synthesis and release of proinflammatory cytokines such as IL-1, IL-6, IL-8, TNF-2, IFN-U, MCP-1, and GM-CSF (Giannella, 1996). This inflammatory response could result to symptoms such as chills, fever, abdominal pain and diarrhoea (Giannella, 1996).

2.8.6. Clinical presentation of Salmonellosis

Salmonellosis has four disease patterns which include: enteric fever, gastroenteritis, bacteraemia and chronic carrier state (Gray and Fedorka-Cray 2002; Pui *et al*., 2011). Hosts respond initially by neutrophil infiltration upon which lymphocytes and macrophages arrive (Cianflone, 2008).

Enteric fever

Enteric fever is a severe form of Salmonellosis (Giannella, 1996) and can occur through the ingestion of food or water that has been contaminated with human waste (WHO, 2003; Pui *et al*., 2011). Typhoid fever is the most common form of enteric fever (Giannella, 1996) and it is caused by *Salmonella* Typhi while *Salmonella* Paratyphi A, B and C cause paratyphoid fever (Pui *et al*., 2011). *Salmonella* Typhi is strictly transmitted by humans and not by animals (Non-complicated Typhoid fever is presented as mild illness with low-grade fever, anorexia and malaise; at an early stage, patients may also experience bronchitic cough (WHO, 2003). Complicated cases are presented as increased abdominal discomfort, perforation of the intestine and encephalopathy (WHO, 2003; Pui *et al*., 2011).

Gastroenteritis

Gastroenteritis is caused by about 150 *Salmonella* serotypes (Pui *et al*., 2011). Infections with non typhoidal *Salmonella* occurs through ingestion of food and water that has been contaminated with animal waste and not human waste (Pui *et al*., 2011). Gastroenteritis manifests as vomiting, nausea and diarrheoa which occurs between 6-48hours of ingestion; fever, chills abdominal pains and headache can also occur (Cianflone, 2008; Pui *et al*., 2011). Gastroenteritis is usually self-limiting but severe diarrheoa and dehydration cases require hospitalization (Cianflone, 2008).

Bacteraemia

This is the most common complication of gastroenteritis owing to the fact that the bacteria crosses the intestinal barrier and enter the bloodstream; this occurs more in immunocompromised patients (Buchwald and Blaser, 1984; Pui *et al*., 2011). Serovars like Cholearaesuis and Dublin are highly invasive and have been known to cause bacteraemia (Pui *et al*., 2011; Cianflone, 2008).

Chronic carrier state

Individuals who are chronic carriers are capable of spreading salmonellosis. This occurs more with those working in food-related industries (Pui *et al*., 2011). Depending on factors like age, 1-5% of patients become carriers as they harbor *Salmonella* Typhi in their gallbladder (WHO, 2003). Non typhoidal serotypes can remain in the gastrointestinal tract 6 weeks to 3 months. This duration depends on what serotype (Pui *et al*., 2011).

2.8.7. *Salmonella* infections in animals

All species of domestic animals can be infected with Salmonellosis but the young and pregnant animals are the most susceptible (OIE, 2010; NADIS, 2016). Poultry animals and pigs may be infected without showing any clinical sign (Wray and Wray, 2000). Such animals may be important in the spread of infection among flocks and herds and could cause human food poisoning when the animals enter the food chain; as such, there could be production of contaminated food products (OIE, 2010). In cattle, salmonellosis occurs more in dairy calves that are 1-10 weeks old; adult dairy cows and beef cattle can also be infected (Randall, 2001). Infection occurs mostly when the cattle feeds have been contaminated with faeces of cattle that are shedding the organism (Randall, 2001; Sheila and Simon, 2003). Animals could also get infected from feeding with contaminated feeding equipments (Randall, 2001).The serovars and species of animal infected determines the clinical signs, post-mortem investigation and epidemiological pattern of the disease (OIE, 2010). Some serovars are host restricted for example, *Salmonella* Gallinarum infects poultry while *S.* Abortus-*ovis* infects sheep (Buxton, 1957; Snoeyenbos, 1994). *Salmonella* Typhimurium and *Salmonella* Dublin commonly infect cattle (Buxton, 1957; Randall, 2001).

The clinical signs of salmonellosis in young calves include: dullness and depression, inability to suck, anorexia and fever, diarrheoa which may contain flecks of blood and mucus; death may occur within 24-48 hours (NYSCHAP, 2016)   Clinical signs in adult calves include: fever, depression, foul smelling diarrheoa containing blood and mucus, decrease in milk production, abortion, dehydration may occur depending on the severity of the disease (Buxton, 1957; Sheila and Simon, 2003).

2.8.8. Virulence of *Salmonella* species

Flagellar are complex motile structures found in members of the Prokarya, Archaea, and Eukarya (Gophna *et al*., 2003). Their presence have been associated with virulence in many pathogens including *Salmonella* species which usually express between five and ten flagella at random positions on the cell surface (Parker and Guard-Petter, 2001, Van Asten and Van Dijk, 2005). Studies have demonstrated that *S.* Enteritidis strains with deletions in major flagellar genes had decreased adherence to chicken intestinal explants and human intestinal epithelial cell lines, suggesting that flagella are important in adherence of *S.* Enteritidis to intestinal epithelial cells prior to invasion (Allen-Vercoe and Woodward, 1999, Dibb-Fuller *et al*., 1999). Fimbriae also contribute to colonization and invasion of the host. Many fimbriae are conserved between the *Salmonella* serovars, while some are unique. As each fimbria is typically specific to a given host receptor, the differences in fimbrial distribution among serovars may contribute to host specificity (Wisner *et al*., 2012).

Pathogenicity islands are forms of Genomic Islands (GI) that encode virulence genes which distinguish pathogenic bacteria from nonpathogenic bacterial strains that are closely related (Gal-Mor and Finlay, 2006). *Salmonella* Pathogenicity islands are specialized loci and prophage elements on which unique genes of *Salmonella* serovars are located (Schmidt and Hensel, 2004). *Salmonella* species possess *Salmonella* pathogenicity Island 1 (SPI-1) which is a virulence associated genomic region that is necessary for invasion of *Salmonella* into the epithelial cells of the host and the initiation of inflammatory responses in the intestine (Coombes *et al*., 2005; Hu *et al.*,2008; Krzyzanowski *et al.*, 2014). *Salmonella* Pathogenicity island 1(SPI-1) contain 41 genes which are very important for virulence of *Salmonella* species as they encode a molecular tool referred to as the type III secretion system (T3SS) (Marcus *et al*., 2000), T3SS regulatory genes, T3SS effectors and a metal transport system (Schmidt & Hensel, 2004, Thomson *et al*., 2008). The SPI-1 system is involved in both systemic infection and gastrointestinal colonization, but may not be essential for both infection process (Jones *et al*., 2007).

The T3SS can interact with the host cells by injecting effector proteins of the bacteria directly into the cytosol of the host cells (Galan, 2001; Marcus *et al*., 2000). The pathogenic genes located on *Salmonella* pathogenicity Island II (SPI-2) are responsible for modification of functions of the host cell which is necessary for survival and replication of *Salmonella enterica* in the macrophages of the host (Eswarappa *et al*., 2008). The *invA* gene is a SPI-1 encoded gene and is of critical importance in the invasion of the host epithelial cells (Garlan and Curtis, 1989). The *invA* gene has been used as a molecular marker in the detection of Enteropathogenic strains of *Salmonella* species (Rahn *et al*., 1992; Chiu and Ou, 1996; Malorny *et al*., 2003; Perelle *et al*., 2004). *Salmonella* species that lack *inv*A gene may not be able to penetrate the host epithelial cells as the gene is needed for full virulence in *Salmonella* and triggers the internalization required for invasion of deeper tissue for salmonellosis to occur (Zahraei *et al*., 2006; Oludairo *et al*., 2013). The *inv*A gene of *Salmonella* contains those sequences that are unique to this genus and has been proved as a suitable PCR target with potential diagnostic applications (Jamshidi *et al*., 2009). However, the presence of *invA* gene is not universal (Krzyzanowski *et al*. (2014) as *Salmonella enterica* serovars lacking the *invA* gene have been isolated from sewadge sludge (Krzyzanowski *et al*., 2014)and from stool samples obtained from a food-borne disease outbreak affecting seven individuals in Shenzehen, Guangdong Province, China (Hu *et al*., 2008).

2.8.9. Antibiotic resistance of *Salmonella*

Antibiotics can be defined as antimicrobial agents that are capable of inhibiting the growth or metabolic activities of microorganisms through a chemical substance derived by natural means (Varley *et* al., 2009; Hamilton and Wenlock, 2016). Antibiotics can be bacteriostatic or bacteriocidal (Varley *et al*., 2009). Bacteriostatic antibiotics might not necessarily get rid of bacterial infections but they prevent further multiplication of the bacteria; Hence the host's immunity contributes to the effectiveness of a bacteriostatic antibiotic (Varley *et al.,* 2009). Bacteriocidal antibiotics on the other hand completely eliminate the bacteria (Varley *et al*., 2009). Antibiotic resistance has been in existence since 1946 where about 14% of *Staphylococcus aureus* cultures were resistant to sulphonamides (Varley *et al*., 2009). The indiscriminate use of antibiotics in treatment of infections, in the agricultural industry, as household disinfectants and as growth promoters has led to increasing antibiotic resistance in different parts of the world (Molt, 2000; Walsh, 2000; Varley *et al*., 2009).

The most common antimicrobials that *Salmonella* has gained resistance to are namely: chloramphenicol, quinolones, tetracyclines, aminoglycosides, β-lactams, trimethoprim and sulfonamides (Hakanen *et al*., 2001; Abatcha *et al*., 2014). Antimicrobial resistance of nontyphoid *Salmonella* has been on the increase since in the early 1990s (Lin-Hui *et al*., 2004). However, the rate of resistance varies with different serotypes and different antibiotics as *Salmonella* Enteritidis which is a prevalent serotype was found to be more susceptible to antimicrobial agents than other serotypes in the 1990s (Lin-Hui *et al*., 2004). *Salmonella* Typhimurium is also a globally prevalent serotype as a multidrug-resistant (MDR) strain of phage type 104 (DT104) as *S.* Typhimurium which was isolated in the early 1990s was found to be resistant to 5 antimicrobial agents which include: sulfonamide, chloramphenicol, streptomycin, ampicillin and tetracycline (Molbak *et al.,* 1999). The increasing distribution and prevalence of MDR among *Salmonella* has led to a switch to the use of fluoroquinolones in the management of enteric fever (Maskey *et al*., 2008). However, the widespread use of fluoroquinolones has also brought about its decreased susceptibility as study carried out by Brown *et al*. (1996) showed that some clinical isolates of *Salmonella* Typhimurium and three other *Salmonella* species had decreased susceptibilities to 4-quinolone drugs due to mutations of the gyrA gene of the isolates. In 2003-2004, there was an outbreak of MDR *Salmonella* Typhimurium definitive type 104 infection which was linked to consumption of commercial ground beef in the northeastern part of the United States (Dechet *et al*., 2006). The isolates were resistant to streptomycin, ampicillin, sulfamethoxazole, chloramphenicol and tetracycline (Dechet *et al*., 2006). In 1997, Cody *et al.* (1999) also reported two outbreaks of MDR *S.* Typhimurium DT104 infection which was linked to the consumption of raw milk cheese in Northern California. In 2000, an outbreak of MDR *S*. Typhimurium infection from milk contaminated after pasteurization occurred in Pennsylvania and New Jersey, 12 of the isolates tested were resistant to ampicillin, kanamycin, streptomycin, sulfamethoxazole, and tetracycline. An antibiotic susceptibility study carried out in Hara, eastern Ethiopia by Reda *et al*. (2011), the *Salmonella* isolates showed resistance to Chloramphenicol (62.3%), Gentamicin (3.6%), Ampicillin (100%), Norfloxacin (7.1%), Amoxycillin (100%) and Tetracycline (71.4%).

Over the years, there has also been widespread of MDR phenotypes among *Salmonella* Typhi, *Salmonella* Paratyphi has shown lower resistance rate (Rowe *et al*., 1997; Gupta *et al*., 2008; Parry and Threlfall, 2008). Surveillance studies have been carried to show the geographic variation of MDR *S.* Typhi isolates within the same region, for example, sites in India, Vietnam and Pakistan have higher rates of MDR than sites in China and Indonesia (Ochiai *et al*., 2008). There have been several reports on the outbreaks caused by MDR strains of *S*. Typhi in China, Egypt, Gulf of Guinea region, India, Singapore, United Kingdom (Prakash and Pillai, 1992; Rowe *et al*., 1992; Mourad *et al*., 1993; Baltazar *et al*., 2015; Phoon *et al*., 2015; Yan *et al*., 2016).

2.8.10. Antibiotic resistance among *Salmonella* isolates in Nigeria

Several studies have shown that antibiotic resistant *Salmonella* are endemic in Nigeria and they have been isolated from various sources including humans, animals, food products and even the environment. A study conducted in Vom, Plateau state by Anyawu *et al*. (2010) revealed that *Salmonella* Paratyphi isolated from day-old chicks was sensitive to Ciprofloxacin, Kanamycin, Chloramphenicol, Gentamycin and Norbafloxacin but resistant to Penicillin. Makut *et al*. (2014) isolated *Salmonella* species from locally processed cow milk products sold in Keffi Metropolis, Nasarawa State .The isolates were highly resistant to Septrin (71.4%), Chloramphenicol (71.4%), Sparfloxacin (85.7%) and Erythromycin (71.4%). Akinyemi *et al.* (2007) studied trends of MDR in *Salmonella* Typhi in Lagos and reported increased resistace of chloramphenicol from 1997 (72.4%) to 2003 (89.2%). The number of MDR *Salmonella* increased from 70% in 1997 to 84.6% in 2003. Susceptible antibiotics include: Ampicillin, Chloramphenicol, Tetracycline, Cotrimoxazole, and Nalidixic acid.

The increasing rate of antibiotic resistance necessitates the need for other antibiotics to be prescribed in cases of *Salmonella* infections as most of the first line antibiotics have been found to be resistant, Hence effective treatment may not be achieved in-vivo.

2.8.11. Detection of *Salmonella*

The detection of pathogenic microorganisms in food is an important aspect in the quality and safety of food (Lopez-Campos *et al*., 2012). Microbiological analysis is an essential tool in monitoring the state of contamination at all production stages in order to detect emerging risks (Lopez-Campos *et al*., 2012; Mata and Vanetti, 2012).It is also an important tool in carrying out laboratory analysis in accordance with the microbiological standards which have been established for all food types (Lopez-Campos *et al*., 2012). Detection of microorganisms involves morphological examination, biochemical, immunological or genetic means (Lopez-Campos *et al*., 2012).

The detection of *Salmonella* in foods has been described in the ISO 6579:2005 method (International Standards Organization, 2005). This involves conventional culture method. The conventional method involves several steps which include: nonselective enrichment, selective enrichment, and selective/differential plating and morphological, biochemical and serological confirmation. This method is quite time consuming as it takes about 5-10 working days to confirm a positive result and at least 3 working days to produce a negative result (Mata and Vanetti, 2012; Zadernowska and Chajecka, 2012). However, the conventional method is still very much in use by many laboratories as it is a harmonized method referred to as the "gold standard" in food diagnostics (Jasson *et al*., 2010; Odumeru and Leon-Velarde, 2012). Moreover, variation in gene expression of microorganisms and environmental factors can give a false result for a biochemical test (Malorny *et al*., 2003).

Traditional Culture Methods

Cultural based methods are based on nutrient acquisition, biochemical characteristics, and metabolic products that are unique to *Salmonella* species. (Ricke *et al*., 1998). Depending on the need for confirmations, culture based methods typically require 5-7 days to obtain results as the method depends on the ability of *Salmonella* to visible colonies which can then be characterized by carrying out additional biochemical tests and or serological tests (Odumeru and Leon-Velarde, 2012; Zadernowska and Chajecka, 2012).

The four basic stages for the detection of *Salmonella* in accordance with the ISO 6579 2003 standard include: Pre-enrichment in non-selective liquid medium, selective enrichment in liquid media, plating on selective media, serological and biochemical identification of suspected colonies (Zadernowska and Chajecka, 2012). The pre-enrichment stage involves the enrichment of a portion of the food sample in a non-selective pre-enrichment medium such as

Buffered peptone water (BPW) (Odumeru and Leon-Velarde, 2012). This is essential for the proliferation and regeneration of damaged cells in the food samples due to heat, cold or osmotic shock (Odumeru and Leon-Velarde, 2012; Zadernowska and Chajecka, 2012). Incubation is done at 37^{o} C for 18-24 hours. The second stage involves inoculating the primary enrichment cultures into secondary selective enrichment broths, such as Selenite Cystine broth (SC), Rappaport Vasiliadis Soy broth (RVS), Tetrathionate Broth (TT), or Muller-Kauffmann Tetrathionate Novobiocin broth (MKTTn) and incubating the cultures at elevated temperatures (37°C or 42°C for 18-24 hours) (Odumeru and Leon-Velarde, 2012). Rappaport-Vassiliadis (RVS) medium is strongly selective and contains malachite green and sodium chloride (which inhibits the growth of accompanying microflora). It also contains Soya peptone, pH 5.2, and increased temperature of incubation (41.5°C) which favour the growth of *Salmonella* species. The medium is dark blue and clear. Strains of *Salmonella* species grow on this medium in the form of milky residue, while the colour of the medium itself does not change (Zadernowska and Chajecka, 2012). Cultures are inoculated onto selective media such as Xylose Lysine Deoxycholate agar (XLD agar), Bismuth Sulphite agar (BIS), Brilliant Green agar (BG) with or without the addition of sulfadiazine or sulfapyridine (BGS), modified semisolid Rappaport Vasiliadis (MSRV), Salmonella Shigella Agar, or Hektoen Enteric agar for individual colonies. Based on morphology or indicative biochemical reactions on selective media, typical *Salmonella* colonies are then cultured onto non-selective medium prior to biochemical confirmation tests (Odumeru and Leon-Velarde, 2012; Zadernowska and Chajecka, 2012). Classical biochemical and serological tests are performed for Preliminary identification of *Salmonella*. These include: glucose fermentation, negative urease reaction, lysine decarboxylase, negative indole test, negative Voges-Proskauer reaction, β-galactosidase detection, H2S production, and fermentation of dulcitol (Odumeru and Leon-Velarde, 2012; Zadernowska and Chajecka, 2012). Serological confirmation tests typically involve the use of polyvalent antisera for flagellar (H) and somatic (O) antigens. Isolates with a typical biochemical profile, which agglutinate with both H and O antisera are identified as *Salmonella* species. Positive isolates are often sent for further serotyping to identify the serovar using specific antisera according to the Kauffman-White (KW) typing scheme recognizing 46 O antigens, and 119 H antigens, thereby permitting the characterization of 2,541 serotypes (Shipp and Rowe, 1980). Other methods for *Salmonella* detection include: immunomagnetic separation, immunological based methods (Rapid agglutination assays, Enzyme-linked immunosorbent assay (ELISA) and Lateral flow immunoassays), molecular methods (Polymerase chain reaction (PCR), Real-time PCR, Multiplex PCR, Reverse transcriptase PCR (RT-PCR) and Nucleic acid hybridization) and Phage based detection methods (Odumeru and Leon-Velarde, 2012; Zadernowska and Chajecka, 2012).

Polymerase Chain Reaction (PCR)

PCR is a molecular tool which involves the rapid detection of food borne pathogens and is highly sensitive and specific (McKillip and Drake, 2004; Odumeru and Leon-Velarde, 2012; Zadernowska and Chajecka, 2012).It is a technique based on the specific amplification of a short target DNA sequence (Mullis *et al*., 1986). The extracted DNA is first subjected to heat denaturation into single stranded DNA after which specific short DNA fragments (primers) are annealed to the single DNA strands, followed by extension of the primers complementary to the single stranded DNA with the aid of a thermostable DNA polymerase, such as Taq polymerase, an enzyme originally isolated from the bacterium Thermus aquaticus (Chien *et al*., 1976). Each new double-stranded DNA is then targeted during a new thermal cycle and thus the exponential amplification of the specific DNA sequence is achieved. The amplified product is then separated by gel electrophoresis and visualized by staining with fluorescent ethidium bromide. However, PCR is limiting in that it is time consuming and labour intensive due to post amplification steps, it is not sensitive enough to measure the accumulated DNA copies accurately, and can only provide a qualitative result (Odumeru and Leon-Velarde, 2012).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Materials

Materials used in this study include: Aluminum foil paper, test tubes, glass slides, sterile universal bottles, disposable hand gloves, face masks, disposable petri dishes, pipettes, hand sanitizers, conical flasks, measuring cylinder, weighing balance, Bunsen burner, cotton wool, inoculating loop, gram staining reagents, masking tape, permanent marker, test tube racks, raw milk samples, fermented milk samples.

Bacteriological culture media

The media used in this study include: Buffered peptone water, Rappaport-Vassiliadis Soy (RVS) broth, Gram negative broth, Salmonella-Shigella Agar (SSA), Xylose Lysine Deoxycholate (XLD) agar, and Nutrient agar and Andrade peptone water.

For biochemical analyses, media used include: Triple Sugar Iron (TSI) agar, Sulphur Indole Motility (SIM) medium, Urea agar base + 40% urea solution, Simmons citrate agar and Methyl red- Voges Proskauer medium.

Sugars and Amino acids

The sugars used for further biochemical analyses include: Mannitol, adonitol, Trehalose, inositol, salicin, sorbitol, lactose, maltose, dulcitol, arabinose, raffinose and rhamnose. The amino acids used include: Lysine, ornithine and arginine.

3.2 Study Area

The study area was Zaria, in Kaduna state, Nigeria, which is located in the northern region of Nigeria, within latitudes 11^0 7’,11^0 12’N and longitudes 7^0 41’E (Mamman *et al*., 2000), with an altitude of 500-700 meters above sea level and a total area of 300km^2. The town is characterized by a tropical climate, a monthly mean temperature ranging from 13.8^{0}C to 36.7^{0}C and an annual rainfall of 1092.8mm (Agbogbu *et al.*, 2006). It has an estimated population of 408, 198 and a density of 1,360.7km^2 (NPC, 2006). Zaria metropolis has two Local Government Areas (LGAs) namely: Zaria and Sabon Gari Local Government Areas (Ministry of Economic Development, 1996). In 2014, cattle population in Zaria was reported to be 1,144,000 and about 99% of these cattle were being managed under semi-intensive or extensive system (Aliyu *et al*., 2014). The major milk production systems in the study area include: the pastoral system, the agropastoral system and the intensive dairy farming. The pastoral system is practiced mainly by the fulanis who are in care of not less than 95% of the cattle population (Yahuza, 2001).Some of these fulanis migrate from one location to another in search of green pasture for their cattle while the agropastoralists are sedentary as they live permanently in homes (Ndambi *et al*., 2007). These pastorialists graze their cattle away from their homes during the dry season. The cattle also graze around and feed on crop residues. Intensive dairy farming involves farmers using part or all of their land to grow fodder crops for their dairy cattle. Milking and milk sales are daily activities in Zaria and its Environs as a large population of the study area love to consume milk and its products.

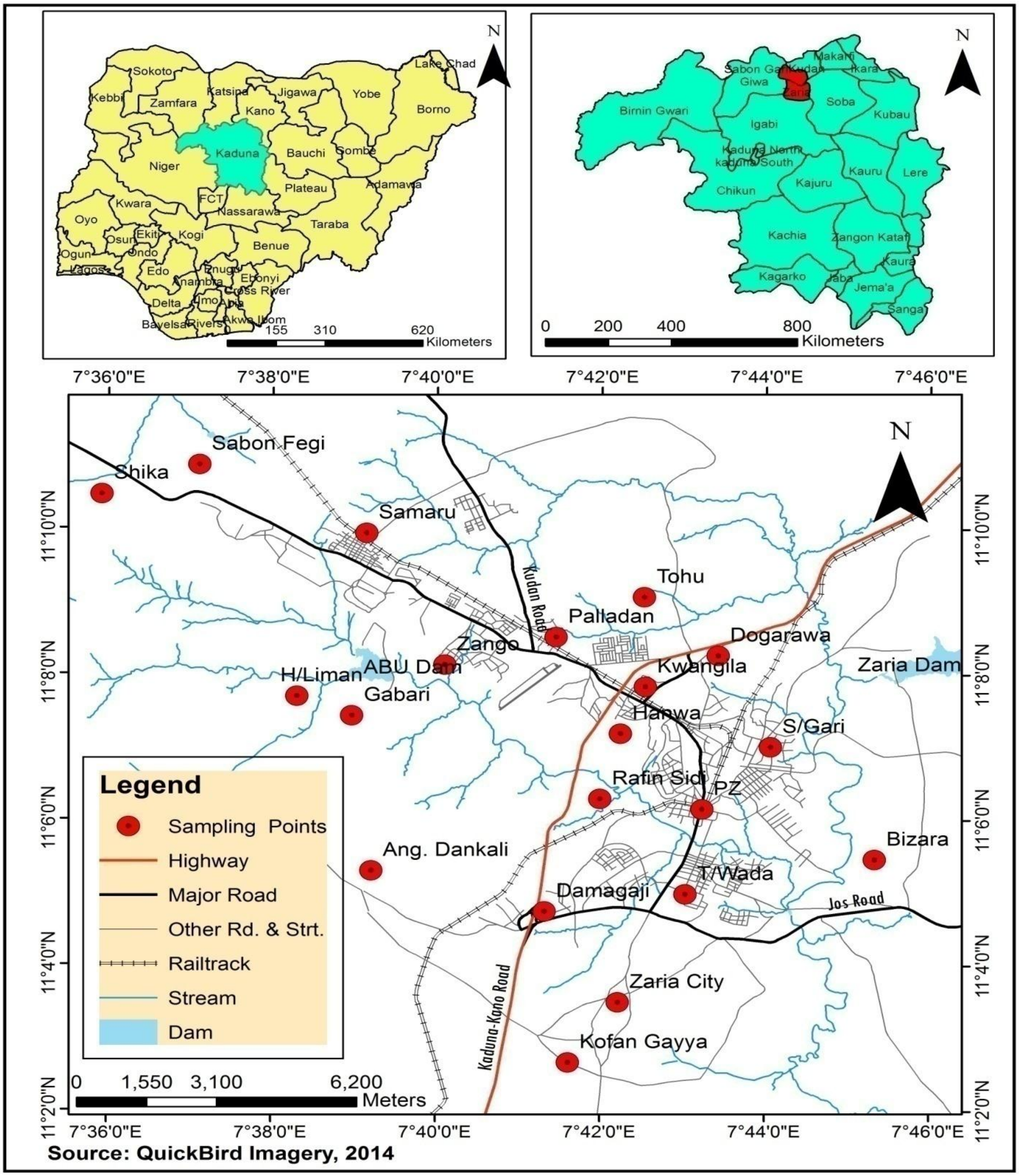

Figure 3.1. Map of Zaria showing sampling locations

3.3 Study Design

Cross Sectional study design was used for this study.

3.4 Sample Size

Sample size was determined using the formula as described by Thrusfield (1997).

Where

N=sample size

Z=Appropriate value for the standard normal deviation of 95% confidence interval=1.96

p= prevalence of 4% for *Shigella* species in raw milk was obtained from Ogbonna *et al*. (2012). Prevalence of 7.6% for *Salmonella* species in raw milk (Ogbonna *et al*., 2012) was used for this study. Also, prevalence of 9.8% for *Salmonella* species in fermented milk and prevalence of 6% for *Shigella* species in fermented milk obtained from Ogbonna *et al*. (2012) was used for the study.

q=1-p

d^2=allowable error of 5%=0.05

= 59 samples for *Shigella* in raw milk

= 108 samples for *Salmonella* in raw milk

= 136 samples for *Salmonella* in fermented milk

= 87 samples for *Shigella* in fermented milk

The sample size which is 244 (108 samples for raw milk and 136 for fermented milk) was adjusted by about 10% giving a total number of samples to be collected to be 268 (128 for raw milk and 140 for fermented milk). Samples for *Shigella* species were obtained from the number of samples collected for *Salmonella* species.

3.5 Sampling Technique

Non-probability sampling technique was implored in which about 25 ml of fresh cow milk samples and fermented cow milk samples were collected from bulk milk into clean sterile sample bottles and sterile polythene bags respectively. This was done based on availability from dairy herds and market places respectively.

3.6 Sample Collection and Transportation

Sample collection was done between January 2016 and June 2016.

Based on availability, fresh milk samples were collected from dairy herds in thirteen Fulani settlements within Zaria and environs between 6:30am – 8:00am. The fresh milk samples were collected from Sabon Fegi, Kwakwaran Manu, Palladan, Hanwa, Tohu, Bizara, Samiyan uku, Dogarawa, Rafin Sidi, Gabari, Hayin Liman, Unguwan Dankali and Unguwan Nassarawa while fermented milk samples were collected between the hours of 1:00am and 2:30am from ten market places/ retail points which include: Samaru, Zango, Kwangila, Sabon Gari, PZ, Tudun wada, Kofan Gayya, Zaria City, Danmagaji and Shika. Twenty-five ml (25ml) of the raw and fermented milk samples were aseptically collected into sterile sample bottles and polythene bags respectively from bulk milk, well labeled and transported in an ice-packed container (cooler) to the Bacterial zoonoses laboratory of the Department of Veterinary Public

Health and Preventive Medicine, Ahmadu Bello University, Zaria for immediate processing and analyses.

3.7 Sample Processing and Laboratory Procedure.

3.7.1 Determination of total aerobic microbial counts in milk and milk products

Standard plate count method was used to determine the Total Aerobic Colony Counts of microorganisms in the samples of the raw and fermented milk (Sanders, 2012). A hundred-fold serial dilution of the milk samples from each of the different locations was performed by taking 0.1ml into 9.9 ml normal saline diluents (10^{-2}) using a Pasteur pipette, then a fresh sterile pipette was used to transfer 0.1ml of the 10^{-2} dilution to another 9.9ml of diluents to give 10^{-4} dilution. This procedure was repeated for further dilutions to attain a suitable dilution (10^{-5} for raw milk and 10^{-7} for fermented milk). 0.1 ml of the final dilution was plated out on Nutrient Agar using spread plate technique. The plates were incubated at 37^{o}C for 24 hours. Following incubation, all colonies within the countable range were considered. Counts exceeding 300 colonies per plate were recorded as too numerous to count (TNTC). The average microbial loads of the raw and fermented cow milk from the different locations were obtained and expressed as Colony Forming Units per milliliter [CFU/ml] (Harrigan and McCance, 1976).

Cfu/ml= colony count (CFUs) on agar plate × final dilution on plate

The logarithm of the counts were obtained by transforming the log to base 10 ($\log_{10}$) of each CFU/ml for statistical analyses.

3.7.2 Culturing and isolation of *Shigella* and *Salmonella* species

3.7.2.1 Enrichment of *Shigella* species

Ten milliliter (10mls) of each milk sample was transferred into 90mls of Gram negative broth (Hajna) for enrichment. These were incubated at 37^{o}C for 24 hours.

3.7.2.2 Selective isolation of *Shigella* species

A loopful of each incubated broth was streaked aseptically onto Salmonella Shigella agar and Xylose Lysine Deoxycholate (XLD) agar plates and incubated at 37°C for 24 hours. Transparent or translucent colonies on Salmonella Shigella agar and pinkish colouration on XLD plates are suggestive of *Shigella* species (Edwards and Erwing, 1962). The transparent or pinkish colonies were subcultured on Nutrient Agar (NA) slants for biochemical characterization of *Shigella* species.

3.7.2.3 Pre-enrichment of *Salmonella species*

Ten mls of each milk sample was transferred into 90mls of buffered peptone water (Merck, Germany) and incubated at 37°C for 24 hours for pre-enrichment of *Salmonella* species.

3.7.2.4 Selective enrichment of *Salmonella* species

For selective enrichment of *Salmonella* spp, 1ml of the pre-enriched sample was transferred into 9ml Rappaport-Vassiliadis Soy (RVS) broth (Oxoid, UK) and incubated at 37^{o}C for 24 hours.

3.7.2.5 Selective isolation of *Salmonella* species

A loopful of each enriched broth was streaked aseptically onto Salmonella Shigella agar plates and Xylose-Lysine Deoxycholate (XLD) agar plates and incubated at 37°C for 24 hours. One or more characteristic colonies appearing transparent or translucent, with or without black centers on SSA agar and colonies appearing pinkish with or without black centers on XLD agar were subcultured on Nutrient Agar (NA) slants for biochemical characterization of *Salmonella* spp.

3.7.2.6 Gram Staining and Microscopy for Morphological identification of *Shigella* and *Salmonella* species

The Gram staining technique was carried out as described by Prescott *et al.* (2009). The stained slides were air-dried for some minutes and viewed with the light microscope using oil immersion at 100× objective lens for microscopic identification. Organisms appearing as gram-negative rods were considered presumptively as *Shigella* or *Salmonella* species.

3.7.2.7 Biochemical Characterization

Appropriate biochemical tests which include Triple sugar Iron Agar (TSI) test, Methyl Red (MR) -Voges-Proskauer (VP) test, Citrate utilization test, Sulphur Indole Motility (SIM) test, Urease test and Carbohydrate fermentation test as described by Odewumi, (1981) and Cheesbrough (2003) were carried out.

Triple Sugar Iron (TSI) Agar Test

The Triple Sugar Iron Agar was prepared according to the manufacturer's instructions. Using a sterile wire loop, the isolate to be cultured was obtained from a 24-hour culture on selective medium and stabbed on the butt then streaked on the surface of the slant. The tubes were incubated at 37^oC for 24 hours. *Shigella* species show red-pink colouration in the slope and yellow colouration in the butt of TSI culture while *Salmonella* species show the same appearance as that of *Shigella* along with hydrogen sulphide production on TSI culture (Odewumi, 1981; Cheesbrough, 2003).

Urease Test

The urea agar was prepared according to manufacturer's instruction. The urea agar slants were inoculated and incubated at 37^{o}C for 24 hours. *Shigella* and *Salmonella* species are urease negative. Hence, no colour change will occur (Odewumi, 1981;Cheesebrough, 2003).

Sulphur, Indole and Motility Test

The Sulphur Indole Motility medium was prepared according to manufacturer's instruction.

It is a medium commonly used to differentiate members of *Enterobacteriaceae* owing to the ability of an organism to reduce sulphur, produce indole and move away from the line of inoculation through the sloppy agar. The isolates were inoculated by stabbing the medium and incubated at 37^{o}C for 24 hours. On 24 hours incubation, they were observed for hydrogen sulphide (H_2S) production which is characterized by a black colouration and motility which is characterized by migration from the line of inoculation. Three drops of Kovac's indole reagent were added and shaken gently. A positive reaction is indicated by the development of a red colour in the reagent layer while for a negative reaction, the indole reagent retains its yellow colour. *Salmonella* species produce hydrogen sulphide, are motile and are indole negative while *Shigella* species do not produce hydrogen sulphide, they are non-motile and could be indole positive or negative depending on the strain (Odewumi, 1981).

Methyl Red- Voges-Proskauer Test

Five mls (5ml) of MR-VP broth was inoculated and incubated for 24hours at 37^{o}C. After incubation, 1ml of the broth was transferred to a small tube. To this quantity, 2-3 drops of methyl red was added. *Shigella* and *Salmonella* are Methyl Red positive and this is indicated by a red colouration.

To the rest of the broth in the original tube, 15 drops of 5% α-Naphthol in ethanol followed by 5 drops of 40% potassium hydroxide were added. It was shaken and placed in a sloppy position. *Shigella* and *Salmonella* are Voges-Proskauer negative. Hence, no color change occurs (Odewumi, 1981; Cheesebrough, 2003).

Citrate Utilization Test

A Simmons' citrate agar was prepared according to manufacturer's instruction. The slant was inoculated and incubated at 37^{o}C for 24 hours. *Shigella* species are citrate negative. Hence, the medium will retain its green color. *Salmonella* species are mostly citrate positive. Hence, the medium becomes blue in color (Odewumi, 1981; Cheesebrough, 2003).

Sugars

One gram (1g) of each sugar was weighed and 1.5g of Andrade peptone water was also weighed and mixed in a conical flask. These were dissolved in 100ml of distilled water and then 5ml were dispensed into sterile test-tubes and steamed at 115^{o}C for 5-10 minutes and allowed to cool. Two to three colonies of isolates which had been sub-cultured on a selective medium were picked and suspended into the test tubes, after which they were kept in the incubator for 24-48 hours at 37^{o}C. Red coloration indicates a positive result while light-pink colouration indicates a negative result depending on whether the organism is able to break down the sugars (indicated as red colouration) or not (indicated as light-pink colouration).

Amino Acid Decarboxylase Test (Arginine, Ornithine and Lysine)

One gram of each amino acid was weighed along with 1.5g of Andrade peptone water and then dissolved in 100ml of distilled water, it was then dispensed into sterile test- tubes and then heated for 5-10 minutes and allowed to cool. Thereafter, the stored isolates were inoculated into the tubes and then 2 to 3 drops of mineral oil were added to them and then the tubes were covered and kept in the incubator for 18-24 hours and read (Moller, 1954).

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

The test was carried out and interpreted as recommended by the manufacturer (Oxoid, UK).

A 24 hour culture of presumptive *Shigella* and *Salmonella* colonies on selective media was obtained of which 1-3 isolated colonies were picked and emulsified in 5ml of sterile saline solution. Using a sterile micropipette, 100µl of the bacterial suspension was added to each well in the set. Using a micropipette, wells 1, 2 and 3 were overlaid with mineral oil. The inoculated rows were resealed and labeled on the end tag with the specimen identification number. Incubation was done at 37 ^{o}C for 24 hours.

On 24 hours incubation, the strip was removed from the incubator; the sealing tape was peeled back and examined. The reactions were evaluated as positive or negative by comparing them with the colour chart provided. Results were recorded under the appropriate heading on the report form. Interpretation was done with the aid of the table of reactions provided.

However, reagents were added to some of the wells as instructed; to well 8, 2 drops of Kovacs reagent was added and evaluated within 2 minutes of the addition of the reagent for indole production. To well 10, 1 drop each of VPI and VPII reagents were added. These were evaluated within 15 to 30 minutes after the addition of reagents for Vogues-Proskauer reaction.

To well 12 (Tryptophan Deaminase), 1 drop of TDA reagent was added. The test was evaluated immediately after the addition of reagent.

Result Interpretation

This was done with the aid of an octal coding system which has been adopted for Microbact in which each group of three reactions produces a single digit of the code. Using the results obtained, the indices of the positive reactions are circled. The sum of these indices in each group forms the code number. The code obtained was then fed into the computer aided identification package. The percentage figure displayed against the organism name is the percentage share of the probability for that organism which is a part of the total probabilities displayed.

3.7.2.9 Serological identification

All biochemically positive *Salmonella* and *Shigella* isolates were confirmed serologically by the use of *Salmonella* polyvalent 'O' group A-S antiserum (Oxoid, UK) and *Shigella* polyvalent 'O' group A-D antiserum respectively in a slide agglutination test. The serological test is based on the principle that antibodies in the serum which when exposed to bacterial antigens will agglutinate with the bacteria carrying the corresponding antigen (Oxoid, 2013). Two drops of saline were put on two glass slides each. A portion of a 24 hour culture of *Salmonella* was emulsified in each drop of saline to give a smooth dense suspension. To one of the suspension which was labeled as the control, one drop (40 µl) of saline was added and mixed. To the other suspension, one drop (40 µl) of undiluted antiserum was added and mixed. The slides were then rocked for one minute and observed against a dark background for agglutination. A positive test result was recorded if agglutination was observed in the test suspension without agglutination in the control (Oxoid, 2013).

The same procedure was carried out for *Shigella* except that the *Shigella* polyvalent antiserum was used.

3.8 Antibiotic Sensitivity Test

Antibiotic sensitivity test was carried out using the Kirby-Bauer disk diffusion method (Bauer *et al*., 1966). Colonies of each isolate was emulsified in normal saline to match 0.5 McFarland turbidity standards in order to standardize the inoculum. Within 15 minutes after adjusting the turbidity of the standardized inoculum, a sterile cotton swab was dipped into the adjusted suspension. The swab was streaked on the entire surface of plain Mueller-Hinton (Oxoid, UK) agar plates and Tetracycline (30µg), Chloramphenicol (30 µg), Amoxicillin-clavulanate (20/10 µg), Ciprofloxacin (5 µg), Amikacin(30µg), Cefotaxime(30µg), Trimethoprim-sulfamethoxazole (1.25/23.75µg), Ceftriaxone (30 µg), Nitrofurantoin (300 µg), Cefotetan (30 µg) and Streptomycin (10 µg) discs were placed on the surface of the inoculated agar plates using antibiotic disc dispenser (Oxoid) and incubated at 37°C for 24 hours. The zones of inhibition were measured and compared with the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2014).

3.9 Polymerase Chain Reaction (PCR) for *Salmonella* species

3.9.1 DNA extraction

An 18-24 hour culture of each isolate was transferred to microtubes containing 400 µl of lysis buffer. 25 µl of proteinase K. Incubation was done at 65^{o}C for 1 hour with vortexing at 20 minutes interval. After incubation, 400 µl of phenol chloroform was added to each suspension, vortexed and centrifuged at 13,000 revolutions per minute (rpm) for 10 minutes. The supernatants were transferred into fresh, sterile tubes and 400 µl of chloroform was added.

These were again vortexed and centrifuged at 14,000 rpm for 5 minutes. The supernatants were then collected in fresh, sterile tubes and 1000 µl each of absolute ethanol was added followed by 40 µl each of 3 molar sodium acetate. These were incubated overnight at -20 °C. The incubated suspensions were centrifuged at 14,000 rpm for 10 minutes and the supernatants discarded after which 400 µl of 70 % ethanol was then added to each residue and centrifuged at 14,000 rpm for 5 minutes. Again, the supernatants were discarded and each residue was allowed to air-dry after which 50 µl of ultra pure water was added.

3.9.2 Primers

The PCR primers used to target the *inv*A gene were:

5' GTG AAA TTA TCG CCA CGT TCG GGC AA-3' Forward

5'-TCA TCG CAC CGT CAA AGG AAC C-3' Reverse

An amplicon of 284bp was expected according to Rahn *et al*. (1992).

3.9.3 Procedure for PCR amplification

$MgCl_2$, Taq (*Thermus aquaticus*) polymerase, and dNTPs which made up 25 µl master mix (Manufactured by Fermentas ™, Germany)was then mixed with 10 µl template DNA, 2.5 µl forward primer, 2.5 µl reverse primer and 10 µl nuclease free water in a PCR microtube. The cycling parameters were: initial denaturation at 95^{o} C for 2 minutes, 30 cycles of amplification consisting of denaturation at 94º C for 30 seconds, annealing at 53^{o} C for 30 seconds, extension at 72^{o} C for 60 seconds and final extension was at 72^{o} C for 7 minutes. PCR was carried out in a DNA thermal cycler.

3.9.4 Electrophoresis of PCR product

After the amplification procedure, 10 µl of the final reaction product was separated by electrophoresis on 2 % agarose gel along with 100 bp DNA ladder. Visualization was carried out through staining with ethidium bromide and the use of Ultra violet (UV) illumination.

3.10 Data Analyses

Total aerobic counts (TACs) were transformed into their logarithmic values and then expressed as logarithmic mean and Standard deviation (mean ± SD) using IBM Statistical Package for Social Sciences (SPSS version 20.0). Unpaired samples t-test was used in assessing the significant difference between the mean TAC of raw milk and fermented milk. A one-way ANOVA was used in assessing differences in mean log TACs across the locations. Statistical difference was established at p-value < 0.05. Post hoc multiple comparisons were carried out using the tukey post hoc multiple comparisons test to indicate locations with significantly different means. The frequency of isolation of *Salmonella* from the raw milk and fermented milk sample was calculated as:

$$\frac{\text{No of positive samples}}{\text{Total number of samples collected}} \times 100$$

CHAPTER FOUR

RESULTS

4.1 Total Aerobic Counts (TACs)

Of the 268 samples examined, 237 (88.4 %) had colonies that were within the countable range(0-300), 13 (5 %) were too numerous to count (>300), while 18 (7 %) had no growth (Fig 4.1).

4.1.1 Mean colony forming units per milliliter of raw milk

The mean colony forming unit per ml of bacteria from raw milk was highest at Samiyan uku than the other dairy herds. The cfu/ml was lowest at Rafin sidi. The mean cfu/ml of raw milk was significantly different across the locations. Mean cfu/ml from Hayin Liman (7.4±0.5) was significantly different from that of Rafin Sidi (6.5±0.4); p=<0.0001. There were also significant differences in the mean cfu/ml of raw milk across the other locations at p=<0.0001as follows: Gabari (6.7±0.6) and Dogarawa (7.7±0.5), : Gabari (6.7±0.6) and Samiyan uku (7.9±0.1), Bizara (6.9±0.5) and Samiyan uku (7.9±0.1), Hanwa (6.6±0.5) and Dogarawa (7.7±0.5), Hanwa (6.6±0.5) and Samiyan uku (7.9±0.1), Dogarawa (7.7±0.5) and Rafin Sidi (6.5±0.4), Samiyan uku (7.9±0.1) and Unguwan Dankali(7.0±0.8), Hayin liman (7.4±0.5) and Rafin Sidi (6.5±0.4) and finally between Samiyan uku (7.9±0.1) and Rafin Sidi (6.5±0.4) (Table 4.1).

4.1.2 Mean colony forming unit per milliliter of fermented milk

The total cfu/ml of bacteria from fermented milk was highest at Sabon gari than the other retail points and market places. The lowest cfu/ml was from Zango. Comparing the mean cfu/ml of fermented milk from the different locations showed significant differences (p=0.0236). The mean cfu/ml of fermented milk from Zango (8.3±1.0) was significantly different from the mean cfu/ml from PZ (9.3±0.6), the mean cfu/ml of fermented milk from Zango (8.3±1.0) was statistically different from the mean cfu/ml from Sabon Gari (9.4±0.5) and the the mean cfu/ml of fermented milk from Zango (8.3±1.0) was also significantly different from the mean TAC from Dan Magaji (9.2±0.5). In the entire study, fermented milk samples had the highest level of contamination based on their high mean counts (Table 4.2).

4.1.3 Overall mean colony forming unit per milliliter of raw milk and fermented milk

The overall mean log cfu/ml for raw milk was 7.1±0.5 while the overall mean cfu/ml for fermented milk was 9.0±0.6 (Table 4.3). The overall mean cfu/ml for raw milk was lower than that of fermented milk. There was significant difference between the mean cfu/ml of raw milk (7.1±0.5) and fermented milk (9.0±0.6); p= <0.0001 (Table 4.3).

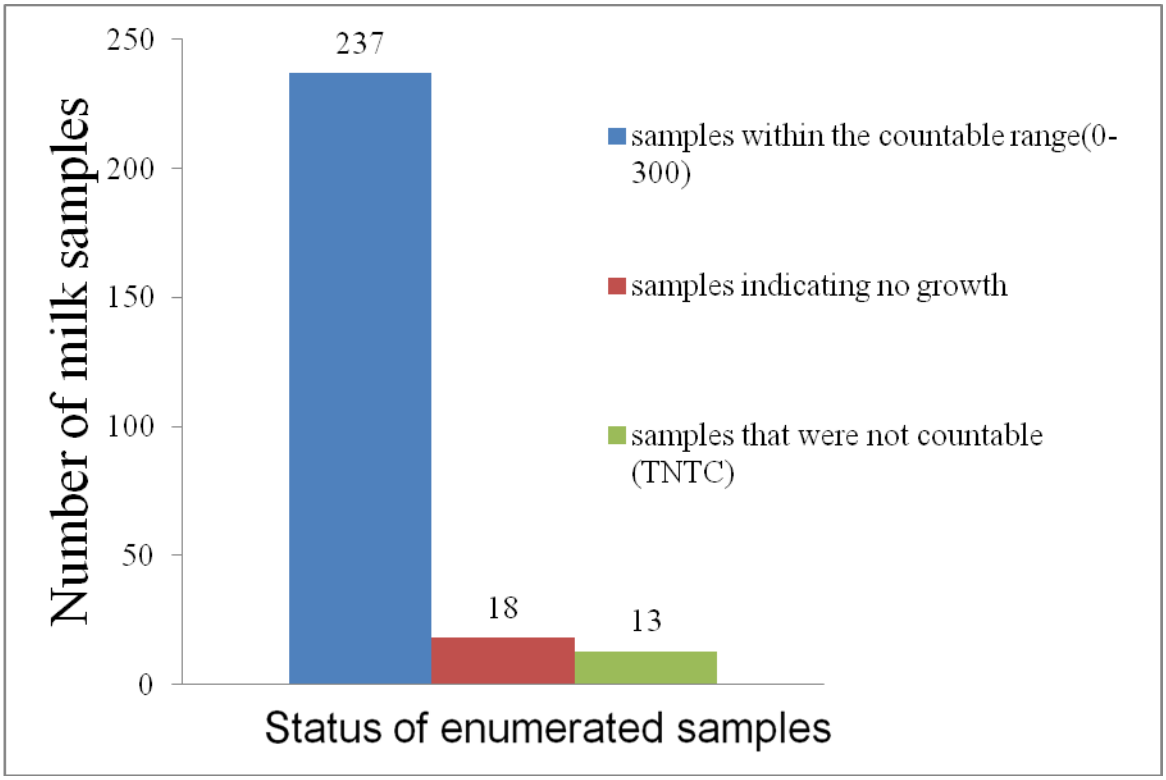

Figure 4.1: Distribution of the 268 milk samples from dairy herds and market places in Zaria and environs with regard to total areobic plate count

Table 4.1: Log$_{10}$ total aerobic count of raw milk samples collected from dairy herds in Zaria and environs

Location	N	Mean±SD	F-value	P-value
Bizara	10	6.9±0.5[a]	4.602	<0.0001
Dogarawa	7	7.7±0.5[a]		
Gabari	9	6.7±0.6[a]		
Hanwa	6	6.6±0.5[a]		
Hayin Liman	10	7.4±0.5[a]		
Kwakwaran Manu	9	7.2±0.7[b]		
Palladan	9	7.3±0.6[b]		
Rafin sidi	8	6.5±0.4[a]		
Sabon Fegi	10	7.2±0.6[b]		
Samiyan uku	8	7.9±0.1[a]		
Tohu	8	7.1±0.7[b]		
Unguwan Dankali	10	7.0±0.8[a]		
Unguwan Nassarawa	10	7.4±0.3[b]		
Overall mean	114	7.1±0.5		

n: number of samples within the countable range,) $F(12,101)=4.602$, Means with superscript a: significantly different ($p<0.05$, superscript b: not significantly different

Table 4.2: Log_{10} total aerobic count of fermented milk samples collected from retail points and market places in Zaria and environs

Location	n	mean±SD	F-value	p-value
Dan magaji	14	9.2±0.5[a]	2.25	0.0236
Kofan gayya	6	9.0±0.4		
Kwangila	14	9.1±0.8		
PZ	14	9.3±0.6[a]		
Sabon gari	13	9.4±0.5[a]		
Samaru	13	9.0±1.0		
Shika	14	9.1±0.6		
Tudun wada	11	8.9±0.3		
Zango	11	8.3±1.0[a]		
Zaria city	13	9.1±0.6		
Overall mean	123	9.0±0.6		

n: Number of samples within the countable range; superscript a: means are statistically significant ($p<0.05$), $F(9,113)= 2.25$,

Table 4.3: Overall mean total aerobic count of raw milk and fermented milk from Zaria and environs

sample type	n	Mean ± SD	t-value	df	p value
Raw milk	114	7.1 ± 0.5^{a}	12.1	21	<0.0001
Fermented milk	123	9.0 ± 0.6^{b}			

n= number of samples within the countable range for each sample type, df=degree of freedom, a,b ; means in the same column with different superscripts are significantly different (p<0.0001)

4.2 Frequency of *Shigella* and *Salmonella* Isolation from Raw Milk and Fermented Milk

Shigella species was not isolated from any of the milk samples examined.

Of the 268 total samples of raw milk and fermented milk from dairy herds, retail points and market places, *Salmonella* was isolated from 3(1.1%) of the milk samples, of which 2(0.7%) were from raw milk and 1(0.4%) was from fermented milk. Based on location, *Salmonella* was isolated from two dairy herds (Gabari and Samiyan uku) and one market place (Samaru). The percentage per sample type and the location from which *Salmonella* was isolated include: Raw milk from Gabari (0.8%), Raw milk from Samiyan uku (0.8%) and Fermented milk from Samaru (0.7%).

4.3 Biochemical Identification of *Salmonella* Using the Microbact ^{TM} System

From conventional biochemical testing, only 7 isolates conformed with *Salmonella* reactions. The 7 isolates were tested with the microbact^{TM} system and 3 yielded typical *Salmonella* reactions. All the 3 isolates were further tested for agglutination using the *Salmonella* polyvalent antiserum and they were found to be positive. The results from the Microbact ^{TM} system are summarized on Table 4.4

Table 4.4 Microbact^{TM} identification of 7 putative isolates from Zaria and environs

Identified Organism (s)	Frequency	Source (no.) of isolates	Percentage (%)probability
Salmonella spp. (unclassified)	2	Raw milk (1) and fermented milk (1)	59.41
Salmonella arizonae	1	Raw milk (1)	64.55
Citrobacter freundi	1	Raw milk (1)	92.82
Serratia marcescens	1	Raw milk (1)	65.81
Morganella morgana	1	fermented milk (1)	76.94
Providencia stuarti	1	Raw milk (1)	96.37
Total	7		

4.4 *In vitro* Susceptibility of *Salmonella* Isolates to 11 Antimicrobial Agents

All the isolates were susceptible to Sulfamethoxazole/Trimethoprim (Folate inhibitor pathway), two of the isolates were susceptible to Cefotetan (Cephem) and Chloramphenicol (Phenicol). Only one of the isolates was susceptible to Nitrofurantoin (Nitrofurantoin). All the isolates were resistant to Amoxicillin/Clavulanate (beta lactamase inhibitor), Streptomycin (Aminoglycoside), Tetracycline (Tetracycline), Ciprofloxacin (Fluoroquinolone), Amikacin (Aminoglycoside) and Erythromycin (Macrolide). The number of *Salmonella* isolates that were susceptible to the 11 antimicrobials tested include: Amoxicillin/Clavulanate; 0, Cefotaxime; 0, Cefotetan; 2, Streptomycin; 0, Tetracycline; 0, Ciprofloxacin; 0, Trimethoprim-Sulphamethoxazole; 3, Chloramphenicol; 2, Nitrofurantoin; 1, Amikacin; 0, Erythromycin; 0. Two resistance patterns were exhibited by the 3 *Salmonella* isolates (Table4.5). Isolates were tested against nine antimicrobial classes but the 3 isolates were susceptible to only one class (Folate inhibitor pathway). All the isolates exhibited multidrug resistance to the other 8 antimicrobial classes with each isolate showing resistance to 8 and to 9 antimicrobials within the class of fluoroquinolones, aminoglycosides, cephem, nitrofurantoin, beta-lactamase inhibitor, tetracyclines, macrolide and phenicols.

Table 4.5. Resistance patterns of 3 *Salmonella* isolates against 11 antimicrobial agents

Resistance pattern	Number resistant to panel
CIP, S, CTX, F, AK, AML, E, TE	2
CIP, S, CTX, F, AK, AML, E, TE, CTN	1

CIP= Ciprofloxacin; S- Streptomycin; CTX=Cefotaxime; F=Nitrofurantoin; AK= Amikacin; AML= Amoxicillin/Clavulanate; E=Erythromycin; TE= Tetracycline; CTN= Cefotetan

4.5. Polymerase chain reaction targeting the *inv*A gene

The *inv*A gene was not detected in any of the 3 isolates (plate I).

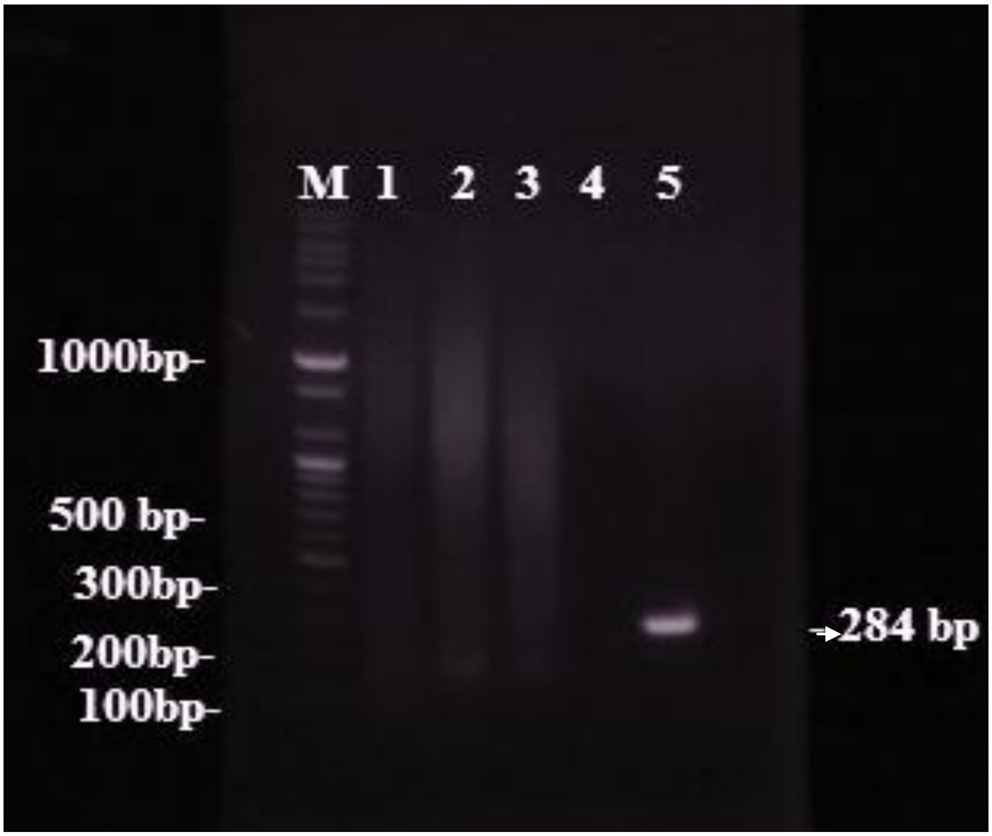

Plate I: Absence of *inv*A gene in 3 *Salmonella* isolates on 1.5% agarose gel. Lane M: 100 base pair marker; Lanes 1 and 2 are raw milk isolates; Lane 3: isolates from fermented milk; 4: Negative control 5: Positive control.

CHAPTER FIVE

DISCUSSION

None of the mean log cfu/ml of raw milk from all the locations was within the acceptable standard as recommended by the New York State milk standards (mean log 4.45 or 3×10^4 cfu/ml) and the European Commission, (2001) which recommends that raw milk should not exceed log 5.0 or 10^5 cfu/ml. The unacceptable levels of the bacterial load in raw milk may be attributed to unhygienic milking practices as most of the milkers in the dairy herds in the study did not wash their hands prior to milking. They were observed to dip their fingers into the milk to moisten their fingers in order to avoid difficulty in milking the cow's udder. The milkers did not disinfect the teats of the udder prior to milking. Hygienic milking procedure involves the proper cleaning and disinfection of the teats of the udder which helps to minimize surface contaminants and dirts (Ajogi *et al*., 2005; FAO, 2016). The lower mean log bacterial counts in Rafin sidi may be because of the better hygiene of the milking environment. The significant difference seen among Rafin sidi, Hanwa, Gabari, Samiyan uku, Dogarawa, Unguwan Dankali, Hayin liman and Bizara could be attributed to the differences in the level of hygiene and environmental cleanliness though the hygienic practices across all the locations are considered to be unsatisfactory. The poor management system by these small scale dairy producers could also have affected the quality of milk production owing to the fact that there are no milking parlours and milking machines as milking was done in an open field where cows feed on grasses and defaecate.

The high mean log cfu/ml of fermented milk obtained across all the locations indicates high level of bacterial contamination. Fermented milk (nono) is locally produced by the Hausa and

Fulani women of Northern Nigeria (Eka and Ohaba, 1977) who do not observe any standard of preparation. Failure of most of these milk handlers to pasteurize their milk can also result in a high TAC as pasteurization reduces the microbial load of food to safer levels. The fulanis who sell fermented milk in Zango claim to boil their milk prior to fermentation; this should have resulted in a far lower TAC as the mean cfu/ml from Zango was the lowest in comparison to samples from the other locations. The significant differences among Zango, PZ, Sabon gari and Danmagaji could be as a result of differences in methods of processing and hygienic practices. The high mean cfu/ml of fermented milk could be as a result of other unhygienic practices in the course of preparation such as addition of milky white supernatant of water obtained from soaked baobab tree seeds in order to increase the quantity of the milk and also improve the milk color (Shehu and Adesiyun, 1990).

The overall raw milk mean log cfu/ml (7.14) observed in the study was lower than the highest (9.84) and lowest (8.52) mean log bacterial counts by Makut *et al*. (2014) in Keffi, Nassarawa, Nigeria. Lawan *et al*. (2012) reported lower mean counts of raw milk from two dairy farms in Zaria, Nigeria as 5.70 and 6.04. Ogbonna *et al*. (2012) reported an overall mean log cfu/ml of 5.34 from raw milk in Maiduguri. Laba and Udosek (2013) reported a range of 6.04-6.41 cfu/ml for raw milk in Ilorin, Nigeria. Milk contamination to unacceptable levels in the other studies and in this present study can be attributed to the fact that poor hygienic practices and poor management systems are common among the milkers in most parts of the country. Poor hygienic practices by milk handlers can lead to introduction of pathogenic microorganisms into the milk (Adeyemi and Umar, 1994).

The overall mean log cfu/ml of fermented milk in this study was far higher than that of raw milk. The significant difference in the mean log of raw milk and fermented milk can be attributed to the fact that raw milk may have had a lower bacterial count as it was analysed in the laboratory shortly after milking had taken place. Hence microorganisms may not have had enough time for multiplication which usually results in high mean TACs. The addition of little quantity of already fermented milk to fresh milk to hasten fermentation can encourage increase in the number of microorganisms, hence, increase in the mean cfu/ml of fermented milk. Also, unfavourable storage temperatures may have resulted in a higher bacterial count for fermented milk as some of the fermented milk that were not sold the previous day may be taken back to the markets without proper refrigeration.

Aerobic count is useful as an indicator for food quality and shelf-life (Pianetti *et al*., 2008). Tamba *et al*. (2016) reported a lower mean cfu/ml of 7.22 in fermented milk from Zaria, Kaduna state, Nigeria. A Study by Mohammed and Abdullahi (2015) revealed that all the hawked fermented milk (nono) samples in Bida, Nigeria had colonies that were Too Numerous to Count (TNTC). Okonkwo (2011) reported a range of log 0 to 8.9 cfu/ml for fermented milk from different markets in Maiduguri, Nigeria. The finding in this study partly disagrees with Egwaikhide *et al*. (2014) who reported mean cfu/ml which was as low as 3.48 to TNTC for fermented milk (nono) consumed within Kaduna, Nigeria. The differences could be due to the different periods in which milking was done and different seasons.

Shigella was not isolated from any of the samples from this study. This could be due to the fact that the isolation of *Shigella* from foods is considered to be more difficult than that of other pathogenic *Enterobacteriaceae* because *Shigella* species are considered to be relatively fragile as they do not survive well outside the host (Bryan, 1978). The classical techniques and media used for detection, isolation and enumeration of Shigellae are neither very specific nor very sensitive which may have resulted in failure of isolation. Contamination of food products by *Shigella* normally comes from human handling as *Shigella* is not an intrinsic flora of animals (Mohammed and Abdullahi, 2015) except for non-human primates like monkeys, chimpanzees, and apes. *Shigella* isolation in the present study is in contrast with the findings from raw milk (2%) and fermented milk (6%) by Ogbonna *et al*. (2012) in Maiduguri, Nigeria. Okonkwo (2011) isolated *Shigella* from fermented milk (8%) in Maiduguri although *Shigella* was not detectable in three areas under study. Akabanda *et al*. (2010) reported a psrevalence of 2.5% for *Shigella* in fermented milk in Ghana. Reported prevalences of *Shigella* from raw milk and fermented milk are generally low across the study areas indicating that the presence of the organism in foods mostly come from handling by an infected individual (Smith, 1987)

The isolation of *Salmonella* in the present study indicates the presence of the organism in both raw milk and fermented milk. The overall prevalence (1.1%) from raw milk and fermented milk is very low considering the level of unhygienic practices. *Salmonella* species can be introduced into raw milk as a result of faecal contamination which may have come from the milker or the cow. The isolation rate from raw milk (1.6%) is lower in comparison to raw milk prevalence (6.4%) reported by Karshima *et al*. (2013), raw milk (7.6%) by Ogbonna *et al.* (2012) and raw milk (4%) by Tamba *et al*. (2016). Laba and Udosek (2013) also reported a prevalence of 4% from raw milk in Ilorin, Nigeria. Fermented milk has a lower pH which is acidic and does not favour the growth of spoilage bacteria (Tamba *et al*., 2016) but *Salmonella* has been reported to be resistant to low pH which may have brought about its survival in fermented milk.

Finding from fermented milk (0.7%) in the present study is similar to findings from fermented milk (0.8%) by Karshima *et al*. (2013) in Kanam, Plateau state, Nigeria. Mhone *et al*. (2012) reported an overall prevalence of 0.0% from both raw milk and fermented milk in Zimbabwe.

Study carried out by Gran *et al*. (2003) reported that *Salmonella* was not detectable in both raw milk and fermented milk.

From the results of the Microbact ™ 12E Gram Negative System, *Salmonella arizonae* and *Salmonella* species (unclassified) were isolated from raw milk and fermented milk following biochemical tests. The impact of *Salmonella* is higher in developing countries that do not practice hygiene or have access to proper public health facilities (Ateba and Moichawa, 2014) and the chances of salmonellosis is higher especially for individuals who are immunocompromised. *Salmonella arizonae* are mostly found in reptiles, particularly snakes (Libby *et al.*, 2002). Snakes and reptiles transmit this organism to humans which results in gastroenteritis and systemic infections especially in immunocompromised individuals and young children (Mermin *et al*., 1997). Other members of the *Enterobacteriaceae* were also detected, some of which have been shown to cause gastroenteritis (Kirov, 1997). Members such as *Serratia marcescens* are opportunistic pathogens and may cause diarrheoa (Gran *et al*., 2003). *Citrobacter freundi* may be a normal inhabitant of the intestine but some strains can be potentially hazardous (Schauber *et al*., 1995). The presence of members of the *Enterobacteriacae* in raw and fermented milk is of public health significance indicating that more emphasis needs to be placed on both personal and environmental hygiene.

All the 3 isolates of *Salmonella* were resistant to Amoxicillin/Clavulanate, Streptomycin, Tetracycline, Ciprofloxacin, Amikacin, Erythromycin and Cefotaxime. Resistance was also shown to the following classes of antibiotics: Phenicol (1 isolate), Nitrofurantoin (2 isolates), Cephem (1 isolate). Antimicrobial resistance among enteric pathogens in developing countries is of public health concern as resistance by bacterial enteric pathogens to most commonly used antibiotics is on the increase (Isenberger *et al*., 2002). This increase can pose a threat to public health especially susceptible populations like the young children, the elderly and the immunocompromised patients (Shea, 2003; Hitti and Wolff, 2005). *Salmonella* has various mechanisms for antibiotic resistance which can be categorized as modification or destruction of the antimicrobial agents, pumping the antimicrobial agent out from the cell by efflux pumps, modification or replacement of the antibiotic target and decrease in cell permeability (Abatcha *et al*., 2014). There is possibility of transmission of resistant organisms from food animals to humans especially in a population where people have close contact with food animals (Okeke *et al*., 2005). The global trend of antimicrobial resistance of *Salmonella* is of great concern as most countries do not have strict regulations regarding purchase of antibiotics without doctor's prescription and incomplete treatment course (Beyene *et al*., 2011). This could have attributed to the resistance exhibited by the isolates in this study. Similar trends of *Salmonella* resistance have been reported in India (Rowe *et al*., 1997), Iran (Jomezadeh *et al*., 2014), Washington (Villar *et al*., 1999), and Gulf of Guinea Region (Baltazar *et al*., 2015). The increasing antibiotic resistance of enteric pathogens to most commonly used antibiotics necessitates the need for continuous monitoring of antibiotic use and more efforts towards preventing antibiotic resistance in the animal populations too as food animals can transmit antibiotic resistant organisms to humans.

The invasion gene (*inv*A) was not detected in any of the *Salmonella* isolates in this study. PCR for *inv*A has been shown to be highly sensitive for the detection of *Salmonella* species and it has been widely used in the detection of bacteria belonging to this genus (Amini *et al*., 2010) as the *inv*A gene is present in most serotypes of *Salmonella* (Galan and Curtiss, 1991; Rahn *et al*., 1992). *inv*A gene is essential for full virulence in *Salmonella* and is thought to trigger the internalization required for invasion of deeper tissue (Khan *et al*., 1999). Hence, serotypes of

Salmonella that do not possess the *inv*A gene may lack the ability to invade host epithelial cells as they lack the ability to express *inv*ABC genes (Galan *et al*., 1992) although there was a report that *Salmonella enterica* serovar Senftenberg isolated from stool samples of human diarrhoea patients from a food-borne outbreak was SPI-1 deficient (Hu *et al*., 2008). Many of the virulence factors of *Salmonella enterica* are encoded by genes organized on *Salmonella* Pathogenicity Islands (SPIs) that have been acquired by horizontal transfer and believed to be self-mobile (Ochman *et al*., 2000). The *invA* gene is located on the SPI-1 and is acquired by horizontal gene transfer, it may be genetically unstable or absent in some serotypes (Ginocchio *et al*., 1997). Even though the *invA* gene was not found in this study, this does not rule out the fact that other virulence genes may be present as it was not within the scope of the study to check for the presence of other genes. Study by Krzyzanowski *et al*. (2014) showed that 2 *Salmonella enterica* serovars (*Salmonella* Molade and *Salmonella* Muechen) lacked *inv*A gene but possessed the *sse*L gene and harboured a plasmid. Oludairo *et al.* (2013) isolated 8 *Salmonella* out of which 5 isolates were positive for *inv*A gene and 3 lacked *inv*A gene.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

This study has shown the overall bacterial load of raw milk to be 7.1±0.5 mean log cfu/ml from 13 dairy herds in Zaria and Environs which exceeded the acceptable standard of 5.0 mean log cfu/ml by the European Commission. The overall mean log bacterial count from fermented milk (9.0±0.6) was higher than that of raw milk.

No *Shigella* species was isolated from the study.

Salmonella was isolated from raw milk and fermented milk at an overall frequency of 1.1%. *Salmonella* was isolated from raw milk in Samiyan Uku (0.8%), Gabari (0.8%) and from fermented milk from Samaru (0.7%).

Resistance of all three isolates to Amoxicillin-Clavulanic acid, Cefotaxime, Streptomycin, Tetracyclin, Ciprofloxacin, Amikacin, and Erythromycin necessitate the need for continuous monitoring of antibiotic use to combat resistance.

The *inv*A gene was not detected in any of the 3 isolates.

6.2 Recommendations

The following recommendations are made based on the findings of the present study:

1. There is need for public health organizations at state and local government levels to educate all dairy herdsmen in Zaria and Environs on the necessity of hygiene before milking, when milking and after milking. The public health scientists should also demonstrate how to implement hygienic measures for the milk handlers to see.

2. It is recommended that raw milk should always be pasteurized before consumption or processing.

3. Regulations regarding irrational purchase and administration of antibiotics should be enforced in the country.

4. The entire public should be enlightened by relevant authorities on the dangers of indiscriminate use of antibiotics in both humans and animals.

References

A guide to Udder health for Dairy Sheep. (2014). Bacterial Contamination of Milk as Measured by Standard Plate Count. SectionV-. Retrieved on 28th, November, 2015 from http://www.uoguelph.ca/~pmenzies/Dairy_Sheep/Pdf/V-3_Bacteria.pdf

Abatcha, M.G., Zakaria, Z., Kaur, D.G. and Thong, K.L. (2014). A trend of *Salmonella* and antibiotic resistance. *Advances in Life Science and Technology*, 17: 1-21.

Abdu, A.B., Aboderin, O.A., Elusiyan, B.J., Kolawole, O.D. and Lamikanra, A. (2014). Multiresistant *Shigella* species isolated from cases of Childhood diarrheoa in Ile-Ife, Southwest Nigeria. *Journal of Microbiology Research and Reviews*, 2(3):19-29.

Abdulrasheed, A.B., Aboderin, O. A., Elusiyan, B. J., Kolawole, O. D. and Lamikanra, A. (2014). Multiresistant *Shigella* spp. Isolated from Cases of Childhood Diarrhoea in Ile-Ife, Southwest Nigeria. *Journal of Microbiology Research and Reviews*, 2(3):19-29.

Acuri, E.F., Brito, M., Brito, J.R.F., Pinto, S.M., Angelo, F. F and Souza, G.N. (2006). Microbiological quality of refrigerated milk on farms. *Arquivo Brasileiro de Medicina Veterinaria e Zootecnia*, 58: 440-446.

Adams, M.J. and Moses, M.O. (1995). Food Microbiology. *(*pp: 104). New Delhi: *New Age International Publisher Limited*.

Adeneye, J.A. (1989). Variations in yield and composition of milk from different quarters of lactating White Fulani cattle in a tropical environment. *Nigerian Journal of Animal Production*, 16: 8-15.

Adesina, K. (2012). Effect of Breed on the Composition of Cow Milk under Traditional Management Practices in Ado-Ekiti, Nigeria. *Journal of Applied Sciences and Environmental Management*, 16 (1): 55 – 59

Adeyemi, L.A. and Umar, S. (1994). Effect of method of manufacture on quality characteristic of kunun zaki, a millet based beverage. *Nigerian Food Journal*, 12: 34-41.

Agbogu,V.N., Umoh,V.J., Okufo, C.A., Smith, S.I. and Ameh, J.B. (2006). Study of the Bacteriological and Physiological indicators of population of Surface Waters in Zaria. *Nigeria African Journal of Biotechnology*, 5:732-737.

Aggad, H., Bridja, M., Aek, B., Benaouali, M. and Djebli, A. (2010). Some quality aspects of pasteurized milk in Algeria. *World Journal of Dairy and Food Sciences*, 5: 21-24.

Ajogi, I., Kabir, J., Okolocha, E.C., Luga, L.I., Dzikwi, A.A., Egege, S.C., … Oni, O.O. (2005). Manual for Clinic in Veterinary Public Health and Preventive Medicine (pp: 1-10). Zaria, Nigeria: Ahmadu Bello University Press Ltd.

Akabanda, F., Owusuz-Kwarteng, J., Glover, R. L. K. and Tano-Debrah, K. (2010). Microbiological Characteristics of Ghanaian Traditional Fermented Milk Product, *Nunu. Nature and Science*, 8(9):178-187

Akinyemi, O.K., Smith, S.I., Oyefolu, A.O., Fasure, K.A. and Coker, A.O. (2007). Trends of Multiple Drug Resistance in *Salmonella Enterica* Serovar Typhi in Lagos, Nigeria. *East and Central African Journal of Surgery*, 12 (1): 83-88.

Ali, A.A. and Abdelgadir, W.S. (2011). Incidence of *Escherichia coli* in raw cow's milk in Khartoum state. *British Journal of Dairy Science*, 2(1):23-26.

Aliyu, A.A., Ajogi, I.A., Ajanusi, O.J., Reuben, R.C. (2014). Epidemiological studies of *Fasciola gigantica* in Cattle in Zaria, Nigeria using Coprology and Serology. *Scholars Journal of Agriculture and Veterinary Sciences*, 1(1):13-19.

Allam, M.F. and Lucane, R.A. (2004). Selenium supplementation for asthma. *Cochrane Database of Systematic Reviews*, 2: CD003538.

Allen-Vercoe, E. and Woodward, M. J. (1999). The role of flagella, but not fimbriae, in the adherence of *Salmonella enterica* serotype Enteritidis to chick gut explant. *Journal of Medical Microbiology*, 48 (8): 771-780.

Allos, B.M . (2002) *Campylobacter jejuni* Infections: update on emerging issues and trends. *Clinical Infectious Diseases,* 32:1201-1206.

Altekruse, S.F., Timbo, B.B., Mowbray, J.C., Bean, N.H. and Potter, M.E. (1998). Cheese-associated outbreaks of human illness in the United States, 1973 to 1992: Sanitary manufacturing practices protect consumers. *Journal of Food Protection*, 61: 1405-1407.

Alves, C. (2006). Effect of seasonal variations in the quality of raw milk refrigerated for two properties of Minas Gerais. Dissertation (Master of Technology and Inspection of Animal Products), Federal University of Minas Gerais, Belo Horizonte 65.

American Public Health Association. (1993). Standard Methods for the Examination of Dairy Products, 16th ed. APHA, Washington, DC.

Amini, K., Zahraei, T.S., Gholamreza, N., Reza, R., Javid, A. and Shahrnaz, B.A., (2010). “Molecular detection of *invA* and *spv* virulence genes in *Salmonella enteritidis* isolated from human and animals in Iran”. *African Journal Microbiological Research,* 4: 2202-2210.

Anonymous-Pathogen Profile Dictionary-PPD, (2010). *Shigella*. *Journal of Undergraduate Biological Studies*. www.pathogenprofiledictionary.html.Retrieved on 11th June,2015.

Anshumala, K.M. and Niranjan, B. (2012). A comparative microbiological quality assessment of rural and urban milk samples. *African Journal of Food Science*, 6(21): 519-523.

Anyanwu, A. L., Fasina, F. O., Ajayi, O.T., Rapu, I. and Fasina, M. M. (2010). Antibiotic Resistant *Salmonella* and *Escherichia coli* Isolated from Day-Old Chicks, Vom, Nigeria. *African Journal of Clinical and Experimental Microbiology*, 11(1): 129-136.

Armstrong, H.C. (1995). *Food and nutrition*. Pp. 20 London.

Ashkenazi, S., Levy I., Kazaronovski, V. and Samra, Z. (2003). Growing antimicrobial resistance of *Shigella* isolates. *Journal of Antimicrobial Chemother*apy, 51: 427-429.

Association of Official Analytical Chemists. (1990). Official Methods of Analysis, 15th ed. AOAC, Arlington, VA.

Ateba, C.N., and Mochaiwa, B. (2014). Use of *inv*A gene specific PCR analysis for the detection of virulent *Salmonella* species in beef products in the North West Province, South Africa. *Journal of Food and Nutrition Research,* 2(6): 294-300.

Baltazar, M., Ngandjio, A., Holt, K.E., Lepillet, E., Pardos de la Gandara, M., Collard, J., Bercion, R., Nzouankeu, A., Le Hello, S., Dougan, G., Fonkoua, M. and Weill, F. (2015). Multidrug-Resistant *Salmonella enterica* Serotype Typhi, Gulf of Guinea Region, Africa. *Emerging Infectious Diseases*, 21(4)

Bánóczy, J., Petersen, P.E. and Rugg-Gunn, A.J. (2009). Milk fluoridation for the prevention of dental caries. World Health Organization. http://www.who.int/oral_health/publications/milk_fluoridation_2009_en.pdf. Retrieved on 24th November, 2015.

Barney, H J. and VanHorn, H.H. (2003). Water and its Importance to Animals. Institute of Food and Agricultural Sciences, University of Florida, Gainesville. pp1 - 8.

Barros, L.S.S., Sóglia, S.L.O., Ferreira, M.J, Rodrigues, M.J. and Branco, M.P.C. (2011). Aerobic and anaerobic bacteria and Candida species in crude milk. *Journal of Microbiology and Antimicrobials*, 3: 206-212.

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

Bäumler, A.J., Tsolis, R.M., Ficht, T.A. and Adams, L.G. (1998). Evolution of host adaptation in *Salmonella* enterica. *Infection and Immunity*, 66:4579–4587.

Bayemi,P. H., Bryant, M. J., Pingpoh, D., Imele, H., Mbanya, J., Tanya, V., Cavestany, D., Awoh, J., Ngoucheme, A., Sali, D., Ekoue, F., Njakoi, H. and Webb, E. C. (2005).

Participatory Rural Appraisal of Dairy Farms in the North West Province of Cameroon. Livestock Research for Rural Development 17:59. Retrieved 16th Feburary, 2015 from http://www.cipav.org.co/lrrd/lrrd17/6/baye17059.htm

Bertu, W.J., Dapar, M., Gusi, A.M., Ngulukum, S.S., Leo, S. and Jwander, L.D. (2010). Prevalence of *Brucella* antibodies in marketed milk in Jos and environs. *African Journal of Food Science*, 4: 62-64.

Beyene, G., Nasir, S., Asrat, D., Mengistu, Y., Engers, H. and Wain, J. (2011). Multidrug resistant *Salmonella* Concord is a major cause of salmonellosis in children. *Ethiopian Journal of Infections in Developing Countries,* 5:23-33.

Borková, M., Snášelová, J. (2005). Possibilities of different animal milk detection in milk and dairy products – a review. *Czech Journal of Food Sci*ences, 23: 41–50.

Bradley, A. (2002). Bovine mastitis: an evolving disease. *The Veterinary Journal*, 164:116–128.

Bramley, A.J. and McKinnon, C.H. (1990). The microbiology of raw milk. pp. 163-208. In R.K. Robinson (Eds.). *Dairy Microbiology*, Vol. 1. Elsevier Science Publishers, London.

Bramley, A.J., C.H. McKinnon, R.T. Staker and D.L. Simpkin. (1984). The effect of udder infection on the bacterial flora of the bulk milk of ten dairy herds. *Journal of Applied Bacteriology*, 57:317.

Brenner, F.W. and McWhorter-Murlin, A.C. (1998). Identification and serotyping of *Salmonella*. (Centers for Disease Control and Prevention, Atlanta, Ga).

Brown, J.C., Thomas, C.J. and Amyes, S.G. (1996). Mutations of the gyrA gene of clinical isolates of Salmonella typhimurium and three other Salmonella species leading to decreased susceptibilities to 4-quinolone drugs. *Journal of Antimicrobial Therapy*, 37 (2): 351-356.

Bryan, F.L. (1978). Factors that contribute to outbreaks of foodborne disease. *Journal of Food Protection*, 41: 816.

Bryne, R. (2004). Microbiological hazards that need to be managed during and after processing: An overview. *Proceeding of the IDF/FAO International Symposium on Dairy Safety and Hygiene*, March 2-5, 2004, Cape Town, South Africa.

Buchwald, D.S. and Blaser, M.J. (1984). A review of human salmonellosis: II. Duration of excretion following infection with nontyphi Salmonella. *Reviews of Infectious Diseases*, 6:345–356.

Buxton, A. (1957). Salmonellosis in Animals: A Review. Commonwealth Agricultural Bureax, Farnham Royal, Bucks, England.no.64. Retrieved on 20 September, 2016 from http://krishikosh.egranth.ac.in/bitstream/1/2048237/1/0193_6081A.pdf

Buxton, A., and G. Fraser. (1977). Animal microbiology. Blackwell Scientific Publications, Oxford, United Kingdom.

Buzby, J. and Roberts, T. (2009). "The Economics of Enteric Infections: Human Foodborne Disease Costs. *Gastroenterology*, 136 (6): 1851-1862.

Centre for Disease Prevention and Control (CDC). (1984). *Salmonella* dublin and Raw Milk Consumption –California. *Morbidity and Mortality Weekly Report*, 33(14): 196-198.

Centre for Disease Prevention and Control (CDC) (2015a). Multidrug-resistant Shigellosis Spreading in the United States. Retrieved on 25th January, 2017 from https://www.cdc.gov/media/releases/2015/p0402-multidrug-resistant-shigellosis.html

Centre for Disease Prevention and Control (CDC) (2015b). Food safety. Raw milk questions and answers. Retrieved on 3rd December, 2015 from http://www.cdc.gov/foodsafety/rawmilk/raw-milk-questions-and-answers.html

Chatterjee, S.N., Bhattacharjee, I., Chatterjee, S.K, and Chandra, G. (2006). Microbiological examination of milk in Tarakeswar, India with special reference to coliforms. *African Journal of Biotechnology*, 5(15):1383-1385.

Cheesbrough, M. (2003). *Medical Laboratory Manual. Tropical Health Technology*, Low priced Edition.Doddington,Cambridgeshire, England, pp. 20-35.

Cheuk, D.K., Chau, T.C. and Lee, S.L. (2005). A meta-analysis on intravenous magnesium sulphate for treating acute asthma. *Archives of Disease in Child*, 90:74–77.

Chien, A., Edgar, D.B. and Trela, J.M. (1976). "Deoxyribonucleic acid polymerase from the extreme thermophile Thermusaquaticus", *Journal of Bacteriology*, 127(3): 1550-1557.

Chiu, C. H., and Ou, J. T. (1996). Rapid identification of Salmonella serovars in feces by specific detection of virulence genes, invA and spvC, by an enrichment broth culture-multiplex PCR combination assay. *Journal of Clinical Microbiology*, 34:2619-2622.

Cianflone, N. F. C. (2008). Salmonellosis and the GI Tract: More than Just Peanut Butter. *Current Gastroenterology Reports*, 10(4): 424–431.

Clare, D.A and Swaisgood, H.E. (2000). Bioactive milk peptides: a prospectus. *Journal of Dairy Science*, 83:1187–95.

Clarke, R.C. and Gyles, C.L. (1993). *Salmonella*. In: Gyles, C.L., Thoen, C.O. (Eds.), *Pathogenesis of Bacterial Infections in Animals* (2nd edn., pp. 133–153)., Iowa State University Press, Ames, Iowa.

Clinical and Laboratory Standards Institute (2014). Performance Standards for Antimicrobial Susceptibility Testing; Twenty-fourth Informational Supplement, 34(1): 50-57.

Cody, S.H., Abbott, S.L., Marfin, A.A., Schulz, B., Wagner, P., Robbins, K., Mohle-Boetani, J.C. and Vugia, D.J. (1999). Two outbreaks of multidrug-resistant *Salmonella* serotype Typhimurium DT104 infections linked to raw-milk cheese in Northern California. *Journal of the American Medical Association*, 281(19):1805-1810.

Coombes, B.K., Coburn, B.A., Potter, A.A., Gomis, S., Mirakhur, K., Li, Y. and Finlay, B.B. (2005). Analysis of the contribution of Salmonella pathogenicity islands 1 and 2 to enteric disease progression using a novel bovine ileal loop model and a murine model of infectious enterocolitis. *Infection and Immunity*, 73 (11): 7161-7169.

Da Silva, M.C.D., Hofer, E. and Tibana, A. (1998). Incidence of *Listeria monocytogenes* in cheese produced in Rio de Janeiro, *Brazilian Journal of Food Protection*, 61: 354-356.

Dabassa, A. and Bacha, K. (2012). The prevalence and antibiogram of *salmonella* and *shigella* isolated from abattoir, Jimma Town, South West Ethiopia. *International Journal of Pharmaceutical and Biology Research*, 3: 143-148.

Daniela, L., Silvana, M., Angela, S., Claudia, L., Ida, L. and Giancarlo, R. (2014). *Salmonella enterica* serovar Virchow meningitis in a young man in Italy: a case report. *Journal of Medical Case Reports*, **8**:139

Davies, D.T. and Law, J.R. (1980): The content and composition of protein in creamery milks in south-west Scotland. *Journal of Dairy Research*, 47: 83–90.

Dechet, A.M., Scallan, E., Gensheimer, K., Hoekstra, R., Gunderman-King, J., Lockett, J., Wrigley, D., Chege, W., Sobel, J. and Multistate Working Group. (2006). Outbreak of multidrug-resistant *Salmonella enterica* serotype Typhimurium Definitive Type 104 infection linked to commercial ground beef, northeastern United States, 2003-2004. *Clinical Infectious Disease*, 42(6):747-52.

Dibb-Fuller, M. P., Allen-Vercoe, E., Thorns, C. J. and Woodward, M. J. (1999). Fimbriae and flagella mediated association with and invasion of cultured epithelial cells by *Salmonella enteritidis*. *Microbiology*, 145 (5): 1023-1031.

Diop, P. E. H and Mazouz, A. (1995). Production laitière en Afrique au sud du Sahara : problématique et stratégie. In Reproduction et production laitière. Actualité Scientifique. Universités Francophones. pp 19 -26 http://www.bibliotheque.refer.org/livre68/l6800.pdf

Dodig, S. and Cepelak, I. (2004). The facts and controverses about selenium. *Acta Pharmaceutica*, 54:261–76.

Downes, F. P., and Ito, K. (Eds.). (2001). *Compendium of Methods for the Microbiological Examination of Foods* (4th ed.). Washington, DC, USA: American Public Health Association.

Dunford, M. and Doyle, J.A. (2008). Nutrition for sport and exercise. Belmont: Thomson Wadsworth.

DuPont, H.L. (2000). "*Shigella* species (bacillary dysentery)". In Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases, (5th ed., pp. 2363-2369)

DuPont, H.L., Myron, M.L, Richard, B.H. and Samuel, B. (1989). "Inoculum Size in Shigellosis and Implications for Expected Mode of Transmission," *The Journal of Infectious Diseases*, 159, (6): 1126-28.

EC (European Commission). 2001. *Overview of Microbiological Criteria for Foodstuffs in Community Legislation in Force*. Retrieved on 29, September, 2016 from http://europa .eu.int/comm/food/fs/sfp/mr/mr_crit_en.pdf

Edwards, P. R. and Ewing. W. H. (1962). Identification of *Enterobacteriaceae*, 2nd ed. Burgess Publishing Co., Minneapolis.

EFSA (European Food Safety Authority) Panel on Dietetic Products Nutrition and Allergies (NDA) (2009a). Scientific Opinion on the substantiation of health claims related to calcium and growth, development and maintenance of the normal structure and function of bones and teeth (ID 224, 230, 231, 354, 3099), muscle function and neurotransmission (ID 226, 227, 230, 235), blood coagulation (ID 230, 236), energy-yielding metabolism (ID 234), normal function of digestive enzymes (ID 355), and maintenance of a normal blood pressure (ID 225, 385, 1419) pursuant to Article 13(1) of Regulation (EC) No 1924/2006. *European Food Safety Authority Journal*, 7(9):1210

EFSA (European Food Safety Authority) Panel on Dietetic Products Nutrition and Allergies (NDA). (2009b). Scientific Opinion on the substantiation of health claims related to iodine and thyroid function and production of thyroid hormones (ID 274), energy-yielding metabolism (ID 274), maintenance of vision (ID 356), maintenance of hair (ID 370), maintenance of nails (ID 370), and maintenance of skin (ID 370) pursuant to Article 13(1) of Regulation (EC) No 1924/2006. *European Food Safety Authority Journal*, 7(9):1214

EFSA (European Food Safety Authority) Panel on Dietetic Products Nutrition and Allergies (NDA) (2009c). Scientific Opinion on the substantiation of health claims related to phosphorus and function of cell membranes (ID 328), energy-yielding metabolism (ID

329, 373) and maintenance of bone and teeth (324, 327) pursuant to Article 13(1) of Regulation (EC) No 1924/2006. *European Food Safety Authority Journal*, 7(9):1219

EFSA (European Food Safety Authority) Panel on Dietetic Products Nutrition and Allergies (NDA). (2010c). Scientific Opinion on the substantiation of health claims related to calcium and maintenance of normal bone and teeth (ID 2731, 3155, 4311, 4312, 4703), maintenance of normal hair and nails (ID 399, 3155), maintenance of normal blood LDL-cholesterol concentrations (ID 349, 1893), maintenance of normal blood HDL-cholesterol concentrations (ID 349, 1893), reduction in the severity of symptoms related to the premenstrual syndrome (ID 348, 1892), "cell membrane permeability" (ID 363), reduction of tiredness and fatigue (ID 232), contribution to normal psychological functions (ID 233), contribution to the maintenance or achievement of a normal body weight (ID 228, 229) and regulation of cell division and differentiation (ID 237) pursuant to Article 13(1) of Regulation (EC) No 1924/2006. *European Food Safety Authority Journal*, 8(10):1725

EFSA (European Food Safety Authority) Panel on Dietetic Products Nutrition and Allergies (NDA). (2010d). Scientific Opinion on the substantiation of health claims related to iodine and contribution to normal cognitive and neurological function (ID 273), contribution to normal energy-yielding metabolism (ID 402), and contribution to normal thyroid function and production of thyroid hormones (ID 1237) pursuant to Article 13(1) of Regulation (EC) No 1924/2006. *European Food Safety Authority Journal*, 8(10):1800

Efuntoye, M. O. and Adenuga, A. (2011). *Shigella* Serotypes Among Nursery and Primary School Children With Diarrhoea In Ago-Iwoye And Ijebu-Igbo, Southwestern Nigeria. *Journal of Pharmacy and Clinical Science*, 2: 28-32.

Egwaikhide, P. A., Malu, P. S., Lawal, U., Adelagun, R. O. and Andrew, C. (2014). Physico Chemical and Microbiological Analysis of Fermented Cow Milk (Nono) Consumed Within Kaduna Town, North Western Nigeria. *Food Science and Quality Management*, 29:44-48

Eka, O.U. & Ohaba, J.A. (1977). Microbiological examination of Fulani milk (nono) and butter (manshanu). *Nigerian Journal of Science, 11*: 113-122.

Ekici, K., Bozkurt, H. and Isleyici, O. (2004). Isolation of some pathogens from raw milk of different milch animals. *Pakistan Journal of Nutrition,* 3(3):161-162.

Eliana, N. Castiglioni, T., Ana Maria, I. K, Greice, F. Z. S., Renato, L. L., Antonio, G. M. D. and Ana Lucia, S. P. C. (2012). Important Aspects of *Salmonella* in the Poultry Industry and in Public Health. In Dr. Dr. Barakat S. M. Mahmoud (Ed.). *Salmonella - A Dangerous Foodborne Pathogen.* ISBN: 978-953-307-782-6, InTech, Available from: http://www.intechopen.com/books/salmonella-adangerous-foodborne-pathogen/important-aspects-of-salmonella-in-the-poultry-industry-and-in-public-health

Erqou, S. A., Teferra, E., Mulu, A., and Kassu, A. (2007). A case of shigellosis with intractable septic shock and convulsions. *Japanese Journal of Infectious Diseases*, 60(5): 314-316.

Eswarappa, S.M., Janice, J., Nagarajan, A.G., Balasundaram, S.V., Karnam, G., Dixit, N.M. and Chakravortty, D. (2008). Differentially Evolved Genes of *Salmonella* Pathogenicity Islands: Insights into the Mechanism of Host Specificity in *Salmonella*. PLoS ONE, 3(12):3829.

EUFIC (European Food Information Council). (2006). Some Animal Diseases and their possible impact on Food Safety. Review 04/2006. Retrieved on 4th March, 2017 from http://www.eufic.org/article/en/food-safety-quality/animal-health/expid/review-animal-diseases/

Extension. (2010). How milk Quality is assessed. Retrieved 28th November, 2015 from https://articles.extension.org/pages/21197/how-milk-quality-is-assessed

FDA (Food and Drug Administration). (2015). The Dangers of Raw Milk: Unpasteurized milk can Pose a serious Health Risk. Retrieved on 3rd December, 2015 from http://www.fda.gov/Food/ResourcesForYou/Consumers/ucm079516.htm

FDA (Food and Drug Administration). (2012). Bad bug book: Foodborne pathogenic microorganisms and natural toxins handbook, 2nd ed. US Food and Drug Administration, Silver Spring. pp.25–28. Retrieved on 9th December, 2015 from http://www.fda.gov/Food/FoodborneIllnessContaminants/CausesOfIllnessBadBugBook/ucm2006773.htm

Flowers, R.S., Andrews, W., Donnelly, C.W. and Koenig, E. (1993). Pathogens in milk and milk products in Standard Methods for the Examination of Dairy Products. R.T. Marshall (Ed.), (16th ed., pp103–212). American Public Health Association, Washington, DC.

Foley, R.C., Bath, D.L., Dickinson, F.N. and Tuckers, H.A. (1972). Dairy cattle: principles, practices, problems and profits. Lea and Febiger, Philadelphia, Pennsylvania.pp. 2-15

Food and Agricultural Organization (2015). Dairy Production and Products; Milk composition. http://www.fao.org/agriculture/dairy-gateway/milk-and-milk-products/milk-composition/en/#.Vk48mKaPWSp. Retrieved on 19th November, 2015.

Food and Agricultural Organization (2016). Milking, milk production hygiene and udder health. http://www.fao.org/docrep/004/T0218E/T0218E03.htm. Retrieved on 6th December, 2017.

Foods Standards Agency (2002). The Composition of Foods . *In* McCance and Widdowson's Sixth Summary Edition. Cambridge: *Royal Society of Chemistry*

Frank, J.F. (1997). Milk and dairy products. In M.P.Doyle, L.R. Beuchat, and T.J. Montwille (Eds.), *Food microbiology, fundamentals and frontiers*. (pp. 101–116, 581–594) Washington, DC: American Society for Microbiology.

Frank, O. (1988). Rural Dairy Technology; Milk Chemistry. *International Livestock Centre for Africa*, manual 4. Dairy Technology Unit. ILCA, Addis Ababa, Ethiopia.

Frank, O. and Mahony, J. (1988). Rural Dairy Technology: Experiment in Ethiopia. International Livestock Centre for Africa Ltd., Ethiopia, ISBN: 9789290530923, pp. 64.

Galán, J.E. and Curtiss, R. (1989). Cloning and molecular characterization of genes whose products allow *Salmonella* typhimurium to penetrate tissue culture cells. *Proceedings of the National Academy of Sciences*, 86:6383-6387.

Galan, J.E. (2001). *Salmonella* interactions with host cells: type III secretion at work. *Annual Review of Cell Developmental Biology*, 17: 53-86.

Galan, J.E., and Curtiss, R. (1991). Distribution of the *inv*A,-B,-C,-D genes of *Salmonella typhimurium* among other *Salmonella* serovars: *inv*A mutants of *Salmonella* Typhi are deficient for entry into mammalian cells .*Infection and Immunity,* 59:2901-2908.

Galan, J.E., Ginocchio, C., and Costeas, P. (1992). Molecular and functional characterization of the *Salmonella* Typhimurium invasion gene *inv*A: Homology of *inv*A to members of a new protein family. *Journal of Bacteriology*, 17: 4338-4349

Gal-Mor, O. and Finlay, B.B. (2006) Pathogenicity islands: a molecular toolbox for bacterial virulence. *Cellular Microbiology*, 8: 1707–1719

Gandiya, F. (2001). Where quality begins: Cow management factors affecting the quality of milk. *Bulletin of International Dairy Federation (IDF)*, 361: 6-8.

Giannella, R.A. (1996). *Salmonella.* In: S.Baron (Ed.) *Medical Microbiology*, 4th ed., Ch 21). University of Texas Medical Branch at Galveston.

Ginocchio, C.C., Rahn, K., Clarke, R.C. and Galan, J.E. (1997). Naturally occurring deletions in the centisome 63 pathogenicity island of environmental isolates of *Salmonella* spp. *Infection and Immunity*, 65: 1267–1272.

Gophana, U., Ron, E. Z. and Graur, D. (2003). Bacterial type III secretion systems are ancient and evolved by multiple horizontal-transfer events. *Gene*, 312:151-163

Gran, H.M., Wetlesen, A., Mutukumira, A.N., Rukure, G. and Narvhus, J.A. (2003). Occurrence of pathogenic bacteria in raw milk, cultured pasteurised milk and naturally soured milk produced at small-scale dairies in Zimbabwe. *Food Control*, 14: 539-544.

Grassl, G.A. and Finlay, B.B. (2008). Pathogenesis of enteric *Salmonella* infections. *Current Opinion in Gastroenterol*ogy, 24(1):22-26.

Gray, J, T. and Fedorka-Cray, P.J. (2002) . "*Salmonella*." In Dean O. Cliver and Hans P. Riemann (Eds.), Foodborne Diseases (pp55-68). San Francisco: Academic Press.

Guetouache, M., Guessas, B. and Medjekal, S. (2014). Composition and nutritional value of raw milk. *Issues in Biological Sciences and Pharmaceutical Research*, 2(10): 115-122.

Gupta, S.K., Medalla, F., Omondi, M.W., Whichard, J.M., Fields, P.I., Gerner-Smidt, P., Patel, N.J., Cooper, K.L., Chiller, T.M. and Mintz, E.D. (2008). Laboratory-based surveillance of paratyphoid fever in the United States: travel and antimicrobial resistance. *Clinical Infectious Diseases*, 46(11):1656-1663

Hakanen, A., Kotilainen, P., Huovinen, P., Helenius, H. and Siitonens, A. (2001). Reduced fluoroquinolone susceptibility in *Salmonella enterica* serotypes in travelers returning from Southeast Asia. *Emerging Infecious. Dis*ease, 7**:**996-1003.

Haley, C.C., Ong, K., Hedberg, K., Cieslak, P. R., Scallan, E., Maran, R. and Shin, S. (2010). "Risk Factors for Sporadic Shigellosis, FoodNet 2005," *Foodborne Pathogens and Diseases*, 7: 741–47. Retrieved on 9th December, 2015 from http://www.ncbi.nlm.nih.gov/pubmed/20113209

Hamilton, W.L. and Wenlock, R. (2016). Antimicrobial Resistance: A Major Threat to Public Health. *Cambridge Medicine Journal*, doi:10.7244/cmj.2016.01.001

Hans, C.P., Chaudhary, D.P. and Bansal, D.D. (2002). Magnesium deficiency increases oxidative stress in rats. *Indian Journal of Experimental Biology*, 40:1275–1279.

Harding, F. (1999). Milk quality. In A Chapman and Hall Food Science Book. 1st Ed., pp 23-50). Aspen publishers Inc., Gaithersburg, Maryland.

Harrigan, W.F. and McCance, M. (1976): Laboratory Methods in Food and Dairy Microbiology. Academic Press, London. Pp 206-209.

Hartman, P.A. (1997). The Evolution of Food Microbiology. In M.P. Doyle, and L.R. Beuchat (Eds.), *Food Microbiology: Fundamentals and Frontiers* (3rd Ed., pp: 3-12). ASM Press, Washington DC,.

Haug, A., Hostmark, A.T. and Harstad, O.M. (2007). Bovine milk in human nutrition- A review. *Lipids in Health and Disease*, 6:1–16.

Hayes, M.C., Ralyea, R.D., Murphy, S.C., Carey, N.R., Scarlett, J.M. and Boor, K.J. (2001) Identification and Characterization of Elevated Microbial Counts in Bulk Tank Raw Milk. *Journal of Dairy Science,* 84: 292-298.

Hitti, W. and Wolff, M. (2005). Two cases of multidrug-resistant *Nocardia farcinica* infection in immunosuppressed patients and implications for empiric therapy. *European Journal of Clinical Microbiology and Infectious Diseases*, 24:142-144.

Holt, C., Carver, J.A., Ecroyd, H. and Thorn, D.C. (2013). Invited review: Caseins and the casein micelle: their biological functions, structures, and behavior in foods. *Journal of Dairy Science*, 96(10):6127-6146.

Hornick, R. B., Greisman, S. E., Woodward, T. E.,. DuPont, H. L., Dawkins, A. T. and Snyder, M. J. (1970). Typhoid fever: pathogenesis and immunologic control. *New England Journal of Medicine*, 283:686-691.

Hu, Q., Coburn, B., Deng, W., Li, Y., Shi, X., Lan, Q., Wang, B., Coombes, K.B. and Finlay, B.B. (2008). *Salmonella enterica* Serovar Senftenberg Human Clinical Isolates Lacking SPI-1. *Journal of Clinical Microbiology*, 46 (4): 1330-1336

International Commission on Microbiological Specifications for foods (ICMSF). (2006). *Microorganism in Foods*. In Microbial ecology of food commodities (2nd ed.) Kluwer Academics, Plenum.Publishers. Londres, U.K.

International Dairy Federation (IDF). (1987). Milk and Milk Products: Enumeration of Microorganisms-Colony Count at 3°C. Provisional IDF Standard 100A. IDF, Brussels, Belgium.

Ingersoll, M., Groisman, E.A. and Zychlinsky, A. (2002). Pathogenicity islands of *Shigella*, *Current Topics in Microbiology and Immunology*, 264:49.

Insel, P., Turner, R.E. and Ross, D. (2004). Nutrition. Second American dietetic association, Jones and Bartlett, USA.

International Standards Organization (ISO). (2005). Microbiology of food and animal feeding stuffs – horizontal method for the detection of *Salmonella* spp. 6579.

Isenberger, D.W., Hoge, C.W. Srijan, A. Pitarangsi, C. Vithayasai, N. Bodhidatta, L. Hickey, K.W. and Cain, P.D. (2002). Comparative antibiotic resistance of diarrhoeal pathogens from Vietnam and Thailand 1996-1999, *Emerging Infectious Diseases*, 8 (2): 1- 4.

Jamshidi, A., Bassami, M.R. and Afshari-Nic, S. (2009). Identification of *Salmonella* spp and *Salmonella* Typhimurium by a multiplex PCR-based assay from poultry carcasses in Mashhad-Iran. *International Journal of Veterinary Research*, 3: 43-48.

Jasson, V., Jacxsens, L., Luning, P., Rajkovic, A., and Uyttendaele, M. (2010). Alternative microbial methods: an overview and selection criteria. *Food Microbiology,* 27(6): 710-730.

Jay, J.M. (1996). Modern food microbiology (5th ed.). New York, Chapman and Hall Publishing Company, 137–141, 328–342, 347–352.

Jayaro, B.M., Donaldson, S.C., Straley, B.A., Sawant, A.A., Hegde, N.V. and Brown, J.L. (2006). A survey of food borne pathogens in bulk tank milk and raw milk consumption among farm families in Pennsylvania. *Journal of Dairy Science,* 89:2451–2458.

Jensen, R.G. (1995): Handbook of Milk Composition. Academic Press, San Diego.

Jensen, R.G. (2002). The Composition of Bovine Milk Lipids: January 1995 to December 2000. *Journal of Dairy Science,* 85:295–350.

Jensen, R.G. and Newburg, D.S. (1995). Bovine milk lipids. In R.G. Jensen (Ed). *Handbook of milk composition* (pp. 543–75). London, UK: Academic Press..

Jomezadeh, N., Babamoradi, S., Kalantar, E. and Javaherizadeh, H. (2014). Isolation and antibiotic susceptibility of *Shigella* species from stool samples among hospitalized children in Abadan, Iran. *Autumn*, 7(4): 218–223.

Jones, M.A., Hulme, S.D., Barrow, P.A. and Wigley, P. (2007). The *Salmonella* pathogenicity island 1 and *Salmonella* pathogenicity island 2 type III secretion systems play a major role in pathogenesis of systemic disease and gastrointestinal tract colonization of *Salmonella enterica* serovar Typhimurium in the chicken. *Journal of Avian Pathology,* 36 (3): 199-203.

Julius, A. (2012). Small-scale Farmer's Perception on the Impact of Grazing Livestock Animals on Crop Production in Abuja, Nigeria. *Trends in Agricultural Economics,* 5 (4): 115-123.

Karshima, N.S., Pam, V. A., Bata, S. I., Dung, P. A. and Paman N. D. (2013). "Isolation of *Salmonella* Species from Milk and Locally Processed Milk Products Traded for Human Consumption and Associated Risk Factors in Kanam, Plateau State, Nigeria." *Journal of Animal Production Advances,* 3 (3): 69-74.

Kauffmann, F. (1966) [On the history of *Salmonella* research]. *Zentralblatt fur Bakteriolgie Originale*, 201: 44–48.

Khan, A.A., Nawaz, M.S., Khan, S. and Sernigelia, C.E. (1999). Detection of Multidrug Resistant *Salmonella* Typhimurium DT104 by Multiplex polymerase chain reaction. *FEMS Microbiology Letters*, 182: 355-360.

Kim, A.Y., Goldberg, M.B. and Rubin, R.H. (2004). *Salmonella* infections. In S.L. Gorbach, J.G. Bartlett, and N.R. Blacklow (Eds.), *Infectious Diseases*. (3rd ed., pp. 68). Philadelphia: Lippincott Williams and Wilkins

Kingsley, R. A., and Baumler, A. J. (2000). Host adaptation and the emergence of infectious disease: the *Salmonella* paradigm. *Molecular Microbiology,* 36**:**1006-1014.

Kirov, S. M. (1997). *Aeromonas* and *Plesiomonas* species. In M. P. Doyle, L. R. Beuchat, and T. J. Montville, (Eds.), *Food microbiology fundamentals and frontiers* (pp. 265–287). Washington, DC: ASM Press.

Korhonen, H.M., Pihlanto-Leppala, A., Rantamaki, P. and Tupasela T. (1998). Impact of processing on bioactive proteins and peptides. *Trends in Food Science and Technology*, 8:307–19.

Kotloff, K.L., Winickoff, J.P., Ivanoff, B, Clemens, J.D., Swerdlow, D.L., Sansonetti, P.J., Adak, G.K. and Levine, M.M. (1999). Global burden of *Shigella* infections: implications for vaccine development and implementation of control strategies. *Bulletin of the World Health Organization*, 77 (8):651-666.

Krzyzanowski, F., Zappelini, L., Martone-Rocha, S., Dropa, M., Matté, M.H., Nacache, F. and Razzolini, M.T.P. (2014). Quantification and characterization of *Salmonella* spp. isolates in sewage sludge with potential usage in agriculture. *BioMed Central Microbiology* , 14:263

Kurjak, A., and Chervenak, F. A. (Eds.). (2006). *Textbook of Perinatal Medicine* (2nd ed.). United Kingdom: Informa UK Ltd.

Kurweil, R., and Busse, M. (1973). Total count and microflora of freshly drawn milk. *Milchwissenschaft*, 28:427.

Kweon, M. N. (2008). Shigellosis: the current status of vaccine development. *Current Opinion in Infectious Diseases*, 21(3): 313-318.

Laba, S.A. and Udosek, C.E. (2013). Bacteriological Quality of Raw Cow Milk in Ilorin, North Central Nigeria. *Nature and Science*, 11(10):73-79

Lampel, K.A. (2001). *Shigella* .In *Dowwnes.Compendium of methods for the microbiological examination of foods* (4th ed., pp 381-385). Washington, DC: American Public Health Association,

Lampel, K.A. and Maurelli, A.T. (2003). *Shigella* species. In M.D. Miliotis, J.W. Bier (Eds.), *International handbook of foodborne pathogens* (Ch 11:pp. 167–180). Marcel Dekker, New York

Lawan, M.K., Abdulsalawu, F.O., Suleiman, A., Aluwong, T. and Yaqub, L.S. (2012). Microbial Assessments of Bulk Milk Before and After Pasteurization in Two Different Dairy Farms in Zaria, Nigeria. *International Journal of Dairy Science,* 7: 103-108.

Libby, S. J., Lesnik, M. Hasegawa, P. Kurth, M. Belcher, C. Fierer, J.and Guiney, D. G. (2002). Characterization of *spv* locus in *Salmonella enterica* serovar Arizona. *Infection and Immununity*, 70**:**3290-3294.

Lindmark M. H. and Swedish Dairy Association (2003). Composition of Swedish dairy milk 2001. Report Nr 7025-P (In Swdish)

Lingathurai, S. and Vellathurai, P. (2010). Bacteriological quality and safety of raw cow milk in Madurai, South India. *Webmed Central Microbiology*, 1(10):1-10.

Lingathurai, S., Vellathurai, P., Vendan, S.E. and Prem, A.A.A. (2009). A comparative study on the microbiological and chemical composition of cow milk from different locations in Madurai, Tamil Nadu. *Indian Journal of Science and Technology*, 2(2):51-54.

Lin-Hui, S., Chiu, C., Chishih, C. and Jonathan, T.O. (2004). Antimicrobial Resistance in Nontyphoid *Salmonella* Serotypes: A Global Challenge. *Clinical Infectious Disease*, 39(4):546-551

Lohmann Animal Health (LAH) (2013). Pathogenesis of Salmonellosis in Humans. Global Technical Services. https://www.salmonella360.com/content/files/439/Tech

Lopez-Campos, G., Martinez-Suarez, J.V., Aguado-Urda, M. and Lopez-Alonso, V. (2012). Detection, Identification, and Analysis of Foodborne Pathogens. Microarray Detection and Characterization of Bacterial Food borne Pathogens. *Springer*, 2:13-32

Lorenzetti, D.K. (2006). Influence of time and temperature on the development of Psychrotropic microorganisms in raw milk from two states of the south. Dissertion (Master of Food Technology) Federal university of parana, Curitiba 71.

Makut, M.D., Nyam, M.A., Amapu, T.Y. and Ahmed, A. (2014). Antibiogram of Bacteria Isolated from Locally Processed Cow Milk Products Sold in Keffi Metropolis, Nasarawa State, Nigeria. *Journal of Biology, Agriculture and Healthcare*, 4(4): 19-25.

Malorny, B., Hoorfar, J., Bunge, C. and Helmuth, R. (2003). Multicenter validation of the analytical accuracy of Salmonella PCR: towards an international standard. *Applied Environmental Microbiology*, 69:290-296.

Malorny, B., Tassios, P. T., Radström, P., Cook, N., Wagner, M., and Hoorfar, J. (2003). Standardization of diagnostic PCR for the detection of foodborne pathogens. *International Journal of Food Microbiology*, *83*(1): 39- 48.

Mamman, A.B., Oyebanji, J.O. and Peters, S.W. (2000). *Nigeria A People United. A Future Assured, Survey of States*. Nigeria: Crabumo Publishing Company Limited, Volume 2.

Marcus, S.L., Brumell, J.H., Pfeifer, C.G. and Finlay, B.B. (2000). *Salmonella* pathogenicity islands: big virulence in small packages. *Microbes and Infection*, 2:145-156.

Markus, C.R., Olivier, B., Panhuysen, G.E., Van Der Gugten, J., Alles, M.S., Tuiten, A., Westenberg, H.G., Fekkes, D., Koppeschaar, H.F. and Haan, E.E. (2000). The bovine protein alpha-lactalbumin increases the plasma ratio of tryptophan to the other large neutral amino acids, and in vulnerable subjects raises brain serotonin activity, reduces cortisol concentration, and improves mood under stress. *American Journal of Clinical Nutrition*, 71(6):1536-1544.

Martins, M.L., Pinto, C.L.O., Rocha, R.B., Araujo, E.F. and Vanetti, M.C.D. (2006). Genetic diversity of Gram-negative proteolytic, psychrotrophic bacteria isolated from refrigerated raw milk. *International Journal of Food Microbiology*, 111: 144–148.

Maskey, A.P., Basnyat, B., Thwaites, G.E., Campbell, J.I., Farrar, J.J and Zimmerman, M.D. (2008). Emerging trends in enteric fever in Nepal: 9124 cases confirmed by blood culture 1993-2003. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 102(1):91-5.

Mata, G.M.S.C. and Vanetti, M.C. (2012). Comparison of Conventional and Rapid Methods for *Salmonella* Detection in Artisanal Minas Cheese. *Journal of Food Research*, 1(3):178-183.

McKillip, J.L. and Drake, M. (2004). Real-time nucleic acid-based detection methods for pathogenic bacteria in food. *Journal of Food Protection*, 67: 823-832.

Mcphee, J.D., Griffiths, M.W. (2011). Psychrotrophic bacteria *Pseudomonas* spp. In W.F. John (Ed.). *Encyclopedia of Dairy Sciences* (2nd ed., pp 379–383) Academic Press, San Diego.

Mehta, M.B. (2015). Chemical Composition of Milk and Milk Products. *Handbook of food chemistry*. Doi: 10.1007/978-3-642-41609-5_31-1.

Mermin, J., Hoar, D. and Angulo, F. J. (1997). Iguanas and *Salmonella marina* infection in children: a reflection of increasing incidence of reptile-associated salmonellosis in the United States. *Pediatrics,* 99**:**399-402.

Mhone, T.A., Matope, G. and Saidi, P.T. (2012). Detection of *Salmonella* spp., *Candida* albicans, *Aspergillus* spp., and antimicrobial residues in raw and processed cow milk from selected small holder farms of Zimbabwe. *Veterinary Medicine International*, Article ID 301902, 5 pages doi:10.1155/2012/301902.

Miller, G.D., Jarvis, J.K. and McBean, L.D. (2007). The Importance of Milk and Milk Products in the Diet. In I .Wolinsky (Ed.), *Handbook of Dairy Foods and Nutrition* (3rd ed). Florida, USA: CRC Press, Boca Raton.

Ministry of Economic Development (1996). Kaduna State Statistical Year Book. Kaduna, Nigeria. Ministry of Economic Development, Statistics Division.

Mohammed, A.S. and Abdullahi, M. (2015). Comparative Study of Microbial Quality of Hawked Nono and Packaged Yogurt Sold In Bida Metropolis. *Specialty Journal of Psychology and Management*, 1 (0): 1-4

Mølbak, K., Baggesen, D.L., Aarestrup, F.M., Ebbesen, J.M., Engberg, J., Frydendahl, K., Gerner-Smidt, P, Petersen, A.M. and Wegener, H.C.(1999). An outbreak of multidrug-resistant, quinolone-resistant *Salmonella enterica* serotype Typhimurium DT104. *New England Journal of Medicine*, 341:1420-1425.

Moller, V. (1954). Distribution of Amino Acid Decarboxylases in Enterobacteriaceae1. *Acta pathologica Microbiologica Scandinavica*, 35 (3): 259-277.

Molt, C. (2000). Antidotes for Antibiotic Use on the Farm. As pathogen resistance spreads, researchers look for alternatives to the heavy use of antibiotics in food production. *BioScience*, 50(11): 955-960.

Monika, S., Manishi, M., Ranjit, S.K., Bishnu, P.M. and Balwinder, K.J. (2012). Milk proteins and human health: A1/A2 milk hypothesis. *Indian Journal of Endocrinology and Metabolism*, 16(5): 856.

Mourad, A.S., Metwally, M., Nour El Deen A.N., Threfall, E.J., Rowe, B., Mapes, T., Hedstrom, R., Bourgeois, A.L. and Murphy, J.R. (1993). Multiple-drug-resistant *Salmonella* Typhi. *Clinical Infectious Disease*; 17:135-136.

Mrutyunjay, S., Jonathan, J., Siegfried, H., Marcus, K.,Thomas, S., Paul, A. B. and Wolf-Dietrich, H. (2006). Virulence of Broad- and Narrow-Host-Range *Salmonella enterica* Serovars in the Streptomycin-Pretreated Mouse Model. *Infection and Immunity*, 74(1): 632–644.

Mubarack, M.H., Doss, A., Dhanabalan, R. and Balachander, S. (2010). Microbial quality of raw milk samples Collected from different villages of Coimbatore district, Tamilnadu, South India. *Indian Journal of Science and Technology*, 3(1):61-63.

Muhammad, K., Altaf, I., Hanif, A., Anjum, A.A. and Tipu, M.Y. (2009). Monitoring of hygienic status of raw milk marketed In Lahore city, *Pakistan Journal of Animal and Plant Science*, 19(2):74-77.

Mullis, K., Faloona, F., Scharf, S., Saiki, R., Horn, G. and Erlich, H. (1986). "Specific enzymatic amplification of DNA in vitro: the polymerase chain reaction", *Cold Spring Harbor Symposia on Quantitative Biology*, 51 (1): 263-273.

Murphy, S.C. and Boor, K.J. (2000). Trouble-shooting sources and causes of high bacterial counts in raw milk. *Dairy Food and Environmental Sanitation*, 20: 606-611.

National Animal Disease Information Service (NADIS). (2016). Salmonellosis in Cattle. Retrieved on 20 September, 2016 from http://www.nadis.org.uk/bulletins/salmonellosis-in-cattle.aspx

National Institute of Allergy and Infectious Diseases (NIAID) (2015). Shigellosis. Retrieved on 9th December, 2015 from https://www.niaid.nih.gov/topics/shigellosis/Pages/shigellosis.aspx

National Population Commission (NPC). (2006). Population Census figures for 2006 Official figures Gazette, Abuja.

Ndambi, O.A., Hemme, T. and Latacz-Lohmann, U. (2007). Dairying in Africa - Status and recent developments.*Livestock Research for rural Development,* 19(8).

Nebedum, J.O. and Obiakor, T. (2007). The effects of different preservation methods on the quality of nunu: A locally fermented Nigerian Dairy Product. *African Journal of Biotechnology*, 6: 454-458.

New York State Cattle Health Assurance Program (NYSCHAP) (2016). Salmonellosis – Background, Management and Control. Retrieved on 20 September, 2016. https://ahdc.vet.cornell.edu/programs/NYSCHAP/docs/SalmonellaTrifold.pdf

New York State Department of Agriculture and Markets. New York codes, rules, and regulations.Retrieved on 29, September, 2016 from http://www.agmkt.state.ny.us/DI/PDF%20WebDocs/RawMilkRegsPart2.pdf

National Institute of Open Schooling (NIOS). (2012). Composition of Milk. Retrieved on 3rd December, 2015 from http://oer.nios.ac.in/wiki/index.php/Composition_of_Milk

National Notifiable Diseases Surveillance System (NNDSS). (2013) Notifications for all disease by State & Territory and year. National Notifiable Disease Surveillance System, Department of Health and Ageing, Canberra. Retrieved on 9th December, 2015 from http://www9.health.gov.au/cda/source/cda-index.cfm

Ochiai, R.L., Acosta, C.J., Danovaro-Holliday, M.C., Baiqing, D., Bhattacharya, S.K., Agtini, M.D., Bhutta, Z.A., Canh, D.G., Ali, M., Shin, S., Wain, J., Page, A.L., Albert, M.J., Farrar, J., Abu-Elyazeed, R., Pang, T., Galindo, C.M., Von Seidlein, L., Clemens, J.D. and Domi Typhoid Study Group. (2008). A study of typhoid fever in five Asian countries: disease burden and implications for controls. *Bulletin of the World Health Organization*, 86(4):260-268.

Ochman, H., Lawrence, J.G., Groisman, E.A. (2000). Lateral gene transfer and the nature of bacterial innovation. *Nature,* 405: 299–304.

Odewumi, A.A. (1981). Pathogenic Bacteriology 1. Companion to practical schedule. Department of Microbiology, Ahmadu Bello University, Zaria. pp 5-16.

Odumeru, J.A. and León-Velarde, C.G. (2012). Salmonella Detection Methods for Food and Food Ingredients. In Dr. Dr. Barakat S M Mahmoud (Ed.). *Salmonella - A Dangerous Foodborne Pathogen.* InTech.

Office International des Epizooties Terrestrial Manual (OIE).(2010). Salmonellosis. Retrieved on 20 September, 2016 from http://web.oie.int/eng/normes/MMANUAL/2008/pdf/2.09.09_SALMONELLOSIS.pdf.

Ogbonna, I.O., David, A.B., Waba, J.T. and Eze, P.C. (2012). Microbiological Quality Assessments of Biradon, Kesham and Kindrimo: Milk Products Sold in Maiduguri, Nigeria. *International Journal of Dairy Science, 7: 11-19.*

Okeke, I.N., Laxminarayan, R., Bhutta, Z.A., Duse, A.G., Jenkins, P.O., Brien, T.F. and Pablos-Mendez, A. (2005). Antimicrobial resistance in developing countries. Part I: recent trends and current status. *Lancet*, 5:481-493.

Okonkwo, I.O. (2011). Microbiological Analyses and Safety Evaluation of Nono: A Fermented Milk Product Consumed in Most Parts of Northern Nigeria. *International Journal of Dairy Science*, *6: 181-189.*

Olasupo, N.A., Akinsanya, S.M., Oladele, O.F. & Azeez, M.K. (1996). Evaluation of nisin for the preservation of nono, a Nigerian fermented milk product. *Journal of Food Processing and Preservation*, 20: 71-78.

Oludairo O. O., Kwaga J. K.P., Dzikwi A. A. and Kabir J. (2013). Detection of *inv* A virulence gene by polymerase chain reaction (PCR) in *Salmonella* spp. isolated from captive wildlife. *Bio-Genetics Journal*, 1(1): 12-14.

Olufunke, O.A., Abike, T.O., Kolawole, D.O. (2014). Phenotypic and molecular characterization of *Salmonella* serotypes in cow raw milk and milk products in Nigeria. *African Journal of Biotechnology*, 13 (37): 3774-3789.

Omotosho, A.O., Abdullahi, I.O. and Damisa, D. (2013). Microbiological quality and HACCP concept in the production of “Nono” in a farm settlement in Minna, Niger state, Nigeria. *African Journal of Microbiological Research*, 7(25) : 3234-3239.

Ovsyshcher, I., Rudnik, L., Alkan, M., and Ilia, R. (1987). Atrio-ventricular block associated with *Shigella flexneri* infection. *European Journal of Clinical Microbiology*, 6(4): 504.

Oxoid (2013). *Salmonella* polyvalent agglutinating sera. Available August, 13 2016 from http://m.thermofisher.com/order/catalog/product/R30858201?

Ozrenk, E. and Selcuk, I.S. (2008). The effect of seasonal variation on the composition of cow milk in van province. *Pakistan Journal of Nutrition*, 7: 161-164.

Pal, S. and Ellis, V. (2010). The chronic effects of whey proteins on blood pressure, vascular function, and inflammatory markers in overweight individuals. *Obesity (Silver Spring)*, 18(7): 1354-1359.

Parker, C. T. and Guard-Petter, J. (2001). Contribution of flagella and invasion proteins to pathogenesis of *Salmonella enterica* serovar Enteritidis in chicks. *Federation of European Microbiological Societies (*FEMS*) Microbiology Letters*, 204 (2): 287-291.

Parodi, P. (2004). Milk fat in human nutrition. *Australian Journal of Dairy Technology*, 59:3–59.

Parry, C.M. and Threlfall, E.J. (2008). Antimicrobial resistance in typhoidal and nontyphoidal salmonellae. *Current Opinion in Infectious Disease*, 21(5):531-538

Paulin, S.M., Jagannathan, A., Campbell, J., Wallis, T.S. and Stevens, M.P. (2007). Net replication of *Salmonella* enteric serovars Typhimurium and Choleraesuis in porcine intestinal mucosa and nodes is associated with their differential virulence. *Infection and Immunity*,75: 3950–3960.

Perelle, S., Dilasser, F., Malorny, B., Grout, J., Hoorfar, J. and Fach, P. (2004). Comparison of PCR-ELISA and LightCycler real-time PCR assays for detecting Salmonella spp. in milk and meat samples. *Molecular and Cellular Probes*, 18:409-420.

Perennia (2013). Standard Plate Count. Rerieved on 27th November, 2015 from http://www.perennia.ca/Fact%20Sheets/Livestock%20and%20Poultry/NonRuminant/Mink/SPC%20Mink%20Feed.pdf

Perilla, M. J. (2003). Manual for the laboratory identification and antimicrobial testing of bacterial pathogens of public health importance in the developing world. World health organization. Atlanta Georges U.S.A Pp. 133 – 284.

Phoon, Y.W., Chan, Y.Y. and Koh, T.H. (2015). Isolation of multidrug-resistant *Salmonella* in Singapore. *Singapore Medical Journal*, 56(8): 142-144.

Pianetti, A., Sabatini, L., Citterio, B., Pierfelici, L., Ninfali, P. and Bruscolini, F. (2008). Changes in microbial populations in ready-to-eat vegetable salads during shelf-life. *Italalian Journal of Food Science*, 20: 245-254.

Popoff, M. Y. and Le Minor, L. (1997). Antigenic formulas of the Salmonella serovars, 7th revision. World Health Organization Collaborating Centre for Reference and Research on *Salmonella* (Pasteur Institute, Paris, France).

Prakash, K. and Pillai, P.K. (1992). Multidrug-resistant *Salmonella* Typhi in India. *Alliance for the Prudent Use of Antibiotics Newsletter*, 10:1-3.

Prescott, L. M., Harley, J. P., Klein, D. A., Willey, M.J., Sherwood, L.M. and Woolverton, C.J. (2008). Microbiology (7th Ed.) McGraw-Hill Publishers,America, New York. Ch2:pp 26.

Pui, C. F., Wong, W. C., Chai, L. C., Tunung, R., Jeyaletchumi, P., Noor Hidayah, M. S., Ubong, A., Farinazleen, M. G., Cheah, Y. K. and Son, R. (2011). *Salmonella*: A foodborne pathogen. *International Food Research Journal*, 18: 465-473.

Rahn, K., De Grandis, S. A,. Clarke, R. C., Mcewen, S. A., Galan, J. E., Ginocchio, C., Curtiss, R. and Gyles, C. L. (1992). Amplification of an invA gene sequence of *Salmonella* Typhimurium by polymerase chain reaction as a specific method of detection of *Salmonella*. *Molecular and Cellular Probes*, 6**:**271-*279.*

Rampling, A .(1990) The microbiology of milk and milk products. In M.T. Parker, L.H. Collier, (Eds.). *Topley and Wilson's principles of bacteriology, virology, and immunity* (8th ed., Vol. I, pp. 265-87). Philadelphia: B.C. Decker.

Randall, J.B. (2001). Salmonellosis in cattle. *VET's Corner,* 1(8): 1-2.

Rayman, M.P. (2002). The argument for increasing selenium intake. *Proceedings of the Nutrition Society*, 61:203–15.

Reda, A.A., Seyoum, B., Yimam, J., Andualem, G., Fiseha, S. and Vandeweerd, J. (2011). Antibiotic susceptibility patterns of *Salmonella* and *Shigella* isolates in Harar, Eastern Ethiopia. *Journal of Infectious Diseases and Immunity*, 3(8): 134-139.

Reeves, M. W., Evins, G. M., Heiba, A. A., Plikaytis, B. D. and Farmer J. J. III.(1989). Clonal nature of *Salmonella typhi* and its genetic relatedness to other salmonellae as shown by multilocus enzyme electrophoresis and proposal of *Salmonella bongori*. comb. nov. *Journal of Clinical Microbiology*, 27:313–320.

Richter, R.L., Ledford, R.A. and Murphy, S.C. (1992). Milk and Milk Products. In C.Vanderzant, and D.F. Splittstoesser (Eds.), *Compendium of Methods for the Microbiological Examination Of Foods* (3rd Edn., pp: 837-838) Washington DC: American Public Health Association.

Ricke, S.C., Pillai, S.D., Norton, R.A., MAciorowski, K.G., and Jones, F.T. (1998). Applicability of rapid methods for detection of *Salmonlla* spp. in poultry feeds: a review. *Journal of Rapid Methods and automation in Microbiology*, 6: 239-258.

Robbins, J. (2001). The Food Revolution, how your diet can help save your life and the world. Berkeley, California, USA: Conari Press.

Rosenthal, I. (1991). Milk and Dairy Products Properties and Processing. pp 70-71.New York: Balaban Publishers VCH.

Rowe B, Threlfall, E.J., Ward, L.R. (1992). Ciprofloxacin and typhoid fever. *Lancet*, 339:740.

Rowe B., Ward, L.R. and Threlfall, E.J. (1997). Multidrug-resistant Salmonella Typhi: a worldwide epidemic. *Clinical Infectious Disease,* 24 (1):106-109.

Ryan, M.J., Wall, P.G., Adak, G.K., Evans, H.S. and Cowden, J.M. (1997). Outbreaks of infectious intestinal disease in residential institutions in England and Wales 1992-1994. *Journal of Infection*, 34:49–54.

Ryser, E. (1999) Microorganisms of importance in raw milk. *Michigan Dairy Review*, 8: 7–9.
Salmonella spp. *Federation of European Microbiological Societies (FEMS) Immunology and Medical Microbiology*, 44 (3): 251-259.

Sanders, E.R. (2012). Aseptic Laboratory Techniques: Plating Methods. *Journal of Visual Ex*periments, *63*: e3064. doi: 10.3791/3064.

Schauber, D. B., Zabel, B. A., Pedraza, I. F., Ohara, C. M., Steigerwalt, A. G., and Brenner, D. J. (1995). Genetic and biochemical- characterization of Citrobacter rodentium sp. nov. *Journal of Clinical Microbiology*, 33(8): 2064–2068

Schmidt, H and Hensel, M. (2004). Pathogenicity islands in bacterial pathogenesis. Clinical Microbiology Reviews, 17:14–56.

Schroeder, G. N. and Hilbi, H. (2008). Molecular pathogenesis of Shigella spp.: controlling host cell signaling, invasion, and death by type III secretion. *Clinical Microbiology Reviews*, 21(1): 134-156.

Serman, S. (2001). Symposium : Typhoid Fever; Pathogenesis and Laboratory Diagnosis. *Journal, Indian Academy of Clinical Medicine*, 2 (1 and 2): 17-20.

Shea, K.M. (2003). Antibiotic Resistance: What is the Impact of Agricultural Uses of Antibiotics on Children's Health? *Pediatrics*, 112:253-258.

Shehu, L.M. and Adesiyun, A.A. (1990). Characteristics of strains of *Escherichia coli* isolated from locally fermented milk (nono) in Zaria, Nigeria. *Journal of Food Protection, 53*: 574 – 577.

Sheila, M.M. and Simon, P. (2003). Salmonellosis in Cattle: A review. American Association of Bovine Practitioners 36th Annual Conference, Dairy Herd Problem Investigation Strategies. Retrieved on 20 September, 2016 from http://www.vetmed.wisc.edu/dms/fapm/fapmtools/7health/Salmorev.pdf

Shipp, C.R., Rowe, B. (1980). A mechanised microtechnique for Salmonella serotyping. *Journal of Clinical Pathology*, 33: 595–597.

Silva, T.M., Oliveria, R.L. and Barbosa, L.P. (2010). Body components of young crossbred Boer goats fed licury oil (Syagruscoronata) in the diet. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, 62(6):1448-1454.

Singh, V. (2013). *Salmonella* serovars and their host specificity. *Journal of Veterinary Science and Animal Husbandry*, 1(3): 301

Slack, J. M. and Snyder, I. S. (1978). Bacteria and Human Disease. Chicago: Year Book Medical Publishers, Inc.

Smith, J. L. (1987). *Shigella* as a foodborne pathogen. *Journal of Food Protection*, 50 (9): 788-801.

Sørhaug, T. (1997). Stepaniak, L. Psychroptophs and their enzymes in milk and dairy products: quality aspects. *Trends in Food Science and Technology*, 8: 35–40.

Spreer, E. (1998). Milk and Dairy Product Technology. (pp. 5) New York, USA: Marcel Dekker Inc.

Strickland, M., Johnson, M.E. and Broadbent, J.R. (2001). Qualitative and quantitative analysis of proteins and peptides in milk products by capillary electrophoresis. *Electrophoresis*, 22:1510–1517

Talukder, K.A., Mondol, A.S., Islam, A.M., Islam, Z., Dutta, D.K., Khajanchi, B.K. and Azmi, I.J. (2007). A novel serovar of *Shigella dysenteriae* from patients with diarrhoea in Bangladesh. *Journal of Medical Microbiology*, 56: 654–658.

Tamba, Z., Bello, M., Raji, M.A. (2016). Occurence and Antibiogram of *Salmonella* species in raw and Fermented Milk in Zaria and Environs. *Bangladesh Journal* of *Veterinary Medicine*, 14 (1): 103-107.

The Dairy Council (2015). Vitamins in Milk. Retrieved on 29th November, 2015 from http://www.milk.co.uk/page.aspx?intPageID=71

Thomas, L.H. and Gerald, T.K. (1996). *Shigella*. In S. Baron (Ed.), Medical Microbiology **(**4th ed., Ch 22). University of Texas Medical Branch at Galveston, Galveston, Texas.

Thomson, N. R., Clayton, D. J., Windhorst, D., Vernikos, G., Davidson, S., Churcher, C., Quail, M. A., Stevens, M., Jones, M. A., Watson, M., Barron, A., Layton, A., Pickard, D., Kingsley, R. A., Bignell, A., Clark, L., Harris, B., Ormond, D., Abdellah, Z., Brooks, K., Cherevach, I., Chillingworth, T., Woodward, J., Norberczak, H., Lord, A., Arrowsmith, C., Jagels, K., Moule, S., Mungall, K., Sanders, M., Whitehead, S., Chabalgoity, J. A., Maskell, D., Humphrey, T., Roberts, M., Barrow, P. A., Dougan, G. and Parkhill, J. (2008). Comparative genome analysis of *Salmonella* Enteritidis PT4 and *Salmonella* Gallinarum 287/91 provides insights into evolutionary and host adaptation pathways. *Genome Research*, 18 (10):1624-1637

Thrusfield, M. (1997).Veterinary Epidemiology. (2nd ed., Pp. 182-183). Blackwell publications, Oxford.

Tierrez, A., and Garcı´a-del, P. F. (2005). New concepts in *Salmonella* virulence: the Importance of reducing the Intracellular Growth rate in the Host. *Cellular Microbiology*, 7: 901–909.

Usman, U.B., Kwaga, J.K.P., Kabir, J., Olonitola, O.S. (2016). Isolation and Antimicrobial Susceptability of Listeria *Monocytogenes* from Raw Milk and Milk Products in Northern Kaduna State, Nigeria. *Journal of Applied & Environmental Microbiology*. 4 (3): 46-54.

Uzzau, S., Brown, D. J., Wallis, T., Rubino, S., Leori, G., Bernard, S., Casadesus, J., Platt, D. J., and Olsen, J. E. (2000). Host-adapted serotypes of *Salmonella enterica*. *Epidemiology and Infection*, 125**:**229-255.

Van Asten, A. J. and Van Dijk, J. E. (2005). Distribution of "classic" virulence factors among *Salmonella* spp. *Federation of European Microbiological Societies (FEMS) Immunology and Medical Microbiology*, 44(3): 251-259

Varley, A.J., Sule, J. and Absalom, A.R. (2009). Principles of Antibiotic Therapy. *Continuing Education in Anaesthesia, Critical Care and Pain*, 9(6): 184-188.

Villar, R.G., Macek, M.D., Simons, S., Hayes, P.S., Goldoft, M.J., Lewis, J.H., Rowan, L.L., Hursh, D., Patnode, M. and Meads, P.S. (1999). Investigation of multidrug-resistant *Salmonella* serotype typhimurium DT104 infections linked to raw-milk cheese in Washington State. *Journal of the American Medical Association*, 281 (19): 1811-1816.

Wallace, R.L. (2008). Bacteria counts in Raw milk. Dairy net. Retrieved on 27th November, 2015 from http://livestocktrail.illinois.edu/uploads/dairynet/papers/Bacteria%20Counts%20in%20Raw%20Milk%20DD%202008.pdf

Walsh, C. (2000). Molecular mechanisms that confer antibacterial drug resistance. *Nature*, 406: 775–781.

Walstra P. and Jenness R. (1984). Dairy chemistry and physics. New York: John Wiley & Sons.

Walstra, P., Geurts, T.J., Noomen, A., Jellema, A. and van Boekel, M.A.J.S. (1999). Dairy technology: principles of milk properties and processes. New York: Marcel Dekker, Inc.

Weir, E. (2002). *Shigella*: wash your hands of the whole dirty business. *CMAJ : Canadian Medical Association Journal = Journal De l'Association Medicale Canadienne*, 167(3): 281.

Wisner, A., Desin, T., White, A., Potter, A. and Köster, W. (2012). The *Salmonella* Pathogenicity Island-1 and -2 Encoded Type III Secretion Systems. In Y. Kumar (Ed.), *Salmonella* - A Diversified Superbug, ISBN: 978-953-307-781-9, InTech, Available from: http://www.intechopen.com/books/salmonella-a-diversified-superbug/the-salmonella pathogenicity-island-1- and-2-encoded-type-iii-secretion-systems Retrieved on 20 September, 2016.

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

World Health Organization (WHO). (2003). The diagnosis, treatment and prevention of typhoid fever. Communicable Disease Surveillance and Response Vaccines and Biologicals. Pp1-2. Retrieved from http://www.who.int/rpc/TFGuideWHO.pdf on 1st September, 2016.

World Health Organization (WHO). (2005) Drug-resistant *Salmonella*. Fact sheet 139. Retrieved from http://www.who.int/mediacentre/factsheets/fs139/en/index.html

Wray, C. and Wray, A. (Eds.) (2000). *Salmonella* in Domestic Animals. Oxon, UK: *CAB* International, Wallingford.

Yahuza M. L. (2001). Smallholder Dairy Production and Marketing Constraints in Nigeria. National Livestock Project Division (NLPD) Yakubu Gowon Way, Kaduna, Nigeria. Proceedings of a South – South Workshop held at the National Dairy Development Board (NDDB), Annand, India.

Yan, M., Li, X., Liao, Q., Li, F., Zhang, J. and Kan, B. (2016). The emergence and outbreak of multidrug-resistant typhoid fever in China. *Emerging Microbes & Infections*, 5: 62.

Zadernowska, A. and Chajęcka, W. (2012). Detection of *Salmonella* spp. Presence in Food. In B. S. M. Mahmoud (Ed.), *Salmonella - A Dangerous Foodborne Pathogen* Retrieved on 23 September, 2016 from InTech,http://cdn.intechopen.com/pdfs/26437/InTechDetection_of_salmonella_spp_presence_in_food.pdf

Zagrebneviene, G., Jasulaitiene, V., Morkunas, B., Tarbunas, S. and Ladygaite, J. (2005). *Shigella* sonnei outbreak due to consumption of unpasteurised milk curds in Vilnius, Lithuania, 2004. *EuroSurveillance*, 10(48) retrieved on 10th December, 2015 from http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=2848

Zahraei, T., Mahzoonae, M.R. and Ashrafi, A. (2006). Ampllification of *invA* gene of Salmonella by polymerase chain reaction (PCR) as a specific method for detection of Salmonella. *Journal of the Faculty of Veterinary Medicine University of Tehran*, 6: 195-199

APPENDICES

Appendix I: Media and Reagents

Rappaport-Vassiliadis (RV) Enrichment Broth (Oxoid, UK)

Soya peptone 5.0; Sodium chloride 8.0; Pottasium dihidrogen phosphate 1.6; Magnesium chloride 40.0; Malachite green 0.04.

* Weigh 30g and add 1l of distilled water. Heat gently until completely dissolved. Dispense 10ml volumes into screw capped bottles or tubes and sterilize by autoclaving at 115^{0}C for 15mins.

Nutrient Agar (Oxoid, UK)

Lab-Lemco powder 1.0; Yeast extract 2.0; Peptone 5.0; Sodium chloride 5.0; Agar 15.0.

* Suspend 28g in 1l of distilled water. Bring to the boil to dissolve completely. Sterilize by autoclaving at 121^{0}C for 15mins.

MRVP Medium (Oxoid, UK)

Peptone 7.0; Glucose 5.0; Phosphate buffer 5.0.

* Dissolve 17g in 1l of distilled water. Mix well and distribute into final containers. Sterilize by autoclaving at 121^{0}C for 15mins.

Simons citrate Agar (Guandong, Huakai)

Magnesium sulphate 0.2; Ammonium dihydrogen phosphate 0.2; Sodium ammonium phosphate 0.8; Sodium citrate 2g, Sodium chloride 5g; Bromothymol blue 0.08; Agar 15g; pH 7.0± 0.2. at 25^{0}C

Triple Sugar Iron Agar (Oxoid, UK)

Lab-Lemco powder 3.0; Yeast extract 3.0; Peptone 20.0; Sodium chloride 5.0; Lactose 10.0; Sucrose 10.0; Glucose 1.0; Ferric citrate 0.3; Sodium thiosulphate 0.3; Phenol red 0.024; Agar 12.0; pH 7.4± 0.2 at 25^{0}C.

* Suspend 65g in 1l of distilled water. Bring to the boil. Dissolve completely. Mix well and distribute into containers, sterilize by autoclaving at 121^{0}C for 15mins. Allow to set as slope.

Sulphide Indole Motility Medium (Oxoid, UK)

Tryptone 20.0; Peptone 6.1; Ferrous ammonium sulphate 0.2; Sodium thiosulphate 0.2; Agar 3.5; pH 7.3± 0.2 at 25^{0}C.

* Suspend 30g in 1l of distilled water and boil to dissolve the medium completely. Dispense into final containers and sterilize by autoclaving at 121^{0}C for 15mins.

Mueller Hinton agar (Titan Biotech. India)

Beef infusion from casein 300.0; Acid hydrosylate 17.5; Agar 17.0; Starch 1.5 pH 7.3± 0.2 at 25^{o}C

Kovac's reagent

P-dimethylaminobenzyldehyde – 10g, Isoamyl alcohol (amyl alcohol) – 150ml, Concentrated hydrochloric acid (HCl) – 50ml.

* Add 50ml of concentrated HCl to 150ml of amyl alcohol. Dissolve 10g of P-dimethylaminobenzaldehyde in the solution.

Normal saline 8.5g/l (BDH Chemical, England)

Sodium chloride (NaCl) – 8.5g, Distilled water – 1 litre

* Weigh the NaCl and transfer into a conical flask. Add distilled water to the 1 litre mark. Mix thoroughly until the salt is fully mixed.

Oxidase reagent (KEM light Labs. India)

Tetramethyl-p phenylenediamine dihydrochloride – 0.1g, Distilled water – 10ml

* Dissolve 10g of the reagent in 1l of distilled water. Use fresh.

Appendix II: Biochemical Tests for *Salmonella* and *Shigella* suspects

Milk samples Isolate number	TSI	Sulphur	Indole	Motility	MR	VP	Urease	Citrate
TO6 RVS	K/AG+H_2S	+	-	+	+	-	-	+
GB5 RVS	K/AG+H2S	+	-	+	+	-	-	+
AD4 RVS	A/AG+H2S	+	-	+	-	-	+	+
AN3 RVS	K/A, H2S	+	-	+	+	-	+	+
AN4 RVS	K/AG+H2S	-	-	+	+	-	-	+
SM2 GNB	K/AG	-	-	+	+	-	-	-
SM7 RVS	K/AG+H2S	+	-	-	+	-	-	+

Appendix III: Sugars and Amino acid test for Suspect *Salmonella* isolates with standard reactions

Sugars/Amino acids	TO6 RVS	GB5 RVS	SM2 RVS	Standard (%)
Adonitol	-	-	-	- (0)
Arabinose	+	+	+	+ or (+) (89.2)
Arginine	-	-	-	+ or (+) (58.5)
Dulcitol	-	-	+	d (86.5)
Inositol	-	-	-	d (34.5)
Lactose	-	-	-	- (0.8)
Lysine	-	-	-	+ (94.6)
Maltose	+	+	+	+ (96)
Mannitol	+	+	+	+ (99.7)
Ornithine	-	-	-	+ (92.7)
Raffinose	-	-	+	-(3)
Rhamnose	+	+	+	+ (90.3)
Salicin	-	-	-	- (0)
Sorbitol	+	+	+	+ (94.1)
Trehalose	+	+	+	+ (93.5)

Appendix IV: Microbact TM identification of the biochemically positive isolates

Isolate code	Octal code	Identification	Percentage probability (%)
GB5 RVS	7653	*Salmonella* species	59.41
SM2 GNB	7652	*Salmonella* species	59.41
TO6 RVS	7642	*Salmonella arizanae*	64.55
AD4 GNB	2073	*Providencia stuarti*	96.37
AN4 RVS	5653	*Citrobacter freundi*	92.82
AN3 RVS	6072	*Serratia marcescens*	65.81
SM7 GNB	2432	*Morganella morgani*	76.94

Appendix V: Antibiotic test for the isolates

Antimicrobial agent	SM2 GNB	GB5 RVS	TO6 RVS
Amikacin	R	R	R
Amoxicillin-Clavulanate	R	R	R
Cefotaxime	R	R	R
Cefotetan	20	20	15
Chloramphenicol	22	21	R
Ciprofloxacin	25	27	30
Erythromycin	R	R	R
Nitrofurantoin	15	18	R
Streptomycin	13	10	R
Tetracycline	11	11	R
Trimethoprim-Sulphamethoxazole	22	27	23

Appendix VI: Zone Diameter Interpretative Standards for *Enterobacteriaceae*

		Zone Diameter (Nearest whole mm)			
Antimicrobial agent	Disc content (µg)	S (mm)	SDD (mm)	I (mm)	R (mm)
Amikacin	30	≥ 17	-	15-16	≤ 14
Amoxillin/Clavulanate	20/10	≥ 18	-	14-17	≤ 13
Cefotaxime	30	≥ 26	-	23-25	≤ 22
Cefotetan	30	≥ 16	-	13-15	≤ 12
Chloramphenicol	30	≥ 18	-	13-17	≤ 12
Ciprofloxacin	5	≥ 31	-	21-30	≤ 20
Erythromycin	15	≥ 23	-	14-22	≤ 13
Nitrofurantoin	300	≥ 17	-	15-16	≤ 14
Streptomycin	10	≥ 15	-	12-14	≤ 11
Sulfamethoxazole/Trimethoprim	23.75/1.25	≥ 16	-	11-15	≤ 10
Tetracycline	30	≥ 15	-	12-14	≤ 11