USMANU DANFODIYO UNIVERSITY, SOKOTO

(POSTGRADUATE SCHOOL)

EFFECTS OF MILKING PRACTICES ON BACTERIOLOGICAL QUALITY OF RAW MILK FROM SELECTED DAIRY FARMS WITHIN SOKOTO METROPOLIS

A DISSERTATION

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MASTER OF SCIENCE (ANIMAL PRODUCTION)

BY

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DEDICATION

This work is dedicated to Almighty God, My Sustainer, My Rock, My Enabler and the Lifter of my head for keeping, guiding and providing for me thus far, Naraekele o and to my loving Husband (Engr. Emeka Akobundu) and my family, for their endless support through the years, God bless you all.

CERTIFICATION

This dissertation by EZIRIM, Oluchi Chizaram (Adm. No: 16/210617002) has met the requirements for the award of the degree of Master of Science (Animal Production) of the Usmanu Danfodiyo University, Sokoto, and is approved for its contribution to knowledge.

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LIST OF ABBREVIATIONS

AI	-	Artificial Insemination
CBN	-	Central Bank of Nigeria
CDC	-	Center for Disease Control and Prevention
CFU	-	Colony Forming Unit
EAS	-	East Africa Community Standards
EU	-	European Union
FAO	-	Food and Agriculture Organization
IDF	-	International Dairy Federation
SCC	-	Somatic Cell Count
UHT	-	Ultrahigh Temperature Treatment
USDA	-	United States Development of Agriculture

ABSTRACT

This study was design to determine the effects of milking practices on the bacteriological quality of raw milk from selected dairy farms within Sokoto metropolis. Five (5) dairy farms were selected, 100 raw milk samples were collected from the milking bucket before and after implementing milking practices. The CFU count before and after implementing milking practices ranged from 2.7 - $6.1x10^8$ cfu/mL and 1.4 - $4.5x10^8$ cfu/mL, there was significant ($P<0.05$) reduction in the CFU count. Distribution on bacterial pathogens were, *Escherichia coli* (22%), *Staphylococcus aureus* (23%), *Salmonella spp* (21%), other members of *Enterobacteriaceae* family (21%) and *Coagulase Negative Staphylococcus* (CONS) (12%). Number of isolates before and after implementing milking practices were 24 and 8 (Farm A), 22 and 7 (Farm B), 27 and 7 (Farm C), 24 and 7 (Farm D) and 24 and 5 (Farm E) respectively. Questionnaire survey on milking practices Farm A and B scored (20%) while C, D, E scored (0%). On hygienic practices Farm A and B was (60%), C and D was (20%) while Farm E was (40%). Finally, the economic cost for implementing milking practices was N176 per milking per cow. It's recommended to implement milking practices for production of quality milk.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the Study

Milk is produced and secreted by specialized skin gland known as mammary gland. Mammary gland is composed of alveolar tissue, with the alveoli lined by milk-secreting epithelium. It provides the primary source of nutrition for young mammals before they are able to digest other types of food and also a source of proteins to man (Olatunji *et al*., 2012). Raw milk is composed up of approximately 87% water while the remaining part comprises of total solids (carbohydrates, fat, proteins and minerals) contained in a balanced form and digestible elements for building and maintaining the human and animal body. Other milk components include immunoglobulins which protect the newly born against a number of diseases (Pandey and Voskuil, 2011). Milk has a complex biochemical composition and its high water activity and nutritional value serve as an excellent medium for growth and multiplication of many kinds of microorganisms when suitable conditions exists.

Microbial contamination in milk may result in milk-borne diseases to humans, while others are known to cause milk spoilage. Sources of microbial contamination in milk include primary microbial contamination from the infected or sick lactating animal. The secondary causes of microbial contamination occurs along the milk value chain which may include contamination during milking by milkers, milk handlers, unsanitary utensils and/or milking equipment's and water supplies used in sanitary activities (Pandey *et al*., 2014). Other secondary sources of microbial contamination occur during milk handling, transportation and storage. There is tertiary microbial contamination

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which occurs mainly due to re-contamination of milk after being processed due to unhygienic conditions and/or poor or improper handling (Pandey *et al*., 2014).

Nigeria is a potential market for 1.3 million tons of milk valued at about N450 billion annually (CBN, 2010). Of the estimated total domestic fluid milk production in 2006 for example, only about 600 000 litres (worth about N232.5 million) passed through the formal marketing channels via corporate commercial dairy farms and other private milk collection co-operatives schemes, or public channels from migrant herdsmen. The rest was either consumed by the producing families or traded informally within the producing communities. Imported milk powder and other processed dairy products were estimated at $275 million in 2006. Industry sources also estimate Nigeria's national herd at 14 million heads (including approximately 900 000 milking cows) (CBN, 2010).

Average yield from the traditional system is 0.7 – 1.5 litres of fluid milk per day. During the dry season, this figure drops to about 0.5 litres /day, but with supplementation using cotton seed cake, could increase to go up to between 1.5 to 2.0 litres/day (Annatte 2010). A further increase of up to 4 litres/day have been reported with improved management and appropriate selection. The "White Fulani" or "Bunadji" breeds are the dominant dairy breed. The pastoralists own and maintain the majority of the cattle which are fed on natural grass under the traditional system with little supplementary feeding regimes in some places. Migrant pastorals move flocks over months and many miles to find pasture during the dry season, and this system often results in weight losses, low yields and high morbidity rates. A few commercial livestock farms maintain crossbreeds of Holstein Friesians, Brown Swiss and Monbeliard for milk production and reported average yield varies from 8 to 18 litres /day compared to only 4 litres/day from local breeds. Foundation stocks for the commercial farms are mostly imported

from South Africa, Europe, Australia, and average yield of pure breeds under commercial management conditions could be as high as 30 litres/day. (FAO, 2010).The livestock resource survey of the Federal Department of Livestock and Pest Control Services in 1990 puts the cattle population of Nigeria at 13.9 million (RIM, 1992) of these, 13.5 million (96%) are in the hands of the pastoral fulani. This pastoral herd is the most important source of domestic milk in Nigeria, some imported cattle breed such as Friesians and Brown Swiss and their crosses are being kept for milk production in farms owned by government agencies. Some privately owned commercially oriented dairy farms owned by companies and individuals are known to exist. Some of these commercially owned dairy farms and pastoral fulani herds use unsanitary methods of milking, they use bare hands and unsterilized containers for milking. Forestripping, pre-dipping and post dipping are not carried out (Okeke *et al*., 2014). This lapses in hygienic milking practices result in poor quality milk production and milk contamination which pose health hazards to the populace, especially among urban dwellers who drink fresh milk. This poor milking practices have been reported by Shittu *et al*. (2008) in Sokoto who reported that 68.33% of the dairy farms surveyed lack facilities for milking and 25.42% lack knowledge on proper milking practices. Also a study on dairy production among small and medium scale farmer in Kano and Kaduna, states the unhygienic and poor milking practices implemented in these states (Daniel, 2010).

According to Food and Agriculture organization (FAO, 2011), recommended milking practices for maximum milk quality are:

Proper hand-washing or use of disposable hand-gloves: It is well established fact that proper hand washing and teat end disinfection can reduce teat surface bacteria by 75% (Rasmussen and Frimer, 2000). Contagious mastitis-causing bacteria such as *Staphylococcus aureus,* may live on the hands of milkers and be transmitted between

cows during milking. At minimum, hands should be thoroughly washed with soap and water before and after each milking. Gloves are also easier to disinfect than bare hands and help protect the milkers skin (Pamela *et al*., 2000).

Forestripping involves manually removing few streams (or strips) of milk from each teat. This process allows the milker to examine the milk for any signs of mastitis, including clots, string, or watery milk (Reneau, 2007). Also, forestripping helps to stimulate the teats and udder and encourage milk letdown. Milk may also be stripped onto the floor and washed from the floor immediately. Milk should not be stripped into the hand or towel because this would encourage the spread of mastitis between teats and between cows (FAO, 2011).

Studies have shown that pre-dipping is the most effective way in the control of environmental bacteria such as *Escherichia coli* and *Staphylococcus aureus*. (Pamela *et al*., 2000; Susan, 2017). Teats should be pre-dipped with disinfectant, such as chlorhexidine or iodine solution. Pre-dipping eliminates bacteria on teat ends prior to milking, thereby improving the quality and helps to control mastitis caused by environmental mastitis pathogens. The pre-dip should remain on the teats for at least 30 seconds before drying.

A study carried out in Wisconsin showed a significant reduction in bacterial load when teats were properly dried using cloth towels or paper. The teats should be thoroughly dried with a single serviette, absorbent cloth or paper towel and a towel should not be used on two cows. All debris, manure, and pre-dip residue on the teats should be removed while drying by using a gentle, twisting motion (Lore *et al*., 2006). During the drying process, particular emphasis should be placed on getting the teat ends clean and dry.

As soon as possible after milking, teats should be dipped with a post-dip, an effective germicide e.g. 1% iodine solution. As with pre-dipping, the goal should be to cover at least three quarters of the teat. An effective post-dip kills organisms on teats, prevents microorganisms from colonizing in the teat canal and reduces the rate of new infections from contagious mastitis pathogens (Lore *et al*., 2006; FAO, 2011).

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Milking process actually begins as cows enter the milking area or parlor. Time in the holding pen should be minimized to less than a total of two hours per day. Cows should be brought into the parlor calmly and gently. In addition, cows that are stressed are more likely to slip, fall, or defecate while entering the parlor (Ruegg and Dohoo, 1997; Galton *et al*., 1998)

1.2 Research Problem

It has been reported that milking takes place under unsanitary conditions with poor milking practices and this is true in most developing countries like Nigeria (Yahaya *et al*., 2012). The microbial load of milk is a major factor in determining its quality and it indicates the hygienic level exercised during milking (Tassew and Seifu, 2011). In addition poor milking practices leads to contamination of the milk with several pathogens such as *Staphylococcus spp*, *E. coli*, *Salmonella spp and Enterobacter spp* (Okpalugo *et al*., 2008; Yahaya *et al*., 2012) which can be hazardous to the populace in the study area.

1.3 Justification

A livestock survey showed that Sokoto state has a livestock population of about 1.18 million cattle, 2.90 million goats, 1.98 million sheep, 2.0 million chickens, 45,000 camels, 34,532 horses and 51,388 donkeys (FDLPCS, 2002). Another livestock survey showed the breeds of cattle present in Sokoto state were the indigenous Sokoto Gudali

(69.07%), White Fulani (18.32%), and others which included Rahaji, Buzuwa, Red Sokoto, Jalli and Holstein/Friesian (12.61%), with Sokoto Gudali constituting about 69% of the total cattle population (Shittu *et al*., 2008), this places Sokoto state second to Borno state in milk production. Due to poor production of milk leading the bacterial contamination of the milk, the purchasing power is low thereby reducing the gross income rate within the state and the country at large. (Shittu *et al*., 2008)

Milk can be contaminated directly from dirty udder, unhygienic milking process, poor milk handling and processing, this makes it important to implement good milking practice and assess its effect on the quality and production of milk (De Silva *et al*., 2015). Also, there is no published information on milking practices or the effect of implementing milking practices on quality of the milk within the study area.

In addition, the findings from this study will assist the dairy farm owners and milkers to have a better understanding of milking practices and how to carry out this practices efficiently to improve quality of the milk they produce.

1.4 Aim and Objectives

1.4.1 Aim

The aim of this study is to determine the effect of milking practices on quality of milk collected from selected dairy farms within Sokoto metropolis.

1.4.2 Objectives

1. To assess the knowledge of dairy farm owners and workers on milking and hygienic practices.
2. To determine how milking practices affect the level of bacteriological contamination of raw milk.

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Are you looking at the just bacterial load of the milk?
Are you looking at how each milking practice affect the bacterial load or type in the milk?
etc

3. To determine the common pathogenic bacterial microorganism in the raw cow miSlk using phenotypic and biochemical techniques.

1.5 Research Question

1. What is the knowledge level of dairy farm owners and workers on milking and hygienic practices?
2. Is there any difference in the bacteriological quality of milk before and after implementing milking practices?
3. What are the common bacterial pathogenic microorganisms present in the milk?

1.6 Limitation

Some dairy farms within the study area did not allow us to implement the milking practices in their farms.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Overview on Milk Quality

Milk is a yellowish-white non-transparent liquid secreted by the mammary glands of all mammals. It is the primary source of nutrition and sole food for offspring of mammals before they are able to eat and digest other types of food. It contains in a balanced form of all the necessary and digestible elements for building and maintaining the human and animal body (Pandey and Voskuil, 2011). The main composition of milk is water (87-88%); the remaining part is total milk solids which constitute 3.5% fats, 0.6% salts, 4.8% sugars, and 3.1% protein. This composition is not constant, the average percentages of milk components vary with species and breeds of animal, season, feed and stage of lactation and physiological status of a particular animal (Pandey and Voskuil, 2011). Sometimes the composition might even change from day to day, depending on feeding and climate, but also during milking the first milk differs from the last milk drops (Pandey and Voskuil, 2011). Nevertheless, milk is an excellent source of high quality protein, vitamins, minerals such as calcium and phosphorus. Fresh milk has a pleasant soft and sweet taste and carries hardly any smell.

Milk is a major source of dietary energy, protein and fat, contributing on average 134 kcal of energy/capita per day, 8 g of protein/capita per day and 7.3 g of fat/capita per day in 2009 (FAOSTAT, 2012). However, when different geographic regions are considered, the contribution from milk to the various nutritional components varies considerably. Milk provides only 3% of dietary energy supply in Asia and Africa compared with 8–9% in Europe and Oceania; 6–7% of dietary protein supply in Asia

and Africa compared with 19% in Europe; and 6–8% of dietary fat supply in Asia and Africa, compared with 11–14% in Europe, Oceania and America (Ndambi *et al*., 2008).

2.2 Milk Nutritive Value and Composition

Milk may be defined as a secretion from female mammary gland used for the feeding of her young, and has been described as close to being nature's perfect food (Nickerson, 1999). The substances in milk have been reported to provide both energy and materials necessary for growth and maintenance of health. Bovine milk is commonly consumed by majority throughout the world, however, in some regions goat's milk or sheep milk may be more commonly used (Dogan *et al*., 2002). Fresh milk is neutral or slightly alkaline but on souring becomes acid because of the lactic acid formed by bacterial action on lactose. It has a water content of 87- 88% and 12% of solids which constitute 3.5% fats, 0.6% salts, 4.8% sugars, and 3.1% protein (Stewart, 1978). It has a wide range of positive nutritional benefits and supplies a variety of nutrients including protein for body building, vitamins, minerals (especially calcium), fat and carbohydrate for energy (Medhammar, 2001).

2.2.1 Milk Fat

Milk fat being an animal fat is characterized as being saturated fat, however about 32% of milk's fatty acids are unsaturated, primarily as mono-unsaturated acids like oleic acid. Milk supplies the essential fatty acids linoleic acid (2.1%), lanoleic (0.5%) and arachidonic acid (0.14%) (Lee and Gerrior, 2002). These are required by the human body for normal metabolism and growth. Short (C2 to C6) and medium chain (C8 to C12) fatty acids account for about 12% of the fatty acids of milk and being more readily digested. They do not contribute to the elevation of blood lipids nor are they deposited in adipose tissue (Lee and Gerrior, 2002).

2.2.2 Milk Protein

Proteins are valuable component of milk in terms of their importance in animal nutrition and their influence on the properties of dairy products containing them. The concentration of protein in milk varies from 3.0 to 4.0% (30-40 grams per liter) (Fox, 1995). The percentage varies with the breed of the cow and in proportion to the amount of fat in the milk. There is a close relationship between the amount of fat and the amount of protein in milk—the higher the fat, the higher the protein level.

The protein falls into two major groups: caseins (80%) and whey proteins (20%). Casein is recognized as the micelle framework which comprises a network of alpha casein complex with calcium phosphate. It is the most commonly used milk protein in the food industry and contains 21 amino acids. Acid casein, a granular milk protein, is available in two types - edible and technical. Edible acid casein is highly nutritional, low in fat and cholesterol, and flavorful making it ideal for medical and nutritional applications (Fox, 1995).

When casein is removed from skim milk using precipitation method, the protein remains in the liquid solution and is called whey proteins or milk-serum proteins. It accounts for only about 20% of the total protein found in milk, while casein makes up about 80% of milk protein. Whey proteins are now well known for their high nutritional value and versatile functional properties in food products (De Wit, 1998; Harold, 2004).

2.2.3 Minerals

Many trace elements essential for health and growth, are present in milk. Sodium, calcium, potassium and phosphorus account for about 4%. Some of the trace minerals are, zinc, cobalt, iodine and iron. (Stewart, 1978; Gaucheron, 2005). Minerals in milk provide constancy of osmotic pressure. This property can prevent the depression of freezing point temperature. The amount of minerals in milk can provide the

recommended daily allowance for calcium and 75% for phosphorus (Renner *et al*., 1989). These minerals are widely recognized as important factor for bone development and growth of children (Okolo *et al*., 2000).

2.2.4 Vitamins

Vitamins are complex organic substances that are needed in very small amounts for many of the processes carried out in the body. Usually only a few milligrams (mg) or micrograms (μg) are needed per day, but these amounts are essential for health. Milk is a source of 12 water-soluble vitamins and four fat-soluble vitamins (Harding, 1995).

Vitamins in milk are readily affected by processing. Some of them are heat-sensitive. Storage conditions also affect vitamins, exposure to light or oxygen can cause loss of some vitamins (Varnam and Sutherland, 1994). Both storage and processing condition must be considered to prevent loss of vitamins in the products.

2.2.5 Water

The nutritional value of milk as a whole is greater than the value of its individual nutrients because of its unique nutritional balance. The amount of water in milk reflects that balance. In all animals, water is the nutrient required in the greatest amount and milk does supply a great amount of water, it contains approximately 90% water (Stewart, 1978).

The amount of water in milk is regulated by the amount of lactose synthesized by the secretory cells of the mammary gland. The water that goes into the milk is delivered to production is very rapidly affected by a shortage of water and drops the same day drinking water is limited or unavailable (Burlingame, 2012). This is one reason why the cow should have free access to a plentiful supply of drinking water at all times.

2.2.6 Carbohydrates (Lactose)

The principal carbohydrate in milk is lactose. Although it is a sugar, lactose is not noticeably sweet to taste. The concentration of lactose in the milk is relatively constant and averages about 5% (4.8-5.2%) (Fox, 1995). As opposed to the concentration of fat in milk, lactose concentration is similar in all dairy breeds and cannot be altered easily by feeding practices. The molecules from which lactose is made are found in much lower concentrations in milk: glucose (14 mg/100g) and galactose (12 mg/100g). In a significant portion of the human population, the deficiency of the enzyme lactase in the digestive tract results in the inability to digest lactose (Stewart, 1978; Burlingame, 2012). Most individuals with low lactase activity develop symptoms of intolerance to large doses of lactose, but the majority can consume moderate amounts of milk without discomfort. Not all dairy products contain similar proportions of lactose. The fermentation of lactose during processing lowers its concentration in many dairy products, especially in yogurts and cheeses. In addition, milk pre-treated with lactase, which minimizes the problems associated with lactose intolerance, is now available.

2.3 Milk Production in Africa

In 2013, total cow milk production in Africa was about 39 million tons produced from a total of 46 million dairy cows giving an average milk yield of 461 kg milk per cow over the year, which is only one fifth of world average yield (Ndambi *et al*., 2007).

The top five African milk producing countries in terms of milk volume are Sudan, Egypt, Kenya, South Africa and Algeria. Meanwhile, the first four countries alone produce 52% of total African milk (Ndambi *et al*., 2007) (Plate 2.1).

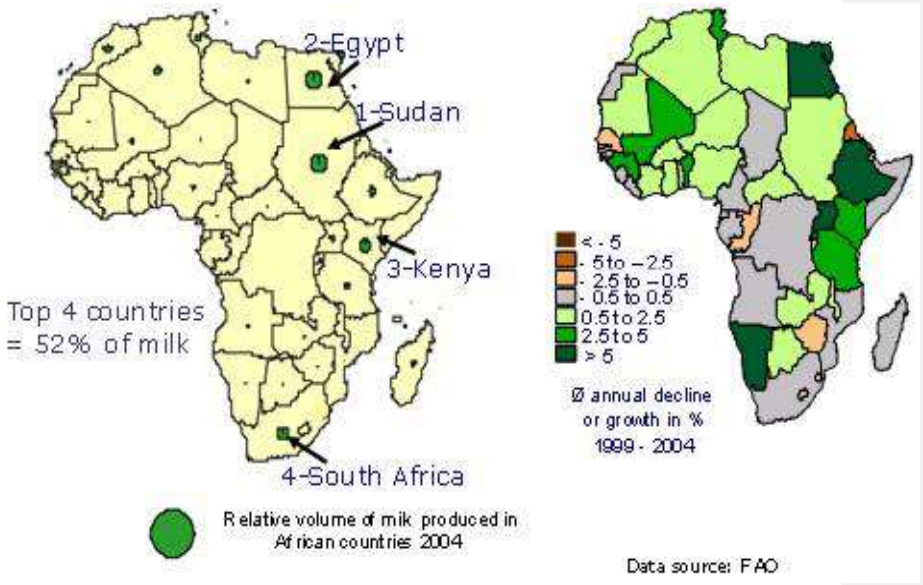

Plate 2.1: Distribution of Milk production in Africa

The milk production in Africa accounts only for 5% of the world milk production and it is not foreseen to be able to cover the demand in the coming decades (Plate 2.1) The milk production has doubled from 1996 to 2013 reaching 40 million milk/ton and in volume terms the major growth has been in the countries in North Africa and in Kenya and South Africa (Ndambi *et al*., 2007). The other countries in Sub-Sahara Africa have all experienced relatively high growth but from an extremely low level. There is therefore a need to increase the total milk production within Africa countries especially Nigeria with the increasing population.

2.3.1 Dairy Systems in Africa

Traditional systems have dominated milk production in Africa for several years and still supply considerable amounts of milk today and also account for above 90% of dairy production in Sub-saharan Africa. Indigenous groups like the Maasai, Borani, Fulani and Tuareg have a strong historic dairy tradition. They share many customs and regard milk as a product of harmony that is offered free to relatives, friends and visitors (Bayé, 2000, Sadou, 2000, Suttie, 2001). Due to population growth, land shortage and increasing interest in production and consumption, market-oriented dairy systems are now evolving, with the use of high performing graded animals (exotic breed and crosses) and/or higher inputs (Ndambi *et al*., 2007).

2.3.1.1 Pastoral systems

Pastoral systems are migratory, transhumant or sedentary. Sedentary farmers live in the same homes all year round while migratory and transhumance farmers moves. One of the oldest pastoral systems is practiced by the Maasai in the sparsely populated semi-arid range-lands of Kenya and Tanzania (Ndambi *et al*., 2008). The Maasai migratory farmers who live in extended families of 10-15 people with herds averaging 100-170

cattle and as many sheep and goats also produce and consume about 0.85 kg of milk per person per day; meanwhile, a greater share of the income comes from sales of livestock, only a few of them grow crops while the majority purchases most of their foodstuff. In this system, milk surplus is shared with neighbors or exchanged in barter, but is rarely sold except by households living close (<5 km) to main roads and urban centers where there is demand for fresh and fermented milk, and butter. The Borana pastoral system is similar to that of the Maasai; here the frequency and amounts of dairy products traded depend on herd size and distance to the market. Milk sales in this system is however of higher interest than is the case with the Maasai (De Leeuw *et al*., 1998; Ndambi *et al*., 2007).

According to Tonah (2002), dairy production by the Fulani in Ghana is characterized by migratory pattern, which is changing over time. Similar to the Maasai, Fulani have a uniqueness which stems from the fact that they are culturally the least known to the indigenous population and share very few practices with the host population (Ndambi *et al*., 2008). Fulani settlements are typically located at the outskirts of the settlement and consist of several concentric huts arranged to form a single housing unit. In a study on Fulani Agro-pastoralists in Central Nigeria, Waters-Bayer (1988) found that dairy production units had household sizes of 7 - 13 persons with almost equal males and females and 45% of members above 18 years.

Contrary to the situation of the Fulani in Ghana, milking by Nigerian Fulani is only done once daily by boys and men and exceptionally by women. The Fulani here have a more sedentary pattern of life. The herd size is usually between 40 - 60 cattle with majority of families keeping sheep and all keeping poultry (Ndambi *et al*., 2008). During dry periods, they graze their animals further away from their homes as

compared to the rainy seasons when sufficient pasture can be gotten closer to their homes. In the dry season, arrangements are made with local farmers for stubble grazing and manuring. During such periods, a woman or one of her children have to spend up to an hour on the way to such farms, taking along cooked food for the herders and returning with milk for the household (De Leeuw *et al*., 1998). In other cases, part or all of the family moves with the herd and only return home when conditions are favourable. Whenever part of the family remains home, a few cows with younger calves are left behind, to supply milk to the household and to prevent the fragile calves from dying during the stressful transhumance period. Another pastoral system is that where herders are pastoralists who act as managers of communal herds with cattle, which are entrusted to them by local farmers who each own a few heads (De Leeuw *et al*., 1998; Ndambi *et al*., 2008).

In an improved system, the agro-pastoral system arises from the pastoral system, whereby cattle owners also cultivate crops in order to diversify production and reduce risks. The farmers here are sedentary, unlike the pastoralists who are mobile. They also graze their animals on communal grazing land, feed crop residues and also feed more supplements to their cattle than pastoralists ((De Leeuw *et al*., 1998).

2.3.1.2 Semi- intensive systems

This system is common in peri-urban zones, having farms which are owned by business men, civil servants and private individuals who employ labour in the catering of their animals, with milk production as their major objective (Diop and Mazouz, 1995). Dairying is done with some degree of intensification by a combination of grazing and concentrate-feeding. Here, there is use of graded cows or crossbreeding, usually between exotic bulls and local cows or through artificial insemination (AI). The aim of

crossbreeding is to upgrade for better milk production and at the same time retaining the adaptability of the animals in changing environmental conditions (Bayemi *et al*., 2005). In such farms where management is moderate, it is important for the animals to have a natural resistance to environmental stress. Milk production here is much higher than in pastoral systems, though still less than in graded cows.

2.3.1.3 Intensive systems

Market infrastructure increases the importance of the dairy component in smallholder dairying. Increasing population growth and urbanization have led to the intensification of dairy systems around urban areas in Africa which is also favoured by a higher demand in such areas. The farms here are small (about 1-2 ha with 1-2 cows generally Holstein Friesian or any other exotic breed). Feeding is mainly cut-and-carry with planted Napier grass *(Pennisetum purpureum)* and crop residues, especially from maize and bananas (Ndambi *et al*., 2007). Most work on the majority of such dairy farms is done by the family. Contrary to pastoral systems where large proportions and sometimes all the daily milk is consumed at home, only a small portion of milk produced in this system is left for home consumption and the rest sold (De Leeuw *et al*., 1998). Larger intensive farms are usually owned by rich individuals, cooperatives or the Government. More investments are also made on buildings and machinery while the use of hired labour is unavoidable. These systems concentrate on the supply of milk in large towns and in most cases have one or more guaranteed delivery sources. There is a higher market orientation in this systems and more emphasis is laid on feeding and breeding management to assure optimal production (Diop and Mazouz 1995). In both intensive and semi-intensive systems Artificial Insemination (AI) plays a major role in breeding, as it is cheaper and less cumbersome than maintaining an exotic bull. Unfortunately, breeding programs are poorly structured in some countries, leading to

ineffectiveness in insemination. Farmers usually complain of poor heat detection and low success rates, leading to long inter calving periods and hence low productivity of animals.

2.4 Milk Production in Nigeria

Milk the most nutritious food known to man is important in the diet and culture of Fulani's in Nigeria, it can be consumed fresh, boiled, or curdled, milk by the Fulani and the rural population. Milk production in Nigeria will not be overemphasized without mentioning the Fulani women monopolize the local dairy production in Nigeria, although they own only a few of the family's cattle (Adholla-Migot and Little, 1998), these women whose liking for milk ranges from mild to excessive, sell milk and cooked millet balls called *Fura* in most parts of the country.

The Fulani's use unsanitary methods of milking, they use bare hands and unsterilized containers for processing. The cow's udder or teat is not properly cleaned before milking and flies can be seen jumping into the milk calabash, often also sick cows are been milked. The lapses in good milking practices result in milk-borne diseases, especially among urban residents who drink fresh milk from the rural areas (Ndambi *et al*., 2007).

Rural inhabitants who do not have refrigerators ferment their milk. More than seventy percent of the milk is converted into sour milk; thirteen percent is drunk fresh; and seven percent is used to make ghee, cheese, and butter. Fresh, liquid milk can only be used by urban residents who use refrigerators. Milk producers cannot sell fresh, wholesome milk except by request (Mallau-Aduli, *et al*., 2009).

Local production of dairy supply in Nigeria is far below the annual demand which was estimated at 1.45 billion litres by 2010 (FAO, 2010), making milk consumption among

Nigerians to be less than 10 litres per head, whereas the world standard was put at 40 litres per head. FAO (2010), reported that dairy industry in Nigerian produces an estimate 450, 000 tons of milk per annum. This production has been found to be inadequate to satisfy the dairy demands of Nigerians (FAO, 2010). Among the problems identified in milk production in Nigeria are:

1. Low milk output of local breeds.
2. Poor grass quality that leads to low milk yield
3. Lack of storage and processing equipment for milk.
4. Unsanitary methods of milking practices and milk handling.
5. Breakdown of processing plants, and inefficient milk collection also impede the performance of the milk industries in Nigeria.

Competition between itinerant milk collectors and official milk collectors, faulty pricing and management policies, and lack of economic incentives from the Government hamper the expansion of Nigeria's dairy industry.

The local cow genotype (*Bos indicus*) can only produce an average milk of 1.27 liters per cow per day during the wet season and less than 0.36 during dry season (Abubakar, 2011), whereas their counterparts in the European and American countries produce an average of 25 litres per day (Mallau-Aduli, *et al*., 2009). High calf mortality (20 - 25%) and long calving interval (20 - 26 months), slow maturation, and low productivity of the local breed of Nigeria's cattle plays a major factor in milk production in Nigeria, consequently, protein deficiencies become a common phenomenon in Nigeria, especially among the poor segment of the society.

2.5 Mammary Gland

2.5.1 Structure and Function of Mammary gland

Mammary gland (udder) is located at and in between hind limbs in inguinal region supported by various ligaments and connective tissues. The mammary gland or udder of the cow should be capacious, leveled and strongly attached with tortuous milk vein (Stewart, 1978). The texture should be soft, pliable and elastic and collapsible after milking. It can be divided into two halves separated by inter mammary groove (Plate 2.2). The half is again divided into two separate quarters by thin membranes. The front portion is called the fore udder and the rear portion is called the rear udder. The four quarters are independent with no communication between them (Plate 2.2). The two rear quarters are larger and produce about 60 percent of the milk whereas the fore quarters produce about 40 percent of the milk. The size, shape and placement of the udder is done by the median and lateral suspensory ligaments (Reece, 2009)

Each quarter of the udder is composed of the secretory tissue and the connective tissues. The alveolus is a microscopic structure almost spherical in shape lined by single layer of epithelial cells or the milk secretory cells. The alveoli are surrounded by a capillary network which provides nutrients. The myo-epithelial cells surrounding the alveolus contract during milking causing milk let down (Akers, 2008). The alveolus is grouped together into lobules, a group of lobules form a lobe. The secretory tissue contains the terminal ducts, intralobular ducts unite to form interlobular ducts, there ducts communicate into gland cistern and teat cisternae. The teat cisterna is joined with streak canal. It is surrounded by teat sphincter responsible for preventing the entry of the pathogens onto teat.

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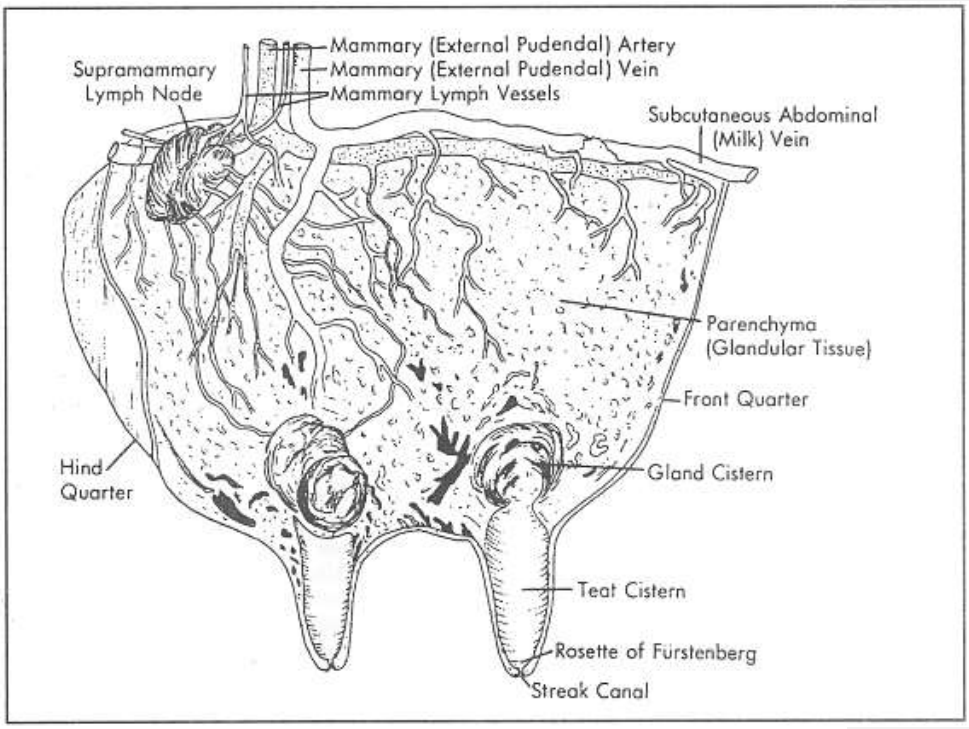

Plate 2.2: Anatomical Structure of the Mammary Gland

Source: https://vetstudentresearch.blogspot.com

2.5.2 Milk letdown

During the milk secretion the alveoli, ducts and gland and teat cisterns are gradually filled with milk. Milk in the cisterns and larger ducts can be removed readily, but the milk in the smaller ducts and alveoli does not flow out easily (Plate 2.3). Milk ejection is an involuntary act on the part of cow. It is a neuro-hormonal reflex that is very important if maximum milk production is to be obtained (Reece, 2009; Ferenc, 2011). Stimulation of the central nervous system by something associated with the milking process is necessary to initiate the reaction. Stimulation of nerve endings in the teats that are sensitive to touch, pressure, or warmth is the usual mechanism (Stewart, 1978; Smith, 2005). Milk ejection is initiated by a stimulus such as washing of udder, manipulating the teats, suckling of a calf. The sucking action of the calf is ideal for this milk let down. Stimulation is carried by the nerves to the brain which is connected with the pituitary gland located at its base (Michael, 2016). The brain causes the release of hormone Oxytocin from posterior pituitary gland into blood system, which carries into mammary gland. Oxytocin acts on the myo-epithelial cells that surround the alveoli and ducts and contracts them. This creates pressure forcing the milk out of the alveoli and smaller ducts as fast as it can be removed through the teat resulting in milk ejection (Stewart, 1978; Senger, 2004) (Plate 2.3). The process occurs in about 45 to 60 seconds after stimulation. The maximum effect starts only 7-8 minutes. Prompt initiation of milking and rapid milking are important in obtaining maximum milk yield (Akers, 2008).

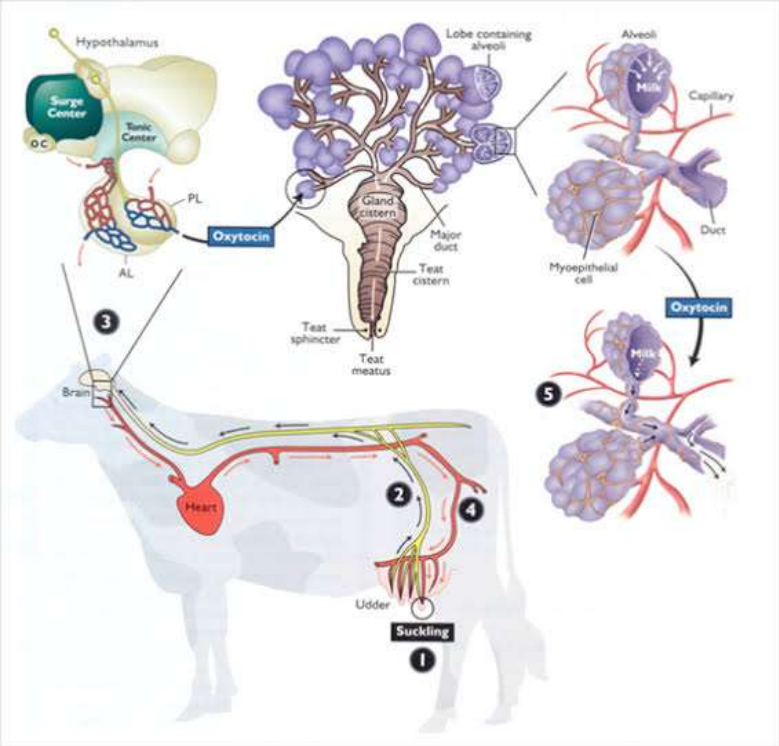

Plate 2.3: Physiology of Milk letdown

Source: Senger, 2003

2.6 Milking

Milking (lactation) is the act of removing milk from the mammary glands of cattle, water buffalo, goats, sheep and more rarely camels, horses and donkeys. Milking may be done by hand or by machine, and requires the animal to be currently or recently pregnant. Lactation includes both milk secretion and storage in alveolar cells and ducts within the mammary gland, followed by milk ejection (let-down) and milk removal (Akers, 2008). Milk secretion is continuous and usually at a constant rate for at least 12 hours resulting in a gradual increase in internal udder pressure (Smith, 2005; Michael, 2016). Milk ejection is a neuro-hormonal reflex initiated by various stimuli at milking time. These stimuli, which reflect good husbandry practices, are either natural (inborn) or conditioned (learned by experience), including, for example, presence of calf, suckling feeding and udder preparation. They cause the alveoli and small milk ducts to contract forcing milk towards the udder sinus. Once this has happened most, but not all, of the milk can be removed when external forces such as suckling or milking open the streak canal (teat duct) at the teat end, but at least 10% will be retained in the udder as residual milk (Akers, 2008).

2.6.1 Milking intervals

The 10 - 20% of the secreted milk which is not expressed from the secretory tissue and is retained in the udder when milking is completed is called ‘residual milk’ and has a much higher fat content than even the end-of-milking stripping. The quantity of residual milk is proportional to total yield, so that with unequal milking intervals there is a larger net carryover of milk fat from the longer night-time to the shorter daytime interval (Akers, 2008). This accounts for the apparent faster secretion rate and higher fat content of afternoon milk production. Milk yields, particularly from higher yielding cows are usually greater when milking intervals are 12 hourly. The effect of uneven intervals is

not large up to 16 and 8 hours, and can be minimized by milking the higher yielders first in the morning and last in the afternoon (Akers, 2008)

2.6.2 Milking methods

Methods of milking influence the quality and quantity of milk produced at the dairy farm. Proper method of milking results in the removal of entire milk present in the udder resulting in optimal milk production in a particular lactation besides avoiding injuries to the teat and udder and improving the udder health (FAO, 2008, FAO, 2011). Disturbances like noises and movement of personnel during milking are to be avoided to cause least disturbance to the milking cows and avoiding with holding of milk at some times. There are basically two types of milking:

- Hand milking
- Machine milking

2.6.2.1 Hand milking

Hand milking should always be done using clean gloved hands. Rear quarters should be milked first as they contain most milk and the milking bucket hooded to reduce contamination from dust and udder hairs (FAO, 2008, Smith, 2005). Cows are milked from left side Teats may be milked diagonally or forequarters together and then hind quarters together. The milk must be squeezed and not dragged out of teats in order to prevent injury to teats. Milking practices such as forestripping, predipping and post dipping should be adhered to in other to reduce the contamination of milk. Hand milking equipment's consist of the following:

- Milking bucket ideally stainless steel pail (Rubber bucket was used by farms within the study area)

- Storage /transport can
- Filters (cloth filters)
- Strip cup with black screen for checking foremilk
- Teat wipes/ cloth towel
- Teat dip (e.g 5% Chlorhexidine)
- Teat dip applicator cup
- And Disposable hand -gloves (FAO, 2008)

2.6.2.2 Machine Milking

Milking is done by using machine and generally adopted for herds with large number of cows and with high yielders. The milk flow is continuous in this method. Advantages are reduced labour cost, short time for milking, less injury to teats and hygienic method of milk production. The parts of a machine milking system are milking unit, pulsator system, vacuum supply system and milk flow system (Jeffery, 2012). The milking unit attached to the udder has a teat-cup assembly, suspension cup, and connecting air and milk tubes (Plate 2:4). The teat cup consists of a steel shell with a liner which fits over teats called as inflation. The inflation squeezes and relaxes on the teat as the pulsator operates causing the milk to flow into the system. The pulsation ratio is the time between milking and resting phases of pulsation cycle. The pulsation ratio refers to the number of pulsations per minute. The pulsation ratio usually varies from 1:1 to 2.5:1. Set the pulsation rate around 48 to 72 cycles per minute to avoid excessive slow or fast speed and subsequent decline in milk flow rate (Pamela *et al*., 2000; Jeffery, 2012). Always maintain the measure of vacuum by operating the milking machine between 10 and 16 inches of Hg. Apply milking machine gently within 30 to 60 seconds of cleaning the udder (Ziegler and Mosimann, 1996). Remove milking machine promptly as soon as milk flow stops by breaking vacuum first. Disinfect the teat ends by dipping them in

antiseptic solution (post-dipping). Milk utensils and teat cups are immediately washed with warm water and detergent.

Plate 2.4: Machine milking

Source: https://ww.123rf.com

2.7 Milking Practices

Milking practices or procedures are the day to day activities carried out in a dairy farm before and after milking to ensure production of quality milk for consumption. The unhygienic and undesirable practices that decrease the quality of raw milk can be classified into three categories based on:

Practices related to the animals:

- Milking of sick animals or mastitic udder.
- Milking of dirty animals, especially the udder, the teats, the hind quarter and the tail.

Practices related to the milker:

- Hands and clothes of the milker are not clean and he/she practices unhygienic personal habits.

Practices related to the milking process:

- Poor milking practices before milking.

From 1962 - 65, scientists with the National Institute for Research in Dairying in England, conducted two large field experiments involving 29 herds and 2200 cows and found that a pre-milking hygiene practice of disinfecting the udder (pre-dipping), use of individual towel (paper towels), use of rubber gloves worn by milkers, and teat dipping reduced new infections by 44% (Fox, 1997, Pankey *et al*., 1989). In addition to these practices, pasteurization of teat-cup clusters with hot water (185 degrees for 5 seconds) reduced new infections by 58%. However, many dairy farms pay too little attention to the importance of proper milking practices (Sahota and Saini, 2001).

2.7.1 Stimulation of the Udder

Milk is produced throughout the day by milk secreting cells (alveoli) located deep within the udder (they resemble bunches of grapes). About 60% of this milk is stored until milking within the alveoli and small ducts that drain the alveoli. The remainder is stored in large ducts and udder cistern (Reneau, 1997; Galton *et al*., 1998; FAO, 2008). For complete, fast milk-out, the cow must be stimulated to let down her milk. Sensitive receptors for stimulating milk let-down are located in the teat skin. After stimulation of these receptors, a signal is sent to the brain, and the pituitary gland releases a hormone, oxytocin, into the blood. Oxytocin is released to the udder and causes contraction of the muscle fibers (myoepithelial cells) that surround the alveoli. (Ruegg and Dohoo, 1997). Contraction forces milk into the large ducts and udder cistern where the milking machine then can remove the milk. A normal milk let-down can be interrupted if cows become frightened or excited either before or during milking. Environmental stress reduce milk yield, increase milking time, and may cause mastitis. Milking routines need to be consistent from day to day and milker to milker and cows need to be handled gently.

2.7.2 Use of Disposable Hand-gloves

Staphylococcus aureus has been isolated from noses and hands of dairy workers which could serve as sources of milk contamination and mastitis infections for cow. English workers included rubber gloves in their hygiene routine, which reduced new infections by 44% (Fox, 1997; Pamela *et al*., 2000). A pack of 100 latex gloves cost from N13 to N15 per glove which is cost effective compare to producing contaminated milk and treating mastitis. Dairy workers should consider using disposable gloves that would be throughout milking and thrown out at the end of every milking, that is single use.

2.7.3 Fore-stripping

Stripping 4-5 squirts/ steams of milk from each quarter is beneficial because it allows you to detect early stages of clinical mastitis, removes foremilk which may have high bacteria count and may serve as the primary stimulus for milk let-down. During foremilk stripping, wipe dry dirt off the teat with the hand (Galton *et al*., 1998)

In USDA (United States Department of Agriculture) (Beltsville) studies, stripping before washing and drying the udder reduced the incidence of new udder infections from 18% to 7%. Stripping after udder preparation was less effective. Foremilk stripping assists in the early detection of clinical mastitis. Look for the presence of flakes, clots or stringiness, or watery secretions. Hard quarters that are warm or enlarged provide an early warning that the cow has clinical mastitis and that her milk should not be added to the bulk tank (Sahota and Saini, 2001).

Use a strip cup with a black surface for forestripping. The detection of a watery secretion may indicate that a problem is developing. When a strip cup is not used, milk often is squirted onto the feet and legs of the cow being prepared, or the adjacent cow (Pankey *et al*., 1989, Lore *et al*., 2006, FAO, 2011). Infections can be transferred via the feet and legs to the bedding, whereby an uninfected cow may use the stall next and become infected. The bacteria can thrive in dirty, wet stalls. If milk is squirted on the parlor floor, flush surface immediately with a water hose, do not squirt foremilk on hands (Sahota and Saini, 2001).

2.7.4 Pre-dipping

Pre-dipping is when cows' teats are dipped in germicidal teat dip prior to milking, has become an important part of the pre-milking practice. It can serve as a substitute for washing teats with sanitizer. However, dirty teats must be cleaned before pre-dipping. Dip should remain on the teat approximately 30 seconds before it is dried-off with a

paper or cloth towel (Galton *et al*., 1998; Jeffery, 2012) Drying is important to avoid increased teat dip residues in milk. Pre-dip will destroy those microorganisms which contaminate the teat skin between milking. Pre-dipping has reduced new cases of mastitis caused by coliforms and environmental streptococci. Also, be sure to continue to teat dip after milking (post dipping). The same teat dip can be used as a pre- or post-dip, but two different dippers should be used. Dippers are preferred over sprayers unless use of sprayers results in adequate coverage of backsides of teats. Teats should be pre-dipped with disinfectant, such as chlorhexidine or iodine solution. If iodine teat dips are used, low iodophor concentrations (0.5% or less). The effectiveness of pre-milking teat dipping is reduced by wet and dirty cows. Such cows should receive a first wash of the teat with either a wet paper towel or hand washing, but don't wet the udder. Pre-dip after the dirt has been washed away (Pamela *et al*., 2000; Susan, 2017).

2.7.5 Drying of Teats and Udder

Teat-cups or milking should be applied to clean, dry teats. When water droplets on the udder drain toward the teat end, they pick up bacteria. This dirty water can be sucked inside the teat-cup and raise the bacteria count. Admitting large amounts of air into the teat-cup causes milk droplets to move backward and up the milk tube. It's similar to a fine aerosol spray, causing milk droplets and any bacteria inside the teat-cup liner (contaminated liners or dirty water on teat ends) to impact against the teat end. Bacteria may strike the teat ends with enough force to cause them to enter the teat canal. These impacts are caused by vacuum loss created when units are attached, when units are removed without shutting off the vacuum, by squawking teat-cups, machine stripping, or unit falloff this is in the case of machine milking (Rasmussen and Frimer, 2000; Pankey *et al*., 1997; Reneau, 1997). Dry wet teats and udders thoroughly, leave no water on the teat or udder. Use single service paper or cloth towel and do not use any towel

on two cows as infection may spread. Do not fold the cloth towel over and use the back side for a second cow. Cloth towels do a better job of getting teats dry are preferred by milkers, and may be cheaper, but they must be laundered before next use.

2.7.6 Post-dipping

Teat dipping is the single most effective practice for reducing infections and contamination, especially by contagious bacteria. Dipping all teats after each milking has a greater impact on reduction of milk somatic cell counts and increased milk yields than any other milking practice. Effective teat dips should destroy microorganisms present on teats at the end of milking. This prevents bacteria from establishing a colony at the teat end or in teat lesions where they could penetrate the teat canal and establish an infection (Pankey *et al*., 1997; Galton *et al*., 1998). In addition, a good teat dip should leave a residue on the teat so the antimicrobial action is still present when the cow lies down in a free stall or any other place where sanitary conditions are less than ideal. Effective teat dips have reduced new udder infections by 50 - 90%.

Cover the entire teat with dip. Don't forget the back sides, especially if you use a spray. Occasionally, check the far side of the rear teats as soon as they've been dipped. In many cases, the back sides are hardly touched. Dip at least the bottom half of all teats after every milking, using similar solution used for predipping. Most effective coverage may be attained by dipping, compared to spraying. A dipper makes covering the whole teat easier. With spraying, more attention is required to assure good coverage (Ruegg and Dohoo, 1997). It is easy to miss the back half of the teat, especially with hand, bottle-type sprayers that spray from the side.

2.8 Sources of Bacterial Contamination of Milk

Microbial quality of milk refers to the cleanness or hygienic quality of milk. This is defined by a number of bacteria present in milk. The high bacterial count as well as the

presence of pathogenic bacteria in milk not only degrades the milk quality and shelf-life of milk or milk related products but also poses a serious health threat to consumers (Yuen *et al*., 2012). Milk being a wholesome food with high nutritive value is often prone to early contamination and spoilage if not handled properly (Minj and Behera, 2012). The fewer the number of micro-organisms in milk the higher the quality of milk. The microorganism may originate from the cow, the environment and milk equipment's.

In ensuring adherence to clean milk production and consumption, there are set standards of legal limits of bacteria in milk for each country. The legal limits are very important to be observed as it provides protection to the health of consumers for milk and other dairy products. The standards are universal with the difference in legal limits. In setting these standards technological level and economic constraints of the region is taken into consideration (Jensen *et al*., 2010). The EU countries are the leading with the highest microbiology quality standard of (<100,000 CFU/mL) for total bacterial count in raw milk (Berry, 2004; Bytyqi *et al*., 2011) of which this is contributed by the presence of all the required resources for clean milk production when compared to the developing countries especially Nigeria where there is no milking quality standards and milking practices are not adhered to, leading to poor milk quality and production. To protect consumers and public health against these milk-borne infections it require proper hygienic milking practices and milk handling procedures.

Common bacteria reported to be isolated from milk include *Staphylococcus spp, Listeria spp, Salmonella spp, E. coli spp, Campylobacter spp, Mycobacterium spp* and *Brucella spp.* All these are pathogenic bacteria that pose serious threat to human health and contribute up to 90% of all dairy related diseases (De Buyser *et al*., 2001; Sivapalasingams *et al*., 2004; Donkor *et al*., 2007). Therefore, proper milking, cleaning

and sanitizing procedures of equipment's and environments (good milking practices) are essential tool to ensure quality milk production. Many countries have implemented laws and regulations concerning the composition of milk and hygienic milking practice to protect both the consumers and the public health (Pandey and Voskuil, 2011). Unfortunately, these laws and regulations are not often adhered in developing countries like Nigeria. This is exemplified by over 75% of milk marketed in many developing countries is sold raw/unpasteurized through informal channels (Bertu *et al*., 2010; Oliver and Murinda, 2011).

The isolation of pathogenic and coliform bacteria from milk indicates that milk may be contaminated from udder of animals, utensils used for milking or the water used as well as handlers (Bonfoh *et al*., 2006). The quality of the starting raw milk has a very definite effect on the yield and quality of products made from it. The compositional quality, the hygienic quality, the health of the cow and the level of contaminants present can all have an impact on the yield and quality, and hence financial return from products made from milk (Harding, 1995). Inadequate cooling of the milk, improper udder preparation methods, unclean milking equipment and the water used for cleaning purposes are considered as the main source of milk contamination (De Graaf *et al*., 1997). In order to produce milk of low bacteriological quality, there is need to be aware of the sources of contamination and importance of proper milk handling, cooling and storage.

2.8.1 Contamination from Cow's Udder

Raw milk as it leaves the udder of healthy cows normally contains very low numbers of microorganisms and generally will contain less than 1000 total bacteria per mL (Murphy, 1996; Godefay and Molla, 2000). Natural flora originating from the cow generally has little influence on total aerobic plate counts (Murphy and Boor, 2000). The bacterial infection of milk taking place inside the udder is called primary infection.

The main groups of microorganisms for this infection are the aerobic mesophilic micro-flora, and they contribute little to the deterioration of good quality raw milk (<5000 cfu/mL) (International Dairy Federation (IDF), 1996). The exterior of the udder can be an important source of contamination. But the exterior of the udder is influenced by the environment of the cows, in which cows are housed and milked (Murphy, 1996). In temperate regions, cows are housed in winter and pastured in summer. Differences in teat contamination can be found between housing and pasturing (IDF, 1994a). Both total aerobic plate and aerobic spore counts are lower when cows are at pasture. When cows are housed, bedding material and feedstuffs can be contamination sources, in either cases (housing and pasturing) dung is also an important contamination source. Teat surfaces are also a source of bacterial spores in milk (IDF, 1996). Damaged teats can affect milk quality in that any break in the skin can become a reservoir for bacteria and give rise to a significant increase in bacterial count.

2.8.2 Contamination from Environment

This includes the skin of the animal, feeds, containers and water facilities. It is important to keep the environment around the dairy animals as clean as possible. Also, bedding materials must be kept dry and clean at all times (Maunsell and Donovan, 2008**).** The environment around the farm is a good source of contamination. The milking place, dirt and even air can be sources of microbes in milk. Contamination of environment varies from place and season. However, the contaminated microbes should be pathogens such as *Salmonella spp* and *E. coli*. Milking area should be cleaned before milking commence. Most of the dirt and soil should be removed. The milk reservoir should be in close vessel to prevent contamination from dust. Furthermore, milk should be filtered with sieve or fabric before pouring into storage tank for further processing.

2.8.3 Contamination from Milking Equipment and Storage

Bacteria are present in the air, dust and water, especially any water containing traces of milk residues which may have been left in the milking plant overnight, as such residues provide a very good source of food for bacteria, thereby enabling the bacterial counts to increase rapidly. Cleaning regimes are based on removing visible dirt, removing milk residues (fat, protein, milk-stones) which harbours bacteria, then sterilization of the cleaned surfaces using heat or chemical disinfectant such as sodium hypochlorite (Harding, 1995). Cleaning and sanitizing procedures can influence the degree and type of bacterial growth on milk contact surfaces by leaving behind milk residues that support growth, as well as by setting up conditions that might select for specific bacterial groups. More resistant bacteria may endure in low numbers on equipment surfaces that are considered to be efficiently cleaned with hot water.

The influence of cleaning and disinfection on the survival of bacteria on milk contact surfaces is not yet fully understood. Attachment of bacteria to different surfaces (Husmark and Ronner, 1990) and possible scaling may cause problems with cleaning and disinfection. In most cases not all bacteria are killed and removed during cleaning and disinfection. The multiplication of bacteria in milk has been observed to be dependent on the temperature and time of storage. After production, milk can be stored in cans and in bulk tanks before collection. The storage temperature influences the types of bacteria which grow and their spoilage characteristics. Spoilage of raw milk has been reported to be due to coliforms, resulting in souring of milk. During storage in bulk tanks and transport, the microflora of the milk changes from micrococci to psychrotrophic gram-negative rods. There are many different microorganisms (mainly bacteria), which can find access to milk, and there are three broad temperature ranges classifying their optimum growth rates (Husmark and Ronner, 1990). Organisms with

an optimum growth rate at low temperatures (0-15°C) are psychrophiles, example, *Pseudomonas spp*, at medium temperatures (20-40°C) are called the mesophiles, example, *Salmonella spp* and at high temperatures (45- 55°C) the thermophiles, example *Bacillus spp* (IDF, 1996).

2.9 Bacteriological Quality of Milk

Milk has been considered as one of the most important primary foods, however, several bacterial pathogens have been detected in milk including entero-haemorragic *Escherichia coli*, *Staphylococcus aureus*, *Salmonella specie*, and *Yersinia enterocolitica* (CDC, 2003; Mazurek *et al*., 2004; Tekinşen and Özdemir, 2006). Bacterial pathogens from milk also include psychotropic microorganisms, mainly belonging to the genus *Pseudomonas*, that are responsible for the spoilage of milk and dairy products owing to their ability to produce heat-resistant proteolytic and lipolytic enzymes at chill temperatures. Their enzymes can withstand heat treatments of pasteurization and ultrahigh temperature treatments (UHT). These pathogens have been linked to livestock, feed, and storage environment (Marco and Wells-Bennik, 2008). The bacteriological quality of milk is strictly related to the management and milking practice, such as equipment and environmental hygiene, pre-dipping, post- dipping, forestripping, packaging and handling (Little *et al*., 2008).

2.9.1 *Escherichia coli*

Escherichia coli a common inhabitant of the human and animal intestinal tract, is a gram- negative, facultative aerobic organism, and a member of *Enterobacteriaceae* family (Nys *et al*., 2004; Von Baum and Marre, 2005). Organisms of this species are generally lactose fermenters, but sometimes the lactose fermentation is delayed. Most strains of *E. coli* are harmless; however some are pathogenic causing severe intestinal and diarrheal diseases. These potentially harmful *E. coli* are classified into categories

based on the production of virulence factors and on the clinical manifestations that they cause. They have been reported in raw milk and milk products by several authors (Chye *et al*., 2004). In addition to the presence of *E. coli* denoting fecal pollution, the presence of virulence – related genes in *E. coli* strains refer to the pathogenicity of the isolates.

2.9.2 *Salmonella* species

Salmonella species includes more than 2500 different serotypes and represents a leading cause of milk-borne infections worldwide (Chen *et al*., 2004; Magistrali *et al*., 2008). *Salmonella* species cause a wide range of human disease such as enteric fever, gastroenteritis and bacteremia. A variety of foods have been implicated as vehicles transmitting salmonellosis to humans, including raw meats, poultry, milk and dairy products (Forsythe, 2000). Contamination has been reported to be through poor temperature control and milking and handling practices, or cross-contamination of processed foods from raw ingredients. The primary reservoir is the intestinal tract of humans and animals. This pathogen is excreted in the faeces and can remain viable in the faecal material for several years. The principal source of Salmonella infection has been reported to be ingestion of contaminated feed. Consumption of contaminated milk may lead to a number of gastrointestinal bacterial infections (CDC, 2003; Magistrali *et al*., 2008). Gastroenteritis has been attributed to species of *Salmonella* especially *Salmonella typhimurium* and *Salmonella enteridis* and symptoms occur 7-72 hours following ingestion of contaminated milk.

2.9.3 *Staphylococcus aureus*

These are Gram-positive, facultative anaerobic, non-spore forming cocci. They were described in 1897 (Forsythe, 2000). This pathogen produces a wide range of pathogenicity and virulence factors like staphylokinase, hyaluronidases, coagulases and haemolysins (Forsythe, 2000). Staphylococcal food poisoning has been reported to be

caused by the ingestion of food containing pre-formed toxins, named enterotoxins secreted by *S. aureus*. It is considered one of the leading food-borne illnesses in human worldwide and is associated with contaminated food of animal origin such as milk and dairy products, meat and meat products (Tsegmed *et al*., 2007). *Staphylococcus aureus* has been a major causative agent of mastitis which is the most economically important diseases for the dairy industry so more effective therapeutic treatment and prophylactic approaches are surely needed (Chiang *et al*., 2007; Oviedo-Boyso *et al*., 2008). *Staphylococcus aureus* can gain access to milk either by direct excretion from udders with clinical and subclinical staphylococcal mastitis or by environmental contamination during the handling and processing of raw milk.

2.10 Bacteriological Quality Tests for Milk

Hygienic methods of milking and handling milk must be strictly adhered to rigidly in order to provide safe milk for human consumption. Furthermore, since milk is a good growth medium, even a small number of non-pathogens can multiply considerably if the milk is not kept refrigerated. A number of standard tests are carried out periodically on milk since consumers cannot determine milk contamination during purchase.

From the results of these tests, milk is classified into grades designated as A, B, and C (Volk and Wheeler, 1980). Tests commonly employed to determine the quality of milk include dye-reduction (Methylene blue reduction and resazurine reduction), Alcohol test, Standard plate count, Coliform count, Somatic cell count, Titrable acidity, and phosphatase tests (Marshall, 1992).

2.10.1 Dye-reduction Tests

Dye- reduction tests have been employed to check for the overall microbial load and quality control of milk and other liquid foods (Impert *et al*., 2002). These tests have been successfully employed to quantify viable cell count in milk within a very short

time. The tests are less precise criterion for classifying milk according to its bacteriological quality. This calls for the need to periodically verify the quality of milk with more precise microbiological tests such as standard plate count (Ombui *et al.*, 1995).

2.10.1.1 Methylene Blue Reduction Test

Methylene blue is a blue-colored reagent used to estimate the bacterial population of a given milk sample (Nandy *et al*., 2007). A known dilution of the methylene blue solution is added to the milk sample and observation is made at fixed intervals until the blue color disappears. The number and species of organisms present in the milk determines the time required for the disappearance of the blue color in the milk (May *et al*., 2003; Nandy *et al*., 2007). Normally if the number of bacteria increases, the time required to decolorize the blue color is shorter. This test is usually used for grading the quality of raw milk before pasteurization. On the basis of this test, raw milk is graded as follows (Kurwijilla *et al*., 1992):

- Very good: not decolorizing in 5 hours.
- Good: decolorized in less than 4 hours, but not less than 3 hours.
- Fair: decolorized in less than in 2 hours, but not less than 1 hour.
- Poor: decolorized in less than ½ hour.

2.10.1.2 Resazurin reduction test

This test is also used for grading the hygienic quality of milk by applying the chemical reagent resazurin. The procedure is similar to that of the methylene blue test, except that this test is quicker and the result is obtained in much less time. Resazurin imparts blue color to milk which when reduced to resorufin changes to pink and finally to white on reduction to dihydroresorufin. The time required for complete decolorization, reduction of the resazurin and the degree of colour change is directly related to the

number of bacterial organisms in the milk (Ombui *et al*., 1995; Teka, 1997). A comparator disc reading value of 4 and above at 10 minutes with resazurin test indicates good quality while a comparator disc reading value of less than 4 at 10 minutes indicates poor quality milk (Ombui *et al*., 1995).

2.10.2 Alcohol Test

When milk contains more than 0.21% acid, or when calcium or magnesium compounds are present in greater than normal amounts, it coagulates on the addition of alcohol. This fact is the basis of alcohol test, which furnishes a means of judging the quality of milk (Ombui *et al*., 1995).

2.10.3 Standard Plate Count (SPC)

The standard plate count of milk gives an indication of the total number of aerobic bacteria present in the milk at the time of pick up. Obviously, very clean milk will have lower bacterial counts than milk collected or handled under unsanitary conditions. The standard plate count has been reported to be a good basis for grading the quality of milk (Volk and Wheeler, 1980). Milk samples are plated on standard plate count agar media and then incubated for 48 hrs at 32°C to encourage bacterial growth. Single bacterium or clusters of bacteria visible colonies are then counted. All plate counts are expressed as the number of colony forming units (CFU) per milliliter (Murphy, 1996). This method has been used to estimate the bacterial population in milk. This method has a limited value in that it doesn't indicate the quality of microbial populations in terms of pathogens and non -pathogens (Teka, 1997). The standard plate count is generally accepted as the most accurate and informative method of testing bacteriological quality of milk (Kurwijilla *et al*., 1992; Godefay and Molla 2000). It is sensitive but also labour intensive and is inaccurate for bacteria high count in milks. Plate count standards have been developed to ensure satisfactory production hygiene and that the product is safe.

The plate count method has been conducted as a valuable adjunct to guide sanitarians in correcting sanitation failures and improving milk quality (IDF, 1996).

2.10.4 Coliform Bacteria in Milk

Coliforms are group of bacteria, which inhabit the intestinal tracts of human and animals. They are excreted in large number with human excreta and animal droppings. They may be found in the soil, on vegetables and in untreated water (Teka, 1997). It includes all aerobic and facultative anaerobic, Gram-negative, non-spore forming rods able to ferment lactose with the production of acid and gas at 35°C within 48 hours. Most of them belong to the genera *Escherichia*, *Enterobacter* and *Klebsiella* (Godefay and Molla, 2000). The presence of coliform organisms in milk indicates unsanitary conditions of production, processing or storage. Hence their presence in large number in dairy products is an indication that the products are potentially hazardous to the consumers' health (Volk and Wheeler, 1980; Godefay and Molla, 2000). Coliform organisms contaminate milk from unclean milker's hands, improperly cleaned and unsanitized or faulty sterilization of raw milk utensils especially churns, milking machines, improper preparation of the cows' flecks or dirt, manure, hair dropping into milk during milking, udder washed with unclean water, dirty towels and udder not dried before milking (Ombui *et al*., 1995).

2.10.5 Tests for Specific Pathogens

Unless there is some evidence that a particular disease is being transmitted through milk, tests for specific pathogens are not run. The procedure to be followed depends on the specific organism in question, it usually involves the culture and isolate of the bacteria using specific media and procedures (Quinn *et al*., 1999).

2.10.6 Somatic Cell Counts (SCC)

Somatic cell count refers to the total cells per millitre in milk. The somatic cell count (SCC) has been internationally recognized as a parameter for assessing milk quality and udder health (De Graaf *et al*., 1997). European Union (EU) standards require that the milk should not contain more than 100,000 somatic cells/mL. Milk markets routinely rely on somatic cell counts to ensure a quality product. Somatic cell counts levels are monitored to ensure compliance with set milk quality standards. Today, most markets in developed countries pay a premium for low SCC, good quality milk. One can appreciate the reasons, for paying a bonus for quality milk when the relationship between mastitis (high SCC) and milk composition is understood. Chemical changes in milk composition due to mastitis reduce milk quality (Rice and Bodman, 1997).

2.10.7 Other Milk Quality Tests

2.10.7.1 Organoleptic tests

Bacteria cause various undesirable and detectable organoleptic and physical changes in milk. Generally, when actively growing types of organism capable of causing changes in flavor and physical appearance reach population levels of 5-20 million per mL; organoleptic and physical changes are evident or imminent. The general appearance, cleanliness, colour and smell of the fresh milk should be checked at collection before it is blended with milk from other suppliers since the volume and value at risk increases down the chain (Harding, 1999).

2.10.7.2 Sedimentation test

This is performed by leaving milk in flask or any container and kept for 15-30 minutes and observing if there is any sedimentation of dirt. The sediment can be examined bacteriologically for the presence of bacteria (Warner, 1975).

2.10.7.3 Clot on boiling test

Acidity decreases the stability of milk. If the concentration of hydrogen ion is more than the normal amount, then casein will get precipitated on heating immediately. The clot on boiling test is used to determine whether milk is suitable for processing, as it indicates whether the milk is likely to coagulate during processing (usually pasteurization). It is performed when milk is brought to the processing plant. If the milk fails the test, it is rejected (O'Mahony**,** 1998).

2.10.7.4 Catalase test

This measures the activity of the enzyme catalase. The catalase content of milk primarily depends upon the number of cells in milk. Hence the increased activity of this enzyme indicates mastitis (Cheesbrough, 2003).

2.10.7.5 Specific gravity

To test adulteration, specific gravity is measured and calculated. The specific gravity of milk will be measured using lactometer. The specific gravity of normal unadulterated cow's milk is between 1.026 and 1.032 at 20°C (Ombui *et al*., 1995)

2.10.7.6 Freezing test

The normal freezing point of milk is between -0.50ºC and -0.61ºC. The soluble constituents, lactose and ash determine the freezing point of milk and are responsible for its being lower than that of water. This fact makes it possible to determine whether or not milk has been watered. It had been shown that with addition of 1% of water to milk, the freezing point is raised approximately by 0.055°C (Hansen, 1994).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

The study was conducted in Sokoto metropolis, Sokoto state, Nigeria (Figure 3.1). Sokoto metropolis is the capital of Sokoto state, the State is semi-arid, located in the extreme North-western Nigeria (between longitudes 4º 8 E and 6º 54 E and latitudes 12º N and 13º 58 N). The average annual temperature is 28.4ºC and average annual rainfall is 629mm. There are two major seasons in Sokoto, namely wet and dry; the dry season starts from October, and lasts to to April in some parts and may extend to May or June in other parts. The wet season on the other had begins in most parts of the state in May and lasts up to September, to October. The Harmattan, a dry, cold and fairly dusty winds is experienced in the state between November and February. Heat is more severe in the state in March and April. The vast Fadama land of the Sokoto-Rima River systems dissects the plain and provides the rich alluvial soil fit for a variety of crop cultivation in the state and pasture for grazing livestock (Ismail and Oke, 2012). It covers a total land area of about 32,000 square Km, with an estimated human population of 2.4 million (NPC, 2006). Sokoto metropolis is composed of Sokoto north, Sokoto South, some part of Kware, Wamakko and Dange Shuni Local Governments (Figure 3.1).

Figure 3.1: Map of Sokoto state showing Sokoto metropolis

Livestock survey showed that Sokoto state has a livestock population of about 1.18 million cattle, 2.90 million goats, 1.98 million sheep, 2.0 million chickens, 45,000 camels, 34,532 horses and 51,388 donkeys (FDLPCS, 2002). The cattle breeds are mostly local, exotic and their crosses. The breeds are Gudali, Bunaji (White Fulani) (Plate 3.1), Rahaji (Bororo), Muturu, Kuri and the exotics are mostly Holstein-Friesian (Plate 3.2), Jersey, Ayrshire breed and their crosses.

Plate 3.1: Local breeds of cattle (White Fulani)

Source: c2.staticflickr.com

Plate 3.2: Exotic breed of cattle (Holstein-Friesian)

Source: http://stockagency.panthermedia.net

3.2 Study Design

Dairy farms within Sokoto metropolis were selected using convenient sampling method, a total of 5 dairy farms were selected, ten (10) samples were collected per farm before and 10 samples after implementing milking practices to assess how the milking practices affects the bacteriological quality of the milk using total bacterial count, culture and isolation and biochemical test, done before and after milking practice implementation. Consent of dairy owners was sought before sample collection and milking practice implementation. Three (3) pathogenic bacteria were the target of this study, which are *Escherichia coli*, *Salmonella spp* and *Staphylococcus aureus* which are common bacterial pathogens based previous studies (Tassew and Seifu, 2011; Oliver and Murinda, 2011; Olatunji *et al*., 2012) Two types of data were collected which are sociological (questionnaire) and laboratory based data (milk sample).

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3.3 Sample collection

Milk samples were collected using convenient sampling, five milliliter (5mL) of milk was collected in sterile sample bottles after milking, each sample was labelled, placed in an ice box containing ice packs and was transported to the City Campus Central Laboratory, Usmanu Danfodiyo University, Sokoto for bacteriological analysis. Samples were collected weekly for 4 months from April - July, before and after implementation of the milking practices from each farm.

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Milking practices such as washing and drying of the teats before milking, wearing gloves for milking, pre–dipping, post-dipping and forestripping were implemented as described by Pamela *et al*. (2000), FAO (2008) and Jeffery (2012).

3.4 Questionnaire survey

A closed ended questionnaire modified from the study of Plozza *et al*. (2011) was used to collect information from dairy farmers. Questionnaire was used to access the

knowledge of dairy farm owners on milking practices and hygienic practices, it was divided into three categories which are milking practices, hygienic practices and other farm activities. The questionnaire was made with pre-coded response choices (closed-ended questions) with a few open-ended questions. The data collected include bio-data information, number of animals, number of lactating animals, source of water, hand washing, milking clothes, fore stripping, pre- dipping and post – dipping. The questionnaire was validated in two dairy farms which was not included in the study before implementation.

The questionnaire was administered through face to face interview conversation. While administering questionnaires, observations on general cleanliness and hygienic conditions and milking practices within the farms was noted. The questionnaires were administered after milking and sample collection.

3.5 Media Preparation and Storage

All the media used in this study were prepared according to manufacturer's instructions. Details of the preparations and handling of different types of media used is hereby shown below.

3.5.1 Nutrient agar (NA)

The medium NA (Oxoid ® Ltd., Basingstoke, Hampshire, England, CM0003, Lot 005251) is composed of 5 g/l Gelatin peptone, 2g/l Yeast extract, 5.0g/l Sodium Chloride, 1.0g/l Lab-Lemco powder, 15 g/l Bacteriological agar and final pH of 7.4 ± 0.2 at 25°C. The medium was prepared according to the manufacturer's instructions, briefly 28g of the powdered medium was suspended into 1 litre of distilled water, mixed well and left on the bench to stand until the mixture is uniform (Bio-cabinet, Suzhou), then the mixed solution was heated with gentle agitation and boiled until completely dissolved (Microwave, AKIRA, Japan). The medium was sterilized in the autoclave at

121°C for 15 minutes then allowed to cool to 45ºC and poured onto sterile petri dishes in a laminar flow. The plates were left at room temperature for two hours for the media to solidify then put upside down in the incubator for 24 hours at 37°C to check for sterility and to dry the condensed vapour on the plate cover before inoculation.

3.5.2 Buffered peptone water (BPW)

The medium (BPW powder, Oxoid® Ltd., Basingstoke, Hampshire, England, CM0509, Lot 1442805) is composed of 10 g/l Peptone, 5 g/l Sodium chloride, 3.5 g/l Di-sodium phosphate and 1.5 g/l Potassium di-hydrogen phosphate. The medium was prepared according to manufacturer's instructions, briefly 20 g of the powdered medium was dissolved in 1 litre of distilled water. The culture medium was mixed well and each 10 mL were dispensed into capped test tubes. Then, the test tubes were sterilized by autoclaving at 121°C for 15 minutes and cooled to 25°C before use. All the unused prepared media were being stored under refrigeration temperature.

3.5.3 Eosin methylene blue agar (EMB)

The medium EMB agar (Oxoid ® Ltd., Basingstoke, Hampshire, England, CM0069) is composed of 10g/l Peptone, 10g/l Lactose, 2g/l Dipotassium hydrogen Phosphate, 0.4g/l Eosin Y, 0.065g/l Methylene blue, 15g/l Agar and pH of 6.8 ± 0.2. . The medium was prepared according to the manufacturer's instructions, briefly 37.5g of the medium was dissolved in 1 litre of distilled water. The culture medium was mixed well and left on the bench to stand until the mixture is uniform. Then the mixed solution was heated with gentle agitation and boiled until completely dissolved. The medium was sterilized in the autoclave at 121°C for 15 minutes then allowed to cool to 45ºC and poured onto sterile petri dishes. The plates were left at room temperature for two hours for the media to solidify then put upside down in the incubator for 24 hours at 37°C to check for sterility and to dry the condensed vapour on the plate cover before inoculation.

3.5.4 Xylose –lysine- desoxycholate agar (XLD)

The medium XLD agar (Oxoid ® Ltd., Basingstoke, Hampshire, England, CM0469) is composed of 3g/l Yeast Extract, 5g/l L-Lysine Hcl, 3.75g/l Xylose, 7.5g/l Lactose, 7.5g/l Sucrose, 1g/l Sodium desoxycholate 5g/l Sodium Chloride, 6.8g/l Sodium Thiosulphate Ferric 0.8g/l Ammonium citrate, 0.08g/l Phenol red, 12.5g/l Agar and pH of 7.4 ± 0.2 at 25º C . The medium was prepared according to the manufacturer's instructions, briefly 53g of the medium was dissolved in 1 litre of distilled water. The culture medium was mixed well and left on the bench to stand until the mixture is uniform. The medium was heated with gentle agitation and boiled until completely dissolved then allowed to cool to 45ºC and poured onto sterile petri dishes. The plates were left at room temperature for two hours for the media to solidify then put upside down in the incubator for 24 hours at 37°C to check for sterility and to dry the condensed vapour on the plate cover before inoculation.

3.5.5 Mannitol salt agar (MSA)

The medium MSA agar (Oxoid ® Ltd., Basingstoke, Hampshire, England, CM0069) is composed of 1g/l Lab-Lemco powder, 10g/l Peptone, 10g/l Mannitol, 75g/l Sodium chloride, 0.025g/l Phenol Agar, 15g/l Agar and pH of 7.5 ± 0.2 at 25ºC . The medium was prepared according to the manufacturer's instructions, briefly 111g of the medium was dissolved in 1 liter of distilled water. The culture medium was mixed well and left on the bench to stand until the mixture is uniform. Then the mixed solution was heated with gentle agitation and boiled until completely dissolved. The medium was sterilized in the autoclave at 121°C for 15 minutes then allowed to cool to 45º C and poured onto sterile petri dishes. The plates were left at room temperature for two hours for the media to solidify then put upside down in the incubator for 24 hours at 37°C to check for sterility and to dry the condensed vapour on the plate cover before inoculation.

3.5.6 Simmons citrate agar (SIM)

The medium SIM agar (Oxoid ® Ltd., Basingstoke, Hampshire, England, CM0155) is composed of 0.2g/l Magnesium Sulphate, 0.2g/l Sodium ammonium phosphate, 0.8g/l Ammonium dihydrogen phosphate, 2g/l Sodium citrate, tribasic, 5g/l Sodium chloride, 0.08g/l Bromothymol blue, 15g/l Agar and pH of 7.0 ± 0.2 at 25ºC . The medium was prepared according to the manufacturer's instructions, briefly 23g of the medium was dissolved in 1 litre of distilled water. The culture medium was mixed well and left on the bench to stand until the mixture is uniform. The medium was heated with gentle agitation and boiled until completely dissolved. The medium was sterilized in the autoclave at 121°C for 15 minutes then allowed to cool to 45ºC minutes then allowed to cool to 45ºC and allowed to set in sloped form with a butt about 1 inch deep before inoculation.

3.5.7 Triple sugar iron agar (TSI)

The medium TSI agar (Hi-media ® Ltd., India, CM0277) is composed of 3g/l Lab-Lemco powder, 3g/l Yeast extract, 20g/l Peptone, 5g/l Sodium chloride, 10g/l Lactose, 10g/l Sucrose, 1g/l Glucose, 0.3g/l Ferric citrate, 0.3g/l Sodium Thiosulphate, 0.024g/l phenol red, 12g/l Agar and pH of 7.4 ± 0.2 at 25ºC. The medium was prepared according to the manufacturer's instructions, briefly 65g of the medium was dissolved in 1 litre of distilled water. The culture medium was mixed well and left on the bench to stand until the mixture is uniform. Then the medium was heated with gentle agitation and boiled until completely dissolved. The medium was sterilized in the autoclave at 121°C for 15 minutes then allowed to cool to 45ºC and allowed to set in sloped form with a butt about 1 inch deep before inoculation.

3.6 Culture and Isolation of Bacteria

Cultural examinations were used to isolate and identify bacterial pathogens found in the raw milk samples. Isolation and identification of bacterial species was carried out based on conventional culture technique and biochemical tests. After thorough mixing of each milk samples, a loopful of the milk sample was streaked on the nutrient agar, the plates were incubated at 37ºC and examined for bacterial growth after 48 hours. From culture positive plates, typical colonies were subjected to Gram staining and microscopy, to study the staining properties and cellular morphology. Pure cultures of a single colony type from the nutrient agar were transferred in to selective media plate. From this, a series of biochemical tests that could aid in the final identification of various bacteria was conducted following standard methods (Quinn *et al*., 1999). Identification of bacteria was carried out based on their colony characteristics, Gram's staining and morphological characteristics and growth on differential media's and biochemical tests.

All bacteriological media used (Nutrient agar, Eosin Methylene blue agar, Mannitol Salt Agar, Xylose-lysine deoxycholate agar, Simmon's Citrate agar, Peptone water) were prepared from commercially available powder according to the Manufacturer's instruction and sterilized by autoclaving at 121°C for 15 minutes (Autoclave, SELECTA, Spain). Using automated pipette, 0.1mL of milk sample was aseptically withdrawn from the sample bottle to make ten-fold serial dilutions using distilled water. Using pour plate method, 0.1 mL of the 8^{th} dilution was poured into the petri dish after which melted Nutrient agar (Oxoid Ltd) was poured and mixed properly before incubating at 37ºC for 24 hours (Incubator, SELECTA, Spain). Total viable counts were carried out on nutrient agar plates manually. The number of colony forming units (CFU) per milliliter were counted and recorded after 24 hours.

3.6.1 Gram staining technique

The Gram staining of the bacterial colony was done on a sterile glass slide as described by (Cheesbrough, 2004). A drop of normal saline was placed on a glass slide and loop full of well-isolated bacteria colony was added and made a smear which was dried in air and fixed by gently flaming. A fixed smear was covered with crystal violet stain for about 2 minutes then, rapidly washed with slowly running tap water and again the smear was covered with Lugol's iodine for about 2 minutes and washed again with tap water. Thereafter, acetone-alcohol was used to decolorize the fixed smear and washed for the third time. Then, the fixed smear was covered with counter stain neutral red that stayed for about 2 minutes then washed off with running tap water. The slide with smear was placed on a draining rack for the smear to dry. A drop of oil immersion was added on the smear and examined under the light microscope with 100X objective to visualize the morphology of the bacteria. Gram positive bacteria appeared spherical or cocci in shape with dark purple colour while Gram negative bacteria appeared rod or coccobacilli with dark red colour.

3.7 Selective Plating and Identification of Isolates

Isolation of specific bacteria was done by streaking on selective media (Eosin Methylene blue agar, Mannitol Salt Agar, Xylose-lysine deoxycholate agar) after gram staining and microscopy. Overnight pure cultures (isolates) were grown on Nutrient agar slant and a loopful of isolates was sub cultured in nutrient agar incubated at 37°C for 24 hours. Mannitol salt agar (MSA) (Oxoid Ltd) was used for isolation of *Staphylococcus aureus*, Eosin Methylene Blue agar (EMB) (Oxoid Ltd) for *Escherichia coli* and Xylose lysine deoxycholate (XLD) (Oxoid Ltd) Agar for *Salmonella* species present in the milk sample. On MSA, colonies that appeared yellowish were presumptively identified as *Staphylococcus* species, colonies that produced greenish

metallic sheen on EMB agar were presumptively regarded as *E. coli*, while reddish colonies with black centers on were identified as *Salmonella* species. Presumptively identified organisms were sub-cultured on nutrient agar slant, incubated at 37°C and stored in refrigerator at 4°C (Refrigerator, Haier Thermocool) for biochemical tests.

3.8 Biochemical Tests

Identification of bacterial isolates were confirmed by biochemical tests. The test include, Indole, Methyl Red, Voges-Proskauer and Citrate (IMVIC), Triple Sugar Iron, Catalase, and Coagulase test following standard methods (Cowan and Steel, 1993). The purity of the isolates was ascertained by plating on the different selective agar before carrying out biochemical tests (Appendix V). Colour changes were observed, recorded according to Cowan and Steel manual.

3.8.1 Indole test

Pure bacterial isolates was grown in sterile peptone water for 24- 48 hours at 37°C. Following incubation, 1-2 drops of Kovac's reagent was added to the tubes. A positive indole test is indicated by the formation of a pink to red color in the reagent layer on top of the medium within seconds of adding the reagent.

3.8.2 Methyl red-voges proskauer test

Bacteria isolates were grown in peptone water for 24 -48 hours at 37°C in two different tubes one for the Methyl Red (MR) test and one for the Voges-Proskauer (VP) test. The pH indicator Methyl Red was added to one tube and a red color indicated positive test. The VP test uses alpha-naphthol and potassium hydroxide to indicate a positive or negative test.

3.8.3 Citrate test

This test uses Simmon's citrate medium to determine the ability of a bacteria to use citrate as its sole carbon source. Bacteria colonies are picked up by a straight wire and inoculated into slope of Simmons citrate agar and incubated overnight at 37°C. If the organism has the ability to use citrate, the medium changes its color from green to blue.

3.8.4 Triple sugar iron (TSI) test

The TSI agar slants were inoculated with pure culture by streaking over the entire surface of the slant and then stabbing deep into the butt. This was incubated at 37ºC for 24 hours. Glucose fermentation was indicated by the butt of the slants becoming yellow and the slant remaining red (K/A). Glucose, Lactose and Sucrose fermentation was indicated by both slant and butt becoming yellow in TSI agar (A/A). No color change indicated that no sugar was fermented. The development or appearance of one or several bubbles in the butt indicated gas formation. Formation of H_2S was determined by the blackening of the whole butt or a streak or ring of blackening at the slant butt.

3.8.5 Catalase test

A drop of 3% hydrogen peroxide solution was placed on a glass slide. A loopful of the test organism was emulsified in the hydrogen peroxide. A positive test was indicated by prompt bubbling and frothing.

3.8.6 Coagulase test

A drop of distilled water was placed on a clean glass slide and a colony picked from the solid medium was emulsified in the saline. A loopful of sheep plasma was added to the bacterial suspension and mixed using the wire loop. The slide was then held up and tilted back and forth for one minute. A positive test is indicated by clumping of cells in the mixed suspension.

3.9 Data Analysis

Data generated from the survey and laboratory were entered into MS excel spread sheets and analyzed using In Vivo stat version 3.7.0.0. The questionnaire data and bacteriological analysis was described using descriptive statistics such as Standard deviation (SD), frequency distribution and percentage and was presented in tables and charts. Colony forming unit (CFU) counts was analyzed using the Paired T- test procedure of In Vivo stat. Means were compared and declared significant at $p \leq 0.05$.

CHAPTER FOUR

4.0 RESULTS

4.1 Questionnaire Survey

Milking practices which compose of 5 items (use of disposable hand-gloves forestripping, predipping, post-dipping and drying) were assessed in the selected farms. A grading system of 20% for those farms practicing only one of the practices, 40% for practicing two of the practices, 60% for practicing three of the practices, 80% for practicing four of the practices and 100% for practicing all the five practices was adopted. The result indicated that Farm A had (20%), Farm B (20%), Farm C (0%), Farm D (0%), Farm E (0%) (Table 4.1).

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Similarly for hygienic practices which compose of 5 items (frequency of cleaning the barn, cleaning of the milking equipment's, frequency of washing milking equipment and use of milking garment during milking), similar grading system was adopted and the result showed that Farms A and B ranked best (60%), followed by Farm E (40%), while Farms C and D had poorest hygiene practices (20%) (Figure 4.1).

Table 4.1: Score on Milking Practices from Selected Farms within Sokoto Metropolis

Farms	Milking practice score	Freq. (%)
A	1	20%
B	1	20%
C	0	0%
D	0	0%
E	0	0%

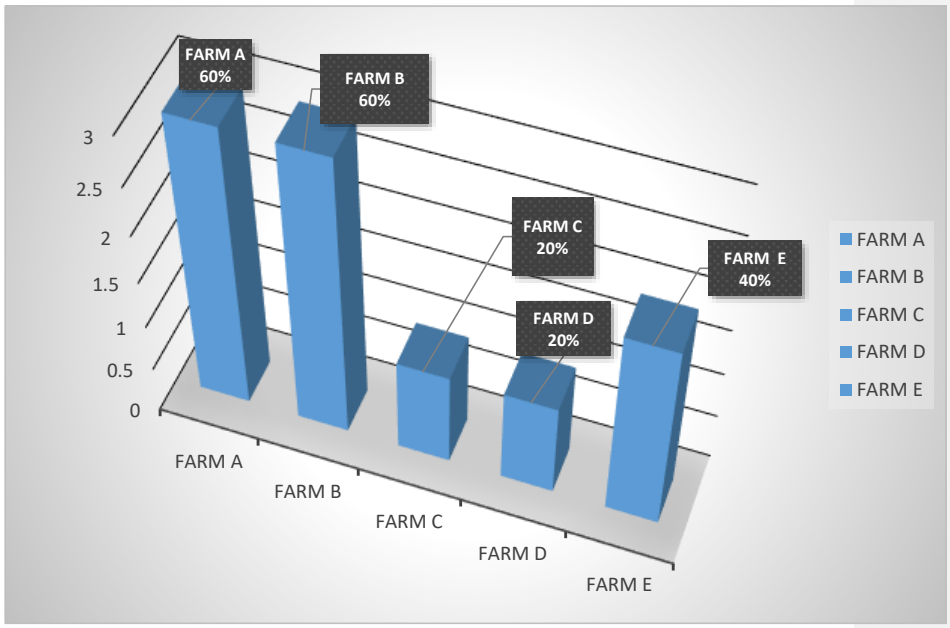

Figure 4.1: Distribution of Scores on Hygienic Practices from Selected Farms within Sokoto Metropolis

4.2 Bacterial and Mean Count

The bacterial load of the raw milk samples obtained from Farms A, B, C, D and E before implementing the milking practices were 2.7 – 5.2 × 10^8 cfu/mL, 2.9 – 5.6 × 10^8 cfu/mL, 4.5 – 6.1 ×10^8 cfu/mL, 3.9 – 6.0 ×10^8 cfu/mL and 3.2 – 5.0 ×10^8 cfu/mL respectively, while the mean counts were 4.7 ± 0.96 ×10^8 cfu/mL, 4.23 ± 0.95 ×10^8 cfu/mL, 5.36 ± 0.60 ×10^8 cfu/mL, 5.20 ± 0.59 ×10^8 cfu/mL and 4.4 ± 0.69 ×10^8 cfu/mL, respectively. The mean counts from the five farms showed that Farm C had the highest count followed by Farm D and the least count is from Farm B (Table 4.2).

After implementing the milking practices, the bacterial load for Farms A, B, C, D and E was 1.4 – 2.9 ×10^8 cfu/mL, 1.8 – 2.7 ×10^8 cfu/mL, 2.2 – 4.1 ×10^8 cfu/mL, 3.1 – 4.5 ×10^8 cfu/mL and 2.0 – 4.5 ×10^8 cfu/mL, respectively, while the mean counts were 2.13 ± 0.45, 2.23 ± 0.34, 3.07 ± 0.59, 3.78 ± 0.32 and 3.02 ± 0.85 respectively. The mean counts from the five farms showed that Farm D had the highest count followed by Farm B and the least count is from Farm A (Table 4.2). The colony forming unit (CFU) before and after applying good milking practices at the farms in this study revealed that implementing good milking practices had a statistically significant reduction ($p < 0.05$) on CFU values of raw milk (Table 4.2).

Table 4.2: Bacterial Count Before and After Implementing the Milking Practices from Selected Farms within Sokoto Metropolis

Farms	Cfu/mL $\times 10^8$ (mean count) Before Implementation	Cfu/mL $\times 10^8$ (mean count) After Implementation	p. values
A	2.7 – 5.2 (4.07 ± 0.96)	1.4 – 2.9 (2.13 ± 0.45)	0.0001
B	2.9 – 5.6 (4.23 ± 0.95)	1.8 – 2.7 (2.23 ± 0.34)	0.0001
C	4.5 – 6.1 (5.36 ± 0.60)	2.2 – 4.1 (3.07 ± 0.59)	0.0001
D	3.9 – 6.0 (5.20 ± 0.59)	3.1 – 4.5 (3.78 ± 0.32)	0.0001
E	3.2 – 5.0 (4.40 ± 0.69)	2.0 – 4.5 (3.02 ± 0.85)	0.0063

(P <0.05)

4.3 Isolation and Identification of Bacteria

Three (3) bacterial species were targeted in this study which are *E. coli*, *Salmonella* spp and *Staphylococcus aureus.* Out of the one hundred (100) samples collected, one hundred and fifty five (155) isolates were obtained following gram staining and microscopy, culture on differential and selective media and biochemical tests, of which 34/155 (22%) were for *E. coli*, 33/155 (21%) were for *Salmonella* spp, 36/155 (23%) were for *S. aureus*, 33/155 (21%) were for other members of the family *Enterobacteriaceae* which was obtained and 19/155 (12%) were for Coagulase negative *Staphylococcus* specie obtained (Table 4.3)

Before and after implementing the milking practices, the number of bacterial isolates from Farm A was 24 and 8, Farm B was 22 and 7, Farm C was 27 and 7, Farm D was 26 and 7, finally Farm E was 24 and 5 respectively, this shows a reduction in the bacteria isolates from all the Farms after implementing the milking practices (Table 4.4).

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Table 4.3: Distribution of Bacterial Isolates from Milk Samples

Isolates	Number of Isolates	Freq. No (%)
E. coli	34	22%
Salmonella spp	33	21%
Staphylococcus aureus	36	23%
Others	33	21%
CONS	19	12%
Total	**155**	**99%**

Keywords:

Others – Includes other members of *Enterobacteriaceae* family

CONS – Coagulase Negative *Staphylococcus*

Table 4.4: Number of Bacterial Isolates Before and After Implementing the Practice from Selected Farms within Sokoto Metropolis

Farms	Number of Isolates Before	Number of Isolates After
A	24	8
B	22	7
C	27	7
D	24	7
E	24	5
Total	**121**	**34**

4.4 Economic Effectiveness of Implementing Milking Practices

The items required for implementing the recommended milking practices includes, disinfectant, disposable hand-gloves, stripping cups, dipping cups and serviette paper or cloth towel. The total cost for purchasing these items in bulk is about four thousand four hundred naira (#4,400). As shown in Table 4.5, the total cost of implementing milking practices per cow per milking shows cost effectiveness, with N176 used to the implementing the practice per cow.

Table 4.5: Total Cost of Implementing Milking Practices per Cow per Milking

Items	**Items Cost (N)**	**Cost per cow per milking (N)**
Disinfectant	600 (125mL)	30 (@ 20mL/cow)
Disposable Hand-gloves	1300 (per pack of 50 pairs)	26 /pair of 50 pairs
Stripping Cup		
Dipping Cups	1200 (24 pcs)	50
Serviette paper	1200 (24 pcs)	50
	100 (per pack of 50 pcs)	20
Total	4400	176

CHAPTER FIVE

5.0 DISCUSSION

5.1 Questionnaire Survey

The result of the questionnaire survey on milking practices showed Farm A and B had a score of 20% (drying of the teat after milking) and Farm C, D, E having the lowest of score. This shows that all the dairy owners lack knowledge on what milking practice is all about and also possess little or no idea on basic hygienic practices that should be implemented in the farm. Similar observation have been reported by Shittu *et al*. 2008 who reported that 68.33% of the dairy farms surveyed lack facilities for milking and 25.42% lack knowledge on proper milking practices.

Also result of questionnaire survey and observation in the study site on hygienic practices showed that milk was generally produced by dairy producers under unhygienic environmental condition. This study further revealed that most dairy farmers milk their cattle in the barn which is not cleaned regularly and may have implications on sources of pathogens for mastitis and other diseases to animals. Meanwhile, such dirty environments are also likely to be sources of milk contaminations. Similar observations have been reported by Shija, 2013 and Bukuku, 2013 in survey carried out in Tanzania, who observed that most small-scale livestock keepers managed their cattle in dirty animal houses that are full of cow dung which are sources of milk contaminations

The present study showed that most of the persons involved in milking activities were also not clean with unhygienic milking environments and milking utensils. All these possibilities predisposed milk to microbial contaminations at farm level.

It was further found that factors that were likely sources of microbial contamination in milk include hand milking in a dirty barns, not dipping the teats before milking and use of poor quality water for cleanliness (hands and milk equipment's).

5.2 Effect of Milking Practices on the Bacteriological quality of raw milk from selected Dairy Farms

In this study, the results obtained from the bacteriological analysis of milk samples from Farm A, B, C, D and E showed that the products were contaminated with bacteria of public health concern. The bacterial count of raw milk samples before implementing the milking practices in this study ranged from 2.7×10^8 cfu/mL to 6.1×10^8 cfu/mL. After implementing the milking practice the bacterial load count of raw milk samples was 1.7×10^8 cfu/mL to 4.5×10^8 cfu/mL. This shows a significant reduction in the total bacterial count, although this exceeds the standard limit (1.0×10^5 cfu/mL) recommended by Bergdoll (1998) and $2.0 – 4.0 \times 10^5$ cfu/mL as recommend by America and European Community on milk production (APHA, 1992).

The Colony forming unit (CFU) before and after applying the milking practices at the farms in this study revealed that implementing these milking practices have significantly reduced CFU values of raw milk. The Food and Agriculture Organization (2008) that recommended these practices be applied to each level, as a result the CFU values were decreased. The CFU values before and after the practice at five farms were analyzed using paired t- test and the mean of the values were significantly different ($p < 0.05$). Similar result was reported by De Silva *et al*. 2015, when milking practice was implemented in five (5) different farms in Sir Lanka, there was a significant reduction in the total colony forming unit from 7.08×10^{10} cfu/mL – 5.94×10^{10}cfu/mL. Also studies done by USDA (Beltsville) showed that pre-milking practice reduced the incidence of new udder infections from 18% to 7%, another studies showed a reduction

in the bacterial counts from (19% -14%) after implementing pre-milking practices (Yuni *et al*., 2015). Similarly, it was well established in a study that proper teat- end disinfection is important in reducing the number of teat surface bacteria by 75% thereby reducing the bacterial contamination in the milk and the rate of mastitis infection (Galton *et al*., 1998).

Milking practices such as use of disposable hand gloves, forestripping, predipping, post dipping and proper drying of the udder and teats should be practiced to ensure quality milk production and reduce bacterial contamination of raw milk within the study area.

5.3 Bacterial contamination of raw milk from selected dairy farms

The result revealed that all the raw milk sampled were contaminated with bacteria pathogens such as *Staphylococcus aureus*, *Salmonella* spp, and *E .coli*. The variation in frequency of occurrence showed levels of contamination in the raw milk analyzed. The microbes might have got into the milk through various sources including, the skin of animal, infected dirty udder, the milkers' hand, milking bucket and feaces. This observation confirms the findings of Frazeir and Westhoof (1998) that these microorganisms grow well in milk and hence endanger its keeping quality. The high numbers of the isolated bacteria observed in this study could be due to the fact that milk being a good nutritive medium enhanced the growth of bacteria contaminants in the milk investigated (IDF, 1994a; Adesiyun *et al*., 1997b).

The detection of bacteria from *Enterobacteriaceae* family such as *E .coli* (22%), *Salmonella* spp (21%) and other *Enterobacteriaceae* species (21%) seen in the raw milk, probably indicates possible faecal contamination (Talaro and Talaro, 2006). Similar bacteria isolates from raw milk have been previously reported by Olatunji *et al*. (2012) which revealed the presence of *E. coli* (24.39%), *Salmonella spp* (17.06%) and *S. aureus* (17.06%) from raw cow milk after milking in Abuja Metropolis. *E. coli* being

an enteric bacteria may indicates poor hygienic practices among milker's which will recommend the use of gloves and milking gowns during milking to reduce bacterial contamination. The presence of these bacteria in milk also suggests contamination from various sources, which may include animal faeces, environment, utensils and others (Murphy and Boor, 2000). Isolation of *E. coli* could be due to faecal contaminated water used in milking, milking bucket, teat/udder of the cow and from the milker's bare hands. The detection of *Salmonella spp* also indicates poor milking procedure and hygienic practice in the selected farms. The 21% prevalence established in this work is of public health importance, since the presence of one *Salmonella* species can lead to recall of food items from the market following the WHO standard (Codex Alimentarius Commission). The microbial contamination of milk is multifactorial, originating from sources like feed, faeces, grasses and milking cow itself. Other possible sources include; improper teat end disinfection teats (predipping), utensils (milking bucket) and unsafe water used in milk processing (Karshima *et al.*, 2013). The fact that milk contains a lot of nutrients made it havens for growth and development of *Salmonella spp* (Ademola and Effiong, 2013). Several reports have documented the prevalence of *Salmonella* in milk. The prevalence of *Salmonella* observed in raw milk was 33%. This is not in agreement with the findings of Rastegar *et al*. (2013) who reported a prevalence of 11% in Iran, and 20% reported by Tadesse and Dabassa (2002) in Ethiopia and higher than 8.7% reported by Karshima *et al.* (2013) in Kanam local government area of Plateau State, Nigeria. These variations may be explained by the differences in management system of dairy farms, milking procedures and hygienic practices. This indicated that milk from some farms in the study area can pose potential risk to public health when consumed or used in production of dairy products such as cheese, yoghurt and ice cream without being subjected to sufficient treatment.

Also another bacteria isolated in this study was *Staphylococcus aureus*. Isolation of *Staphylococcus aureus* from milk products have been reported in other works (Teale, 2002; Jayarao and Wolfgang, 2003; Sato *et al*., 2004). *Staphylococcus aureus* is associated with mastitis; a predominant farm animal disease confronting dairy industries. Mastitis is an inflammation and a highly communicable disease of the bovine udder (Bergdoll, 1998 and Olatunji, 2009).

Some strains of *Staphylococcus aureus* according to Adesanya *et al*., (1995) produce a potent exotoxin. Consumption of a product containing toxin producing strains may result in severe gastroenteritis. Most entero-toxigenic strains of *Staphylococcus spp* are members of coagulase positive group (Adams and Moss, 1995). Thus, only coagulase positive strains are considered potentially entero-toxigenic.

5.4 Conclusion and Recommendations

5.4.1 Conclusion

a The farm owner's and milker's lack knowledge on the proper milking and hygienic practices.

b The CFU count ranged from 2.7 - 6.1×10^8 cfu/mL and 1.4 - 4.5 $\times10^8$ cfu/mL before and after implementing milking practices, showing a reduction in CFU count after.

c From the findings in this study it's therefore concluded that: raw milk samples gotten from the milking bucket immediately after milking from the selected farms within Sokoto metropolis were contaminated with bacteria majorly of *Enterobacteriaceae* group and *S. aureus*.

d The cost of implementing proper milking practices per cow per milking is affordable when implemented.

5.4.2 Recommendations

Based on the results obtained from this study, proper milking practices and hygienic measures should be applied during milking, processing and distribution of milk and its products to avoid contamination by bacteria pathogens. Production of quality milk is not only the responsibility of dairy producers, but it should also concern Government; non-Governmental organizations and consumers in general should be responsible. So far, there was no standard practice for milking in dairy farms within the study area. The state regulatory agency should set a milking process standard based on the local condition and routinely control the quality of milk produced by such urban and peri - urban producers. Raw milk should be boiled using available materials at pasteurization time and temperature. In addition to microbial quality testing, drug residue and other tests, and identification of all contaminants at sub species level should be conducted. Adequate hygienic measures should be taken at all stages from milking to consumption to provide wholesome sound dairy products to the needy society.

It is therefore recommended that regular enlightenment campaign by State Government in collaboration with the Stakeholders (Veterinary Professionals) in the State should be embarked upon emphasizing the need to implement proper milking practices and basic farm hygienic measures in the dairy farms.

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APPENDIX I: Questionnaire Survey Format

Number:…… Date:……

1. Farm name_____ Area_____
2. Herd size_____ Breed: Local_____ Cross _____ Exotic_____
3. Source of water for the farm.

   (i) Pipeline water (ii) Well (iii) Others (specify)
4. Where are your cows milked? (i) In barn (ii) In milking room (parlor)
5. How often do you clean milking room or Barn?

   (i) Twice a day (ii) Once a day (iii) Per three days (iv) Once a week
6. How do you milk your cow? (i) Hand milking (ii) Using milking machine
7. Milking frequency per day: (i) Once (ii) Twice (iii) Three or more times
8. Do you wash hands and/or wear gloves during milking? (i) Yes (ii) No
9. If yes, when do you wash? (i) Before milking (ii) After milking (ii) Both.
10. Do you use paper or cloth towel for drying the teat? (i) Yes (ii) No
11. Do you fore-strip before milking? (i) Yes (ii) No
12. Do you pre-dip before milking? (i) Yes (ii) No
13. Do you post –dip after milking? (i) Yes (ii) No
14. How do you keep milking equipment and other utensils clean?

    Washing with: (i) Warm water (ii) Cold water (iii) Both alternatively
15. Use of detergent: (i) Yes (ii) No
16. How regularly are your milking equipment washed?

    a. (i) Daily (ii) Weekly (iii) Monthly
17. What do you wear during milking? (i) Boot and outer garment (gown) (ii) Personal cloth?
18. Where does the milk go? (i) To household consumption (ii) To processing plants

    (iii) To local consumers (iv) To restaurants (iii) Others (specify) _____

Thank you

APPENDIX II: Total Bacterial Count (CFU/mL) of Samples Before and After Implementing Milking Practices from Selected Dairy Farms in Sokoto Metropolis.

FARM A		FARM B		FARM C		FARM D		FARM E	
Lab No.	CFU/ml	Lab No.	CFU/ml	Lab No.	CFU/ml	Lab No.	CFU/ml	Lab No.	CFU/ml
BEFORE		BEFORE		BEFORE		BEFORE		BEFORE	
A1	2.8	B1	2.9	C1	4.5	D1	5.8	E1	4.1
A2	2.7	B2	3.2	C2	5.8	D2	5.3	E2	4.8
A3	3.1	B3	4.0	C3	5.7	D3	5.6	E3	4.7
A4	4.2	B4	3.2	C4	5.1	D4	5.4	E4	4.9
A5	5.1	B5	3.8	C5	6.1	D5	4.9	E5	3.8
A6	4.8	B6	4.5	C6	6.0	D6	4.8	E6	3.2
A7	3.8	B7	5.0	C7	4.8	D7	6.0	E7	3.5
A8	4.0	B8	5.2	C8	4.7	D8	5.1	E8	4.9
A9	5.0	B9	5.6	C9	5.9	D9	5.2	E9	5.1
A10	5.2	B10	4.9	C10	5.0	D10	3.9	E10	5.0
AFTER		AFTER		AFTER		AFTER		AFTER	
A1	2.0	B1	1.9	C1	2.8	D1	3.1	E1	2.2
A2	2.1	B2	2.0	C2	3.2	D2	3.4	E2	2.4
A3	2.1	B3	2.5	C3	3.1	D3	3.8	E3	3.1
A4	2.2	B4	2.2	C4	3.0	D4	4.1	E4	3.5
A5	1.8	B5	2.0	C5	4.0	D5	3.8	E5	2.3
A6	1.7	B6	1.8	C6	4.1	D6	3.7	E6	2.0
A7	1.4	B7	2.7	C7	2.9	D7	3.6	E7	2.8
A8	2.5	B8	2.5	C8	2.2	D8	3.9	E8	3.2
A9	2.6	B9	2.7	C9	2.6	D9	4.0	E9	4.5
A10	2.9	B10	2.0	C10	2.8	D10	4.5	E10	4.2

CFU = Colony Forming Unit

APPENDIX III: Number Bacteria Isolates Before Implementing Milking Practices from Selected Farms within Sokoto Metropolis

Isolates	Farm A	Farm B	Farm C	Farm D	Farm E	Total
E. coli	5	4	5	6	5	25
Salmonella spp	3	4	7	6	7	27
Staphylococcus aureus	5	5	5	7	4	26
Others	7	5	6	5	3	26
CONS	2	4	4	2	5	17
Total	**22**	**22**	**27**	**26**	**24**	**121**

Keywords:

Others – Includes other members of *Enterobacteriaceae* family

CONS – Coagulase Negative *Staphylococcus*

APPENDIX IV: Number Bacteria Isolates After Implementing Milking Practices from the Selected Farms within Sokoto Metropolis

Isolates	Farm A	Farm B	Farm C	Farm D	Farm E	Total
E. coli	2	1	2	2	2	9
Salmonella spp	2	1	2	1	0	6
Staphylococcus aureus	2	2	2	2	2	10
Others	1	3	1	1	1	7
CONS	1	0	0	1	0	2
Total	**8**	**7**	**7**	**7**	**5**	**34**

Keywords:

Others – Includes other members of *Enterobacteriaceae* family

CONS – Coagulase Negative *Staphylococcus*

APPENDIX V: Typical Biochemical Reactions of Bacterial Species from Milk Samples from Selected Dairy Farms

IND	MR	VP	CIT	TSI	CAT	COA	ISOLATES
+	+	-	-	A/AG	NT	NT	*E. coli*
+	+	-	-	A/AG	NT	NT	*Proteus spp*
-	+	-	-	K/A	NT	NT	*Salmonella spp*
-	+	+	+	NT	+	+/-	*Staph. spp*
-	+	-	+	K/AG	NT	NT	*Citrobacter spp*
-	-	+	+	A/AG	NT	NT	*Klebsiella spp*
-	+	-	+	A/A	NT	NT	*Yersinia spp*

KEY: IND = Indole, MR = Methyl-red, VR = Voges-Proskauer, CIT = Citrate, TSI = Triple sugar iron, CAT = Catalase, COA = Coagulase, A = Acid, K = Alkaline, G = Gas, NT = Not tested, + = Positive, - = Negative.

APPENDIX VI

Predipping observed in a study farm when implementing milking practices

APPENDIX VII

Drying of the teat using a serviette paper after milking observed in a study farm when implementing milking practices