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Assessment of Hygiene Practices and Bacteriological Quality of Raw Cow Milk of Selected Dairy Farm in Dessie, Ethiopia

Zakariye Abdifatah Ahmed[1], Mohamedamiin Hasan Kasim[2*], Betelihem Tegegne[3], Hussein Mohamed Salah[1]

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ABSTRACT

A cross sectional study was conducted to assess the hygienic practices and bacteriological quality of milk in Dessie town, South Wollo zone in Amhara regional state, Ethiopia. The data was generated by interviewing 60 dairy cow owners and farm attendants using semi-structured questionnaire regarding milking area and barn hygiene, hygienic practice of milker, cleaning of the udder and milking equipments. The result showed that the majority of the interviewers (98.34%) milked their cows in the barn, about 88.33% clean the barn daily and others clean once, twice and three times a week. All respondents washed their hands before milking. All respondents practiced udder washing before milking but only 30% of the respondents used towel for udder drying. Plastic utensils were mainly used for milking cows and used detergents and warm water for cleaning milking equipments. Lower number of respondents (36.66%) had a habit of consuming raw milk. From a total of 70 milk samples were collected from two milking points (the teat and milking bucket), The mean total bacterial count of milk samples collected directly from the teat and the milking buckets from current finding were 5.087log10 and 5.759log10 respectively. The mean difference (0.6728 ± 0.1579) indicated the increment of bacterial count from teat to the milking bucket. The results showed very significant difference between the two points ($P<0.05$). About 65.71% of milk samples collected directly from the teat and milking buckets at the farm were above the accepted level 105 according to the international milk quality standard. The high count of bacteria in the milk suggests a poor bacterial quality of milk and indicating poor hygienic quality of milk. Keeping the quality of the milk is the responsibility of dairy cow owners, institutions concerned and the government. This needs training of milk producers on the importance of milk hygiene and raising awareness on risks of consumption of raw milk.

INTRODUCTION

Milk is an important source of nutrients to human and animals and it is meant to be the first and the only food for the offspring of mammals as is almost complete food (Pandey & Voskuil, 2011). Cow milk has long been considered a highly nutritious and valuable human food and is consumed by millions daily in a variety of different products in the world (Ali, 2010). It is also an economically important farm commodity and investment option for smallholder farmers in developing countries. (Haile *et al*., 2012). In Ethiopia, milk and milk products are mainly used for home consumption as it have high nutritional value. In addition, it is a source of cash income to purchase farm inputs like feed, fertilizer and improved crop varieties as well as food and non-food items like educational materials for their children (Melese & Tesfaye, 2015).

Raw milk of good hygienic quality meets the nutritional needs of body better than any single food as it contains essential food constituents such as fat, proteins, carbohydrates, minerals and vitamins (Medhammar *et al*., 2012). However due to its high water activity and nutritional value it serves as an excellent medium for growth of many kinds of microorganism under suitable environment (Mesfin *et al*., 2017). Milk is often prone to early contamination and spoilage if not handled properly (Ekici *et al*., 2004).

Microbial contamination might generally occur from three main sources: within the udder, exterior to the udder and from the surface of milk handling and storage equipment's, but the surrounding air, feed, soil, faeces and grass are also possible sources of contamination. Microorganisms may contaminate milk at various stages of procurement, processing and distribution. The safety of raw cow milk is influenced by a combination of management and control measures along the entire dairy supply chain. The quality and safety of raw milk can be evaluated by assessing hygiene indicator microorganisms. In developing countries, the production of milk is said to be taken place below standard sanitary practices, ineffective farm management and hot tropic weather. All these conditions have contributed to spoilage and economic loss to the milk industry (Worku *et al*., 2012; Yuen *et al*., 2012).

Microorganisms present in milk can be classified into two main groups: pathogenic and spoilage organisms. Pathogenic organisms are those capable of inducing food poisoning, thus posing a threat to public health (Logan, 2012). These pathogenic microbial contaminants in milk have been a major factor for public health concern since the early days of dairy industry (AL tug & Bayrak, 2003). Milk meant for human consumption must be free from any pathogenic organisms (Bertu *et al*., 2010). Human may be infected with milk-borne pathogens through

[1] Department of Veterinary Medicine, Somali National University, Mogdishu-Somalia.
[2] School of Veterinary Medicine, Wollo University, Ethoipia
[3] Department of Veterinary Laboratory Technology, School of Veterinary Medicine, Wollo University, Dessie, Ethiopia.
[*] Corresponding author's e-mail: amiinqasim202@gmail.com

consumption of infected raw or unpasteurized milk and milk products. Sometimes consumption of contaminated or spoiled milk and dairy products may cause milk-borne diseases in humans. Bacteria that mostly grow in milk are Lactobacillus, Streptococcus, Coliforms, Staphylococcus and Micrococcus spps (Torkar & Teger, 2008) of which Escherichia coli, Salmonella tyhpi, Pseudomonas aeruginosa and Staphylococcus aureus are the fast rate in milk (Murinda *et al*., 2004: Oliver *et al*., 2005). The growth of microorganisms in milk results in spoilage of milk and milk products which brings infections or intoxications to consumers. Contaminated raw milk and milk products may act as a source of many harmful bacteria leading to various diseases, such as undulant fever, salmonellosis, dysentery and tuberculosis (Oliver *et al*., 2005).

There is a constant challenge to those involved in milk production to prevent or minimize the entry and subsequent growth of microorganisms in milk. These is mainly due to the importance of producing milk of good hygienic quality, which is necessary to milk product of superior quality and prolonged shelf-life thereby to provide a safe and wholesome food for the consumers (O'Connor, 1994).

In Ethiopia, milk hygiene and handling practice is below the standard due to insufficient pre- milking handling practices like washing udder with clean water, cleaning milking barn, drying the udder with individual towel, washing of milkers' hands and milking utensils, using of poor quality and non-boiled water for cleaning of udder and insufficient post handling practice like poor hygiene of milk equipment and storage containers, prolonged storage, transportation and retailing practices predispose the milk to microbial contamination (Tsedey & Asrat, 2015; Fufa *et al*., 2019). Control of animal health, adherence to good milking practices, and control over milking parlour hygiene are important in reducing the microbial load in raw milk (FSA, 2006).

Even though milk and milk products represent an important place in the nutrition of consumers as well as nutrition and income of producers, there is limited work so far undertaken regarding assessment of bacteriological quality of raw cow milk in Dessie town dairy farms. So, determining the current status of bacteriological quality of milk and assessing hygienic practices of the dairy farms would create awareness on the bacterial safety in the milk and support for strengthening the hygienic standards practiced at different levels of the production chain.

Therefore, the aim of this study was to assess hygienic practices and examine the bacteriological quality of milk in selected dairy farms in the study area.

LITERATURE REVIEW

Composition of Milk

Milk is a considerable resource of products whose composition varies. Four components are dominant in quantitative terms: water, fat, protein and lactose; while the minor components are minerals, enzymes, vitamins, and dissolved gases. It satisfies the demand of the consumer who seeks more and more innovative products with consistent quality. The dairy industry needs to utilize all the riches of this raw material, which is both simple in appearance and complex in composition (Guetouache *et al*., 2014).

The nutritional value of milk is particularly high due to the balance of the nutrients that compose it. The composition of milk is extremely complex, consisting chiefly of water, protein in colloidal suspension, lactose and fats in emulsion, inorganic salts in solution, vitamins, enzymes, gases and other substances (Woldecherkos and Yitayal, 2003). Milk is also an outstanding source of calcium and phosphorus for bones and teeth, and contains riboflavin, vitamin B1, vitamin B6, vitamin B12, and vitamin A in significant amounts (Yirsaw, 2004).

The composition varies among animal species and breeds within the same species, and also from one dairy to the other, depending on the period of lactation and diet. The proteins in milk are of great quality, that is to say, they contain all the essential amino acids, and elements that our bodies cannot produce. It is important to remember that proteins are the building blocks of all living tissue. The major groups of milk proteins are caseins and whey proteins. The major lipid component of cow's milk is triglyceride, which makes up about 95% of milk fat. Lactose (milk sugar) is the major carbohydrate in the milk of most mammals; hence mammalian milk is the major source of lactose, one of the most common natural disaccharides. Lactose consists of two molecules, D-glucose and D-galactose and is digested or broken down into these constituents by the enzyme lactase (Cawe, N.B., 2006). Milk also comprises functional elements, such as traces of vitamins, enzymes and dissolved gases, and contains dissolved salts, especially in the form of phosphates, nitrates and chlorides of calcium, magnesium, potassium and sodium (Guetouache *et al*., 2014).

Composition of Cow milk

Constituent	Percentage %
Water	87.2
Fat	4.0
Protein	3.4
Lactose	4.5
Ash	0.9
Total	100.0

Source: (Atkins, 2005)

Milk production in Ethiopia

Ethiopia holds a substantial potential for dairy development mainly due to its large livestock population coupled with the relatively suitable environment for livestock production (Bereda *et al*., 2014). According to the Ethiopian central statistical authority report (2020), Ethiopia has 65.35 million cattle, 39.9 million sheep, 50.5 million goats, 28 million horses, 9.9 million donkeys, o.46 million mules, 4.8 million camels and 60 million poultry.

Milk production in Ethiopia is mainly dependent on

indigenous breeds; more specifically on cattle, goats, camels and sheep. Cattle have the largest contribution (81.2%) of the total national annual milk output, followed by goats (7.9%), camels (6.3%) and sheep (4.6%) (CSA, 2014). The estimate of total cow milk production for the rural and sedentary areas of Ethiopia is about 3.06 billion liters (CSA, 2017).

The average daily milk yield (ADMY) performances of indigenous cows is 1.85 liters/day and ranges from 1.24 liters in rural lowland agro-pastoral system of Mieso to 2.31 liters in rural highland dairy production system of Fogera (Azage *et al*., 2013). For hybrid cows, milk production per day is 8 to 10 liters (Tadesse *et al*., 2015). Currently, in Ethiopia per capital consumption is very low, estimated at about 19 liters per person, but urbanization is driving up consumption in Addis Ababa about 52 liters per person per year (Azage *et al*.,2013).

Milk production systems in Ethiopia are generally classified into three categories based on geographical location: rural, peri-urban and urban. In the rural system, milk is mainly produced for household consumption, and leftovers are sold in local informal markets. The rural system has been reported to account for 98% of the country's milk production. The minority of milk was reported to be produced in peri-urban systems that include smallholder and commercial farms in the suburban areas near cities that have available grazing land. Lastly, a small proportion of milk is produced in urban areas that are limited to farms close to cities, and that have no access to grazing lands (Tadesse, 2018).

Hygienic Quality of Milk and Microbial Contamination

Hygienic practices in milk production

Hygienic production of milk is important for the quality of milk and the safety of consumers. Facility hygiene comprises amongst others the cleanliness of the barn, access alleys and milking parlour, and is an integral part of hygienic milk production and quality control program (Vissers and Driehuis 2008). The cleanliness of cows (e.g. udder and teats) and, thus, microbial contamination of milk via the exterior of teats and the incidence of mastitis are affected by measures related to facility hygiene. They include, for example, regular removal of dung from the barn, regular refreshment of bedding materials, clean entries to the milking parlour, one or more cubicles per cow and non-crossing walking paths ((Ruegg, 2003).

The hygienic conditions are different according to the production system, adapted practices, level of awareness, and availability of resources. In under small holder condition, the common hygienic measures taken during milk production especially during milking are limited to letting the calf to suckle for few minutes and/or washing the udder before milking (Muleta, 2016).

Maintaining a high standard of hygiene is one of today's most important milk production objectives. The hygiene level directly influences the production's economical result and dairies are enforcing this by steadily raising their quality requirements for raw milk. More importantly though, consumers are concerned about the safety of dairy products and the conditions under which these are produced. It is therefore critically important to ensure high quality raw milk can be produced from healthy animals under good hygienic conditions and that control measures are applied to protect human health. Good hygienic practice is very important in the production of clean milk (Girma *et al*., 2014). Clean milk has the following characteristics low bacterial count, pleasant creamy smell and colour, no obnoxious odours, No dirt and extraneous matter and No residues of antibiotics, sanitizers or pesticides (Kurwijila, 2006)

Hygienic practice during milking

An efficient hygiene practice during milking should begin at the farm. Microbiological hazards can be introduced to the milk from the farm environment and the milking animals themselves. Appropriate animal husbandry practices should be respected and care should be taken to assure that proper health of the milking animals is maintained. Further, lack of good agricultural, animal feeding and veterinary practices and inadequate general hygiene of milking personnel and equipment and inappropriate milking methods may lead to unacceptable levels of contamination with chemical residues and other contaminants during primary production Depiazzi, L. and Bell, I. (2002).

Effective handling practice during milking is important and necessary element to produce safe and suitable milk and milk products. Failure to maintain adequate sanitation practices has been shown to contribute to contamination of milk with undesirable or pathogenic micro-organisms or chemical or physical hazards (Anwer *et al*.,2018). Bacteria find accidental access to milk may give rise to consumer's health problems or product faults. Bacterial contamination of milk can all be minimized by starting the manufacturing process with raw milk of good hygienic quality (Mirkena, 2010).

Milk when it emerges from a healthy udder contains only a very few bacteria. However, milk is a perishable product. It is an ideal medium for micro-organisms and as it is a liquid, it is very easily contaminated and invaded by bacteria. Almost all bacteria in milk originate from the air, dirt, dung, hairs and other extraneous substances. In other words, milk is mainly contaminated with bacteria during milking. It is possible to milk animals in such a clean way that the raw milk contains only 500 to 1,000 bacteria per ml. usually the total bacteria count after milking is up to 50,000 per ml. However, counts may reach several millions of bacteria per ml. That indicates a very poor hygienic standard during milking and the handling of the milk or milk of a diseased animal with i.e. mastitis (Pandey and Voskuil, 2011). Poor hygiene introduces additional bacteria that cause the milk to get spoilt very quickly. To ensure that raw milk remains fresh for a longer time, you need to practice good hygiene during milking and when handling the milk afterwards (Lore *et al*., 2006).

Milking procedure

It is important to remember that quality control must begin at the farm. Hygienic milking operations start with a clean and stress-free milking environment, teat cleaning, pre-dipping, fore- stripping, careful attachment of the teats cups and post-milking teat disinfection. Teat cleaning is performed to reduce the microbial load on the teats prior to milking. Pre-dip agents are often used to disinfect the teats prior to milking and reduce the risk of environmental mastitis. Pre-dipping should be applied with care since residues may contaminate milk. Fore-stripping is expressing two or three streams of milk before attachment of the milk liners in order to visibly check the milk quality and to stimulate milk let down. Post-milking teat disinfection is important to increase the hygienic defense against infection of the teats after milking is completed (Vissers and Driehuis, F. 2009). In this operations, the milk will have fewer bacteria that cause spoilage and diseases. In order to ensure good quality and protect the health of consumers, one must always carry out milking in accordance with good hygienic practice (Lore *et al*., 2006). A good milking technique is essential to produce safe, raw milk (FSA, 2006).

Cleaning of Milk Handling Equipment

Equipment used for milk handling, storage, and transportation has an effect on the safety and quality of milk and is a major source of microbial contamination (FSA, 2006; SNV, 2017). Cleaning of milk handling equipment is accomplished by a combination of chemical, thermal and physical processes which when combined have a minimum reaction time to be effective (Carlin, 2011). The milk house is a critical place on a dairy farm for maintaining sanitation to produce high quality milk. Milking machines are usually cleaned at least twice and sometimes three times per day, corresponding with the milking frequency of the herd. Milking machines must be cleaned more frequently than equipment in diary plants because of the increased soil load and greater bacterial load resulting from handling a non-pasteurized product (Reinemann, 2003).

All milking equipment, lines, and utensil surfaces that come into contact with milk or dirt or manure must be thoroughly cleaned and sanitized before the next milking. Bulk milk tanks also must be cleaned after each milk pickup, and sanitized before the next milking. The purpose of cleaning is to remove milk soils, organic and mineral solids that form on equipment surfaces after the milk is removed. The purpose of sanitizing is to kill residual microorganisms present on these surfaces immediately prior to milking. Inadequate or improper cleaning or sanitizing or both allows bacteria to remain on equipment surfaces and to grow and multiply. This results in elevated bacteria counts in milk (Jones, 2009).

A very important item of the milk transport business is the vessel in which the milk is carried (Kurwijila, 2006). In addition, all milk handling vessels should be washed and disinfected immediately after use as follows. Pre-rinse with clean potable water, thoroughly scrub the container with warm water and detergent/soap using a suitable brush or scouring pad (do not use steel wool or sand), Rinse the container with clean running water, Immerse the container in boiling water for at least one minute, Sundry the container upside down on a drying rack.

Source of contamination for Milk

Milk can be contaminated at any point in the milk production process. It is the responsibility of the food business operator (milk producer) to identify these points and implement control measures to protect milk from contamination. Microbial contamination might generally occur from three main sources; within the udder, exterior to the udder and from the surface of milk handling and storage equipments, but the surrounding air, feed, soil, feces and grass are also possible sources of contamination (Mosu *et al*., 2013).

Microorganisms are mainly transferred from the farm environment to milk via dirt (e.g. faeces, bedding and soil) attached to the exterior of teats. In addition, microorganisms attached to the exterior of the teats can enter the teat canal and cause mastitis. Finally, contamination can originate from insufficiently cleaned milking equipment when, during milking, microorganisms adhered to surfaces of the milking equipment are released into the milk (Vissers and Driehuis, 2008). The common predisposing factors of milk contamination by microorganisms are milking environment, cows, milking personnel, milking equipment, milk transportation and water (Bekuma and Galmessa, 2018).

Contamination within the udder Healthy udder

Fresh milk drawn from a healthy cow normally contains bacterial load of less than 10^3 CFU/ML (Chatterjee *et al*., 2006 and Lingathurai, 2009). But when drawn from the udder of a healthy cow, milk gets contaminated at various stages including the cow itself, the milker (manual as well as automated) i.e. the milker's hand or milking equipment, storage vessels and water supply particularly when used for adulteration (Edward and Inya, 2013).

In healthy cows, the teat cistern, teat canal, and the teat apex may be colonized by a variety of microorganisms, though microbial contamination from within the udder of healthy animals is not considered to contribute significantly to the total numbers of microorganisms in the bulk milk, nor to the potential increase in bacterial numbers during refrigerated storage. Natural flora originated of the cow generally has little influence on total plate counts (Murinda t al.,2004)

Infected udder

Mastitis is an inflammation of the mammary glands in the udder caused by infection with disease- causing bacteria. These bacteria can also end up in the milk and result in illness if the milk is consumed. 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 (Yirsaw, 2004). The bacteria which are naturally present on the skin of animal enter into milk from the surface of the udder and teats; these also include the bacteria which are present in milking and housing places of animals (Ali *et al*., 2010).

Exterior of the udder

The exterior of the cows' udder and teats can contribute microorganisms that are naturally associated with the skin of the animal as well as microorganisms that are derived from the environment in which the cow is housed and milked (Nangamso, 2006). The bacteria which are naturally present on the skin of animal enter into milk from the surface of the udder and teats; these also include the bacteria which are present in milking and housing places of animals (Ali *et al*., 2010). Teats and udders of cows inevitably become soiled while they are lying in stalls or when allowed in muddy barnyards. Used bedding has been shown to harbor large numbers of microorganisms. Contamination of bedding material can be very high due to absorption of urine and feces (Yirsaw, 2004).

Udder preparation

Careful cleaning of the cow prior to milking significantly reduces contamination. Cleaning the udder of cows before milking is one of the most important hygienic practices required to ensure clean milk production (Zelalem, 2010). This is important since the udder of the milking cows could have direct contact with the ground, urine, dung and feed refusals. Cleaning and removal of soil particles, bedding material and manure from the udder and flanks is necessary to prevent the entry of many types of bacteria into the milk. Special care must be given to the cloths used for cleaning the udder. The re-use of cloths for cleaning and sanitizing may result in re-contamination of the udder. It is therefore recommended that separate cloths be used for cleaning and sanitizing and, if possible, each cloth should be used for one cow only ((O'Connor, 1995).

A maximum reduction of teat contamination of 90% can be achieved with good udder preparation before milking. This depends on the initial level of contamination and the way of udder preparation. So, with high initial contamination levels this 90 % reduction might not be reached (Bekuma and Galmessa, 2018).

The milker

The milker can be an important source of milk contamination and hence should keep their personal hygiene and be in good health during milking operation. Milk handling personnel (milker) may contribute various organisms including pathogens especially when they are careless, uninformed, or willfully negligent, directly to milk. Organisms may drop from hands, clothing, nose, and mouth and from sneezing and coughing. It is important for milk men to be in good health so that they can be a source of infectious diseases such as tuberculosis (Kurwijila, 2006). Sterile milk from a normal cow's udder becomes contaminated during milking, cooling, storage and processing (Yirsaw, W. (2004).

Milking and handling personnel should be healthy and acknowledge the importance of cleanliness. Wet milking should be avoided as organisms present on the milker's hands, cow's teats and udder are washed into the milking utensil contaminating milk and leading to spoilage. Other sources of microorganisms are nasal cavities, mouth, dirty hands, skin and the gastrointestinal tract of both the milker and the animal (Mbabazi, 2005).

The milking equipments

Contamination of milk via the milking equipment occurs when (a) microorganisms adhere to surfaces of the milking equipment and (b) milk residues that remain in the equipment after the cleaning cycle. Under these conditions, growth of adhered microorganisms may occur, especially in cracked and decayed rubber parts that are sensitive to accumulation of microorganisms. During the next milking, adhered microorganisms can be released into the milk (Vissers and Driehuis, 2008).

Proper cleaning of equipment used for storage, processing and further handling of milk and milk products are essential to keep microbial contamination of the products to a minimum. As a means of protecting milk from contamination and to extend its shelf life, it is a common practice to use stems and leaves of different types of plants for cleaning and smoking of milk handling Equipment (Welearegay *et al*., 2012). Among the factors that affect the quality of dairy products, adequately performing milking procedures and cleanness of the milking utensils is commonly mentioned.

Environment

Maintaining the sanitary condition of the milking area is important for the production of good quality milk. Dirty milking places tend to breed flies, which may fall in milk causing contamination and thus spoilage may occur (Mbabazi, 2005). When a cow urinates or defecates in the course of milking some of its urine or dung particles may drop into the milk.

Microorganisms are mainly transferred from the farm environment to milk via dirt (Faeces, bedding and soil) attached to the exterior of teats; in addition, microorganisms attached to the exterior of the teats can enter the teat canal and cause mastitis (Vissers and Driehuis, 2008). Practices that expose the teat end to organic bedding sources, wet and muddy pens increase the risk of occurrence of mastitis and milk contamination (Ruegg, 2006).

Control of Milk Spoilage

Milk quality across the value chain could be improved through; changing milking practices to ensure better hygienic conditions, improvement of milk handling and improvement of storage conditions maintaining the cold chain (Mbabazi, 2005). Milk should therefore be cooled to 4C0 and transported in insulated trucks for quality delivery. Planners should consider the relative efficiency

of alternative milk marketing systems in terms of costs and marketing margins, product hygiene and quality range to avoid losses due to spoilage (Mbabazi, 2005). Milk should be handled in containers which are made of seamless stainless steel without cracks where bacteria can lodge and multiply leading to spoilage and these containers should be unaffected by milk or by chemicals used in cleansing.

Bacteria in Milk

Milk is considered as a universal food. The nutritive perfection of milk has made it not only a unique food for human being but also a medium most favorable for microbial growth. Microorganisms which may gain entry into milk can multiply and bring about spoilage of milk and milk products and render them unsafe due to potential health hazards (Islam *et al*., 2009).

The list of bacteria which can be responsible for milk-borne diseases is long and it includes Brucella spp, Campylobacter jejuni, Bacillus cereus, Shiga toxin-producing E. coli (E.coli O157:H7), Coxiella burnetii, Listeria monocytogenes, Mycobacterium tuberculosis, Mycobacterium bovis, Mycobacterium avium subspecies paratuberculosis, Salmonella spp, Yersinia enterocolitica, and certain strains of Staphylococcus aureus which are capable of producing highly heat-stable toxins. Microorganisms such as Escherichia coli, Pseudomonas aeruginosa, Citrobacter spp, Klebsiella spp and Proteus mirabilis can multiply in the normal summer temperatures and hence unpasteurized milk has every chance of containing E coli (Dhanashekar *et al*., 2012).

Bacteriological Quality Tests of Milk Alcohol test

Alcohol test is a very rapid test to determine overall quality of milk. It relies on how milk reacts with alcohol. Fresh or normal milk will not react with the alcohol. Milk that is high in acidity contains salt concentrates often coagulates when exposed to alcohol (Ebner *et al*., 2016).

Clot on boiling test

The test is quick and simple. It is one of the old tests for too acid milk. The test is used to determine the overall quality of milk. The test simply boils milk and looks at physical characteristics once cools. When high quality milk is boiled, it remains a fluid with a general milk appearance once it is cooled. Milk that is acidic (an indication of bacterial growth) or adulterated milk cannot withstand boiling and will coagulate and form lumps and flakes once cooled (Ebner *et al*., 2016).

Methyl blue reduction test

The methylene blue reduction test is based on the fact that the color imparted to milk by the addition of a dye like methylene blue will disappear more or less quickly. The removal of the oxygen from milk and the formation of reducing substances during bacterial metabolism cause the color to disappear (Anwer *et al*., 2018).

Methylene blue is a blue-colored reagent which is used to estimate the bacterial population of a given milk sample. 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. 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 (Mahari and Yemane, 2016). 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.

Resazurine reduction test

This test is also used for grading the sanitary quality of raw milk by applying the chemical reagent Resazurine (Yirsaw, 2004). This test is based on the reduction of the oxidation/reduction indicator Resazurine to Resorufine and finally to dihydroresorufine. Resazurine imparts a blue colour to milk which when reduced to resorufin changes to pink and finally to white when reduced to dihydroresorufin. The test is a good indicator of the bacteriological quality of milk. The time required for complete decolorization, reduction of the Resazurine andthe degree of colour change is directly related to the number of bacterial organisms in the milk. A comparator disc reading value of 4 and above for 10 minutes Resazurine test indicates good quality but while a comparator disc reading value of less than 4 at 10 minutes indicates poor quality milk.

Standard Plate Count Test

Standard plate count (SPC) is one of the most commonly used microbial quality tests for milk and milk products. The standard plate count of raw 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 is a basis for grading milk (Mahari and Yemane, 2016). Milk samples are plated in a standard plate count agar media and then incubated for 48 hours at 32°C to encourage bacterial growth. Single bacteria or tight clusters (e.g. chains or clumps) grow to become visible colonies that are then counted. All bacterial plate counts are expressed as the number of colony forming units (cfu) per milliliter (ml) (Count, S.P, 2008).

Aseptically collected milk from clean, healthy cows generally has SPC values of less than 1,000 cfu/ml. Higher counts suggest that contaminating bacteria are entering the milk from a variety of possible sources. 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 (Count, S.P, 2008).

Other tests which can be used to assess the quality of milk are coliform counts, organoleptic tests, sedimentation test, specific gravity, free

METHODOLOGY

The paper used Cross sectional study was conducted from March to August 2021 to asses hygienic practices, magnitude of bacteriological quality of raw cow milk produced in selected dairy farms in the study area. Purposive sampling was used based on the accessibility, willingness to participate by the dairy animal owner and having lactating cow.

The target animals in this study were lactating dairy cows in Dessie and they were sampled in irrespective of their breed and body condition and Semi-structured questionnaire was used to collect information from the selected dairy farms and small dairy holder owners around the study area such as individuals involved in milking as well as handlers of the milk. A total of 60 participants were interviewed. The questionnaire was designed to get information on possible risk factors for bacterial contaminations in milk. Risk factors such as sanitary conditions of the house, udder cleaning, hand washing practices, hygiene of milking equipment's such as milk handling practices, milk containers used for milking were assessed in this study. The questionnaire was administered through face-to-face interview. While administering questionnaires, direct observation on general cleanliness and hygienic conditions and practices about milk was also done and noted.

Raw cow milk samples were collected directly from teats and milking buckets immediately after milking for bacteriological analysis. A total of 70 milk samples were collected. The samples were collected aseptically in sterilized universal bottles and the bottling samples was capped, kept in icebox and transported to Wollo university, school of veterinary medicine microbiological laboratory and then was stored in refrigerator at 4°C before being analyzed within 24hrs of sampling.

The total bacterial count was made by adding 1ml of sample into sterile test tube having 9ml normal saline solution (NSS). After thoroughly mixing; the sample was serially diluted up to give 1:10 dilution 'first dilution. Serial dilutions was made by transferring1ml of the previous dilution in 9ml of normal saline solution (NSS) up to 1:10-6 then the sample was pour plated using standard plate count agar solution. 1ml of milk sample from each dilution was placed in the center of duplicate labelled petri dishes using sterile pipette. Molten cooled plate count agar was then poured into the Petri dish containing the inoculum and mixed well. After the solidification of the agar, the plate was inverted and incubated at 37°C for 48 hours. Dilutions with the total number of colonies on a plates 30 to 300 per plates were selected and colonies were counted ((Welearegay *et al.*, 2012).

Data collected for questionnaire survey and bacteriological quality analysis were entered into excel spread sheet and analyzed using SPSS version 20. Data on the bacterial counts were first transformed to logarithm of colony forming units per milliliter of sample (log CFU/ml). Descriptive statistics like frequencies and percentages were used to express the proportion of bacterial loads of the dairy farms and milk quality grade based on international standards. The differences in bacterial load between the samples from the teat directly and milking buckets were compared. P-value <0.05 will be taken as cut-off for statistical significance.

RESULTS

General information of the farms and farm owners

A total of 60 of medium dairy workers and owners and small holder dairy cow owners were interviewed. 63.33 % (38) of the respondents were males, 23.33% (14) were between 21-30 years, 28.33% (17). The educational level of 36.66 % (22) respondents were illiterate. All the owners interviewed had heads of cattle ranged from 1 to 40. About 76.67 % (46) of the respondents had 1 to 5 cattle, 16.66 % (10) of the respondents had 6 to 15 cattle and 6.68 % (4) had 16 to 40 cattle.

Table 1: Demographic Characteristics of the Respondents

Variables	Category	Frequency (N=60)	Percent (%)
Sex	Male	38	63.33
	Female	22	36.66
Age	21-30	14	23.33
	31-40	17	28.33
	41-50	20	33.33
	Above 50	9	15
Educational level	Illiterate	22	36.66
	Elementary	28	46.66
	High school	8	13.33
	Diploma and above	2	3.33
Herd size	1-5	46	76.67
	6-15	10	16.66
	16-40	4	6.68

Hygienic Practices Followed During Milking

All the participants milked their cows twice (in the morning and afternoon). 98.34 %(59) of the participants stated that they milk their cows in the barn and 1.66 %(1) used separate milking room for their cows. About 88.33% (53), 3.33% (2), 6.66% (4) and 1.66 % (1) of the respondents cleaned the barn daily, twice a week, three times a week and once a week respectively. All the participants used pipeline water as source of water for the farm. All the respondents practiced hand washing before milking. Out of 60 respondents, 68.33 % (41) washed their hands before milking and 31.67% (19) washed before and between milking. About 81.66 % (49) of the respondents used detergent and water for hand washing and 18.34 % (11) used water only. All the respondents washed the udder before milking but 30 % (18) of them used towel for udder drying and 70 % (42) did not use towel.

All respondents used hand milking for the cows. 98.34 % (59) used plastic utensils for milking the cows and only 1.66 % (1) used pot for milking. 80% (48), 10% (6) and 10% (6) of the respondents cleaned the milking utensils with warm water and detergent, cold water and detergent and only warm water respectively.

Table 2: Hygienic practices in the studied farms during milking

Variables	Category	Frequency (N=60)	Percentage (%)
Milking frequency	Twice a day	60	100
Milking room	Barn	59	98.34
	Separate milking room	1	1.66
Barn cleaning	Daily	53	88.33
	Twice a week	2	3.33
	Three times a week	4	6.66
	Once a week	1	1.66
Source of water	Pipeline water	60	100
Hand washing	Before milking	41	68.33
	Before and between milking	19	31.67
Hand Washing by	Water with detergent	49	81.66
	Water	11	18.34
Towel for drying udder	Yes	18	30
	No	42	70
Milking system	Hand milking	60	100
Utensils used for milking	Plastic	59	98.34
	Pot	1	1.66
Cleaning of utensils by	Warm water with detergent	48	80
	Cold Water with Detergent	6	10
	Warm water	6	10

Public Health Awareness and Practices

Out of the 60 respondents, 36.66 %(22) had a habit of consuming raw milk and the remaining 63.34 %(38) did not consume raw milk. 96.66% of the respondents sold raw milk for consumers and 3.4% mainly used for family consumption. About 36.66% of the respondents had processes milk and 63.33% did not use processing milk.

Table 3: Public health importance

Variables	Category	Frequency (N=60)	Percentage (%)
Habit of raw milk Consuming	Yes	22	36.66
	No	38	63.34
Selling raw milk	Yes	58	96.66
	No	2	3.4
Processing milk	Yes	22	36.66
	No	38	66.34

Bacteriological Analysis

The mean total bacterial count of milk samples collected directly from the teat and the milking buckets in the farm are shown in the table below. The average bacterial counts were 5.087log10 (122,180 cfu/ml) and 5.759log10 (574,116.5 cfu/ml) for milk samples collected from the teat

and the bucket in the farm. The bacterial count increased by 0.6728±0.1579log10 (474,116 cfu/ml) from teat to buckets. The results showed very significant difference between the two points. According to international milk bacteriology standards (in which the maximum limit of total bacterial count for producer's milk is 100000cfu/ml) about 60.7% of the samples taken from teats had bacterial count above this number and regarded as poor quality even if, 39.3% have lesser bacterial counts. From the samples taken from the buckets only14.3% were good quality.

Table 4: Bacteriological Analysis

Variables	N	Mean (log10cfu/ml)	Max	Min	Mean Difference ±SEM	95% CI for mean	p-value
Teat	56	5.087	5.968	4.136	0.6728±0.1579	0.3577 to	0.0001
Bucket	14	5.759	6.763	4.732		0.9880	

There was significant difference ($p<0.05$) between counts of bacteria in raw milk collected directly from teats and collecting buckets. zing test (Yirsew, 2004).

CONCLUSION AND RECOMMENDATIONS

The present study indicated that the hygienic conditions and practices of the farms studied in Dessie town can be judged as poor, in which most of the farm hygienic practices and parameters like hygienic condition of the milking environment, sanitation and use of proper type of the milk containers, appropriate cleaning of udder and teats and the personal hygiene of the milkers were not fully performed by most of the farm owners and their workers. In this study, bacteriological quality of milk from medium dairy farms was not at the level required for consumption. The total bacterial count found in the study was higher than the accepted level of 1×105 according to the international standards. The quality of majority of the milk samples were substandard, due to the poor applying of strict hygienic practices during milk handling and milking.

Based on above conclusion the following recommendations are forwarded

- Training of dairy cow owners on hygienic practices during milk, good hygiene and sanitation practice, good husbandry practice is needed
- Raising the public awareness on the risk of the consumption of raw milk is necessary
- Detailed and wider research should be done in Dessie to raise the awareness of milk contamination
- Setting hygienic standards for raw milk and milking practice
- Keeping the quality of the milk is the responsibility of dairy cow owners, institutions concerned and the government, so continuous evaluation should be done to maintain the quality of milk delivered to the public

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