

Thesis Ref. No. _____

**HYGIENIC PRACTICES, BACTERIOLOGICAL QUALITY OF COW MILK
AND IT' S PUBLIC HEALTH IMPORTANCE ALONG THE DAIRY VALUE
CHAIN IN SIDAMA HIGH LANDS OF SOUTHERN ETHIOPIA**

Msc Thesis



By

Mesfin Zewdu

Addis Ababa University, College of Veterinary Medicine and Agriculture, Department of
Microbiology, Immunology and Veterinary Public Health

June, 2015

Bishoftu, Ethiopia

**HYGIENIC PRACTICES, BACTERIOLOGICAL QUALITY OF COW MILK
AND IT' S PUBLIC HEALTH IMPORTANCE ALONG THE DAIRY VALUE
CHAIN IN SIDAMA HIGH LANDS OF SOUTHERN ETHIOPIA**



A Thesis submitted to the College of Veterinary Medicine and Agriculture of Addis Ababa University in partial fulfillment of the requirements for the degree of Master of Science in Veterinary Public Health

By

Mesfin Zewdu

June, 2015

Bishoftu, Ethiopia

Addis Ababa University

College of Veterinary Medicine and Agriculture

Department of Microbiology, Immunology and Veterinary Public Health

As members of the Examining Board of the final MSc open defense, we certify that we have read and evaluated the Thesis prepared by **Mesfin Zewdu** entitled “**Hygienic Practices, Bacteriological Quality of Cow Milk And it’s Public Health Importance along the Dairy Value Chain in Sidama High Lands of Southern Ethiopia**” and recommend that it be accepted as fulfilling the thesis requirement for the degree of Masters of Science in Veterinary Public Health.

Dr Addisu Demeke	_____	_____
Chairman	Signature	Date
Dr Kebede Amenu	_____	_____
External Examiner	Signature	Date
Professor Mahindra Pal	_____	_____
Internal Examiner	Signature	Date
Dr. Bedaso Mamo	_____	_____
Major Advisor	Signature	Date
Dr. Yoseph Mekasha	_____	_____
Co- Advisor	Signature	Date
Dr. Bedaso Mamo	_____	_____
Department chairperson	Signature	Date

STATEMENT OF AUTHOR

First, I declare that this thesis is my *bonafide* work and that all sources of material used for this thesis have been duly acknowledged. This thesis has been submitted in partial fulfillment of the requirements for an advanced (MSc) degree at Addis Ababa University, College of Veterinary Medicine and Agriculture, and is deposited at the University/College library to be made available to borrowers under rules of the Library. I solemnly declare that this thesis is not submitted to any other institution anywhere for the award of any academic degree, diploma, or certificate.

Name: Mesfin Zewdu

Signature: _____

College of Veterinary Medicine and Agriculture, Bishoftu

Date of Submission: _____

ACKNOWLEDGMENTS

First and foremost, I would like to provide thanks to the Almighty God and his mother Virgin Mary for the blessing and supplying strength, believes, hope, patience and protection to me and my families throughout my life. Had not been the willing of them, nothing would have been possible to me.

Next I am very much indebted to my advisors Drs. Bedaso Mammo and Yoseph Mekasha for their unreserved advice, encouragement, useful comments, suggestions and constructive criticisms.

I would like to thank the Bureau of Agriculture of Southern Nations and Nationalities Peoples Region (SNNPR) for granting the study leave and securing my salary throughout the study period. I am also indebted to Livestock and Irrigation Value Chain for Ethiopian Smallholders (LIVES) project for providing the opportunity and financial and technical support to successfully accomplish this MSc study.

I would like to acknowledge the Addis Ababa Regional Laboratory for their cooperation.

My special thanks also goes to Dr. Ashenafi Feysia for his devotion in editing and commenting the manuscript.

I would like to thank the community in the study area for their cooperation during the interview and sample collection.

Last, but most significantly, I would like to express my deepest thanks to my Family for their unfailing encouragement throughout my life.

TABLE OF CONTENTS

ACKNOWLEDGMENTS	iv
LIST OF ABBREVIATIONS	vii
LIST OF TABLES	viii
LIST OF FIGURES	ix
LIST OF APPENDICES	x
ABSTRACT	xi
1. INTRODUCTION.....	1
2. LITERATURE REVIEW	4
2.1. Definition and Composition of Milk.....	4
2.2. Importance of Milk in Human Health	5
2.3. Characteristics and Flavor of Milk.....	5
2.4. Hygienic Quality of Milk and Microbial Contamination	6
<i>2.4.1. Contamination from udder infection</i>	<i>9</i>
<i>2.4.2. Contamination from the external surface of the udder.....</i>	<i>11</i>
<i>2.4.3. Environmental sources of contamination</i>	<i>11</i>
<i>2.4.4. Contamination from milking and storage equipment.....</i>	<i>13</i>
2.5. Public Health Impact	14
2.6. Antimicrobial Agents and Bacterial Resistance	15
2.7. The milk chain	16
3. MATERIALS AND METHODS	17
3.1. Description of the Study Area	17
3.2. Study Design	18
3.3. Study Animals and Population	18
3.4. Selection of Study Districts, Peasant Association's and Households.....	18
3.5. Inclusion and Exclusion Criteria	19
3.6. Sample Size Determination	19
3.7. Data Collection	20
<i>3.7.1. Questionnaire survey.....</i>	<i>20</i>
<i>3.7.2. Milk sample collection.....</i>	<i>20</i>

3.8. Laboratory Analysis of Milk Samples.....	23
3.8.1. Quantitative analysis of raw milk for bacteria.....	23
3.8.2. Qualitative analysis of raw milk for bacteria.....	25
3.8.3. Antimicrobial sensitivity testing	26
3.9. Data Management and Analysis	27
4. RESULTS	28
4.1. Dairy Cattle Housing and Cleaning Practices	28
4.2. Feeding and Watering Practices	29
4.3. Milking and Hygienic Practices	29
4.4. Public Health Aspect.....	32
4.5. Bacterial Count.....	33
4.5.1. Aerobic mesophilic bacterial count (AMBC)	33
4.5.2. Coliform count (CC).....	34
4.6. Bacteriological Quality of Raw Cow Milk	35
4.6.1. Bacterial isolates of milk samples from the cows' udder	35
4.6.2. Bacterial isolates of milk samples from the milking bucket	35
4.6.3. Bacterial isolates of milk samples from the marketed milk containers	35
4.7. Antimicrobial Susceptibility Profile	37
5. DISCUSSION	39
5.1. Questionnaire Survey.....	39
5.2. Bacteriological Count.....	43
6. CONCLUSIONS AND RECOMMENDATIONS.....	49
7. REFERENCES.....	51
8. APPENDICES	61

LIST OF ABBREVIATIONS

BPW	Buffered Peptone Water
EHEC	Enterohemorrhagic <i>Escherichia coli</i>
HACCP	Hazard Analysis Critical Control Point
HUS	Hemolytic Uremic Syndrome
SCC	Somatic Cell Count
SNNP	South Nation and National People
TFTC	Too Few to Count
TMTC	Too Many to Count
VRBA	Violet Red-Bile Agar
VRBG	Violet Red-Bile Glucose

LIST OF TABLES

Table 1. Composition of cow's milk.....	5
Table 2. Overview of pathogens most commonly associated with outbreaks in milk and dairy products.....	9
Table 3. Sampling of raw milk from udder, milking bucket and marketed milk container in the study area	22
Table 4. Types of housing, cleaning practice and calf management in three districts of Sidama zone	28
Table 5 . Feeding and watering practices of dairy cattle in three selected districts of Sidama zone	29
Table 6 . Hygienic practices followed by producers in three selected districts of Sidama zone	31
Table 7. Public health aspects associated with consumption of raw milk	32
Table 8. Mean ($\pm$ s.e.) aerobic mesophilic bacterial count of pooled milk sample (log 10 cfu/ml) in the study area	33
Table 9. Mean ($\pm$ s.e.) coliform counts of pooled milk samples (log 10 cfu/ml)	34
Table 10. Bacterial isolates from raw milk samples of different sources.	36
Table 11. Drug sensitivity test	38

LIST OF FIGURES

Figure 1. Major sources of contamination of milk	7
Figure 2. Geographical map of <i>Sidama</i> zone and the study area.....	17

LIST OF APPENDICES

Appendix I. Questionnaire survey format.....	61
Appendix II . Primary identification of bacterial pathogens.....	63
Appendix III . Differential tests used for <i>Staphylococcus</i> species.....	64
Appendix IV . Differential tests used for <i>Streptococcus</i> species.....	64
Appendix V .Differential tests used for <i>Corynebacterium</i> species.....	65
Appendix VI . Differential tests used for <i>Bacillus</i> species	65
Appendix VII . Differential tests for Gram-negative rods.....	66
Appendix VIII . Media used for isolation and counts of bacteria.....	66

ABSTRACT

*Milk is an important source of nutrients to human and animals, but due to its high water activity and nutritional value, it serves as an excellent medium for growth of many kinds of microorganisms under suitable conditions. The present cross sectional study was conducted to assess hygienic practices, determination of bacterial quantity of milk, isolation and identification of bacterial pathogens in milk and to determine antimicrobial susceptibility of the major isolate at each critical control points throughout the value chain in Arbegona, Bensa and Bona districts, Sidama zone from November 2014 to May 2015. A total of 120 respondents were interviewed and subsequently, 166 milk samples were collected for laboratory analysis including bacterial load assessment and isolation and identification of bacteria. Aerobic mesophilic bacterial counts (AMBC) and coliform counts (CC) from milk were conducted. Isolation and identification of the bacteria in the milk was also conducted following standard methods. Results showed that, majority of small-dairy holders were males, managing their cattle in unclean environments and practicing extensive grazing system on communal grazing area. The mean aerobic mesophilic bacterial counts of raw milk samples analyzed were $5.86 \log_{10}$ cfu/ml (udder), $8.25 \log_{10}$ cfu/ml (bucket) and $9.31 \log_{10}$ cfu/ml (marketed milk container). The mean coliform counts were $3.61 \log_{10}$ cfu/ml (udder), $5.47 \log_{10}$ cfu/ml (bucket) and $7.47 \log_{10}$ cfu/ml (marketed milk container). The increment of both counts at each critical control points was observed statistically significant ($P=0.000$) and there was no significant variation between districts ($P=0.976$) for AMBC and ($P=0.795$) for CC. According to international standards of raw milk quality, both the AMBC and CC have values above the upper limits set. In the course of this study, the frequent bacterial pathogens isolated from raw milk samples taken from different critical points include: *Staphylococcus* spp., *Streptococcus* spp., *Corynebacterium* spp., *Bacillus* spp., and coliforms. Of total isolates, 15 were tested for susceptibility to different eight antimicrobial discs; Gentamycin, Chloroamphenicol, Vancomycine and Kanamycin were the most effective antibiotics where by 93.1%, 75.8%, 72.4% and 58.6%, respectively.*

Key words: AMBC, Antimicrobial sensitivity, Colony counts, Critical control point, Milk

1. 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 and Voskuil, 2011; Pal, 2012). Milk meant for human consumption must be free from any pathogenic organisms. Microbial contamination in milk may cause milk-borne diseases to humans while others are known to cause milk spoilage (Pal, 2012). Many milk-borne epidemics of human diseases are spread through milk contamination (Bertu *et al.*, 2010). 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 equipments and water supplies used in sanitary activities (Pal and Jadhav, 2013). The quality of milk is determined by its composition and overall hygiene. However, consumption of contaminated food like milk may lead to food-borne diseases (FBDs). Specifically, human may be infected with milk-borne pathogens through consumption of infected raw or unpasteurized milk and milk products (Bertu *et al.*, 2010; Pal and Jadhav, 2013). Sometimes consumption of contaminated or spoiled milk and other dairy products may cause milk-borne diseases in humans (Pal, 2012). Indeed, food-borne diseases (FBDs) are a serious threat to people in Africa, responsible for 33-90% cases of deaths in children (Flint *et al.*, 2005).

In many countries of the world, the dairy industry is one of the most important food sectors and it has, by and large, been very successful in providing safe products. Nevertheless, the concern for the safety of these products remains high on the agenda of public health authorities. There are several reasons for this; milk is particularly rich in nutrients and provides an ideal environment for growth of many microorganisms, contamination of these products can occur at different points in the food chain through often complex pathways, and these products have been the source of food-borne outbreaks caused by a broad range of microbial and chemical hazards.

Ethiopia possesses the largest livestock population in Africa. Estimates for farmer holding in rural areas indicate that the country has about 53.99 million heads of cattle, 24.6 million goats, 25.5 million sheep and 0.92 million camels (CSA, 2013). In Ethiopia dairy production depends mainly on indigenous livestock genetic resources; more specifically on cattle, goats, camels and sheep. Cattle has 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, 2013). While the industry is growing at a rapid rate, no milk quality standards currently exist. Therefore, it is important to establish milk quality standards that focus on food safety measures in order to improve public health.

The consumption of raw milk and milk products is common in Ethiopia (Yilma, 2003), which is not safe from consumer health point of view as it is good media for the growth of microorganisms. Provision of milk and milk products of good hygienic quality is desirable for consumers. This is one reason why milk testing and quality control include hygiene as well as microbial qualities in addition to testing for fat content and heat stability (Giangiacomo, 2000). Prior to the discovery and widespread adoption of pasteurization for instance, raw milk and its products were responsible for serious bacterial infections such as diphtheria, scarlet fever and tuberculosis (Spreer, 1998). Consumers all over the world are increasingly concerned about the safety of their food in general and milk and milk products in particular. Therefore, quality should not be ignored at all stages of the dairy value chain from stable to table.

There is limited data on hygienic practices throughout the dairy production system in Ethiopia and standard milking procedures do not exist. A recent study in Ethiopia showed many farmers do not properly clean teats prior to milking. The study also showed a trend of farmers either not using a towel at all for disinfection or using a collective towel for two or more cows (Yilma, 2010). This practice can clearly lead to the spread of contagious pathogens. Raw milk is an important vehicle for the transmission of milk-borne pathogens to humans, as can be easily contaminated during milking and handling (Addo *et al.*, 2011; Pal, 2012). Poor or improper handling of milk can exert both a public health and economic constraints thus requiring hygienic vigilance throughout the milk

value chain (Swai and Schoonman, 2011). In some parts of the world including developing countries like Ethiopia, milk is still a significant source of these infections and other FBDs (Shirima *et al.*, 2003). Therefore, microbiological assessment of milk is essential to establish the degree of contamination and recommend some corrective measures (Parekh and Subhash, 2008).

In Sidama zone, where “*kocho*” (source of carbohydrate and staple diet made from Enset [*Ensete ventricosum*]) is highly consumed, there is also high consumption of raw cow milk along with this local food. However, although there is risk associated with the consumption of raw cow milk, there is lack of information on the extent of raw milk contamination by bacteria in this area. In addition, there has been no established milk quality control system. Therefore, the present study was initiated to generate base-line information on the quality of raw cow milk consumed and potential public health risks associated with the consumption of raw milk.

General objective:

This study was aimed at assessing bacteriological quality of raw cow’s milk and to estimate the public health risks associated with consumption of raw cow milk in Arbegona, Bensa and Bona districts in Sidama highlands of southern Ethiopia.

Specific objectives:

- To assess the hygiene and handling practices of cow milk along the value chain in the study area,
- To evaluate bacteriological quality of raw cow milk across dairy value chain in the study area,
- To isolate major bacteria species contaminating raw cow milk, and
- To determine antimicrobial susceptibility of the common milk-borne bacteria isolated from raw cow milk.

2. LITERATURE REVIEW

The use of milk and milk products as human food has got a very long history. The milk as it is meant to be the first and sole food for offspring of mammals is an almost complete food. Almost 87% of milk is composed of water and the remaining part comprises 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 ingredients include immuno-globulins, 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 serves as an excellent medium for growth and multiplication of many kinds of microorganisms when suitable conditions exists (Parekh and Subhash, 2008; Pal and Jadhav, 2013).

2.1. Definition and Composition of Milk

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. This composition is not constant; the average percentages of milk components vary with species and breeds of animal, season, feeds, stage of lactation and health and physiological status of a particular animal. 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). Moreover, 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.

Table 1. Composition of cow's milk.

No.	Composition of cow's milk	Composition in percent
1	Water	87.2 %
2	Fat	4.0 %
3	Protein	3.4 %
4	Lactose	4.5 %
5	Ash (minerals)	0.9 %

Source: (Atkins, 2005)

2.2. Importance of Milk in Human Health

The use of milk and milk products as human food has got a very long history. It contains in a balanced form all the necessary and digestible elements for building and maintaining the human and animal body. Research has shown that milk and milk products have an immune enhancing property as well, particularly for the benefit of HIV/AIDS affected people. In addition, milk contains various properties, which make it easy to convert into different milk products or to use it as an ingredient for other food items. Various human cultures have their own traditional ways of using milk and preparing different milk products (WHO, 2003).

2.3. Characteristics and Flavor of Milk

Consumer acceptance of milk is greatly affected by its flavor. There are several factors which may produce off-flavors and/or odors in milk (Clare *et al.*, 2005). Some of the more common causes of flavor and odor problems are:

- Feed and weed flavors
- Strong smelling plants, like wild onion or garlic
- Strong flavored feedstuffs such as poor quality silage

- Cow-barn flavors from dung, etc. These are found when milk is obtained from a dirty or poorly ventilated environment or from improperly cleaned milking equipment.
- Rancid flavors. These are caused by excessive agitation of milk during collection and/or transport. Damage of the fat globules in the milk results in the presence of free fatty acids.
- High acidity flavors
- Oxidized flavors, from contact with copper or exposure to sunlight
- Flavors from the use of chlorine, fly sprays, medications, etc.

2.4. Hygienic Quality of Milk and Microbial Contamination

The unhygienic and undesirable practices that decrease the quality of raw milk can be classified into three categories:

Practices related to the animal:

- Animals are not healthy or suffer from mastitis;
- Animals are dirty, in particular 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:

- Wrong milking procedures (like stripping) are used; the utensils and the milkcan are not cleaned properly.

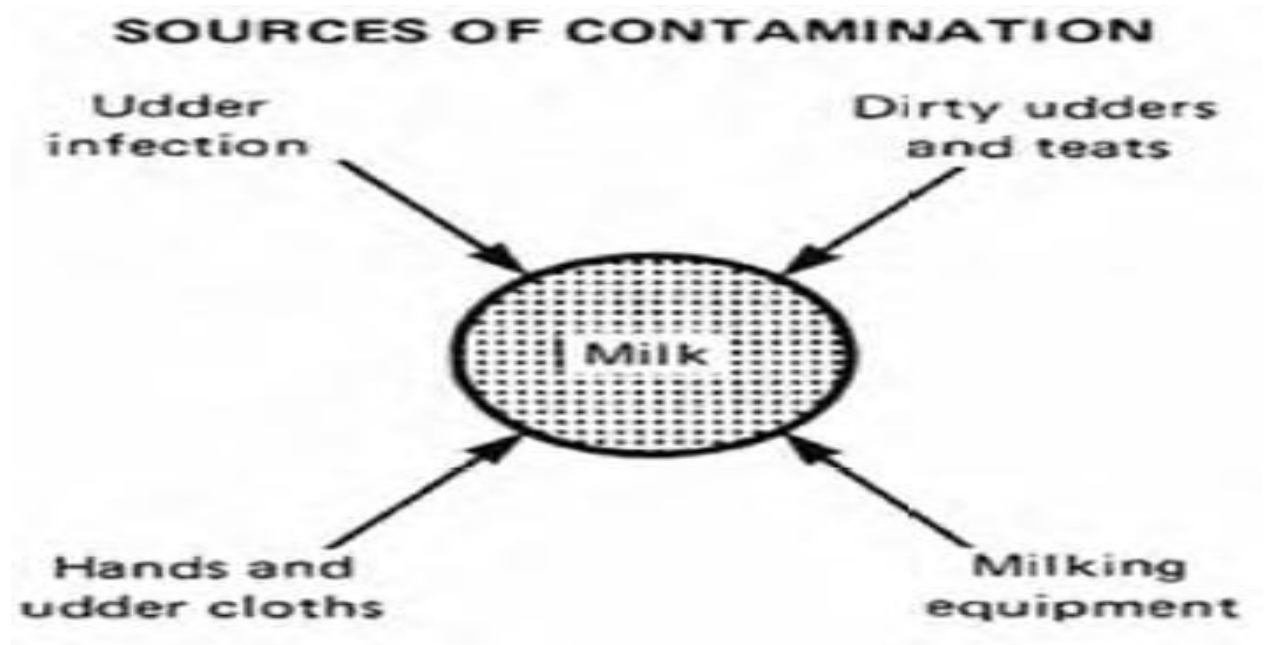


Figure 1. Major sources of contamination of milk

Source: (National Mastitis Council, 2005)

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 microorganisms 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 bacteria per ml (Rodrigues *et al.*, 2005). 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.

Raw milk is one of the most suitable media for the growth of a wide variety of bacteria, especially immediately after milking when it is almost at body temperature. However, milk contains a natural inhibitory system which prevents a significant rise in the bacteria count during the first 2 - 3 hours. If milk is cooled within this period to 4 °C, it maintains

nearly its original quality (van Schaik *et al.*, 2005). Timely cooling ensures that the quality of the milk remains good for processing and consumption. The bacterial load in fresh raw milk should be less than 50,000 per ml when it reaches the collection point or processing plant. To prevent a too high multiplication of bacteria, the milk has to be produced as hygienic as possible and should be cooled or heated at the earliest. Hygienic milk only originates from mastitis free and healthy animals. Cows suffering from a disease may secrete the pathogenic bacteria, which cause their disease, in the milk they produce. Consumption of raw milk therefore might be dangerous to the consumer. Some of these diseases, like tuberculosis, brucellosis and anthrax, can be transmitted to the consumer (O'Reilly *et al.*, 2006; Pal, 2007).

Milk contains proteins, carbohydrates, lipids, vitamins and minerals and its primary role is to provide nourishment to the neonates of the mammalian species from which it was derived. However, milk from a variety of animals has become an important and valuable part of the human diet; these same components that make it nutritious for humans also provide an ideal growth medium for many microorganisms, including potential pathogens (O'Reilly *et al.*, 2006).

Although milk production practices differ greatly throughout the world, in most developed countries milk is collected by machine milking and transferred to refrigerated bulk storage tanks where it is held prior to transportation. These handling methods have resulted in a dramatic change in the microflora of raw milk brought about by selection and adaptation. The microorganisms present in milk can be introduced by a variety of routes (Frisvad *et al.*, 2005; Pal and Jahdavi, 2013).

Table 2. Overview of pathogens most commonly associated with outbreaks in milk and dairy products

Microorganisms	Products	Incidences
<i>Escherichia coli</i>	Milk	95.5
<i>Campylobacter</i>	Dairy products, Milk	15.3
<i>Clostridium</i>	Dairy products	4.8
<i>Cryptosporidium</i>	Dairy products	1 .4
<i>Salmonella</i>	Dairy products, Milk	40.6
<i>Staphylococcus aureus</i>	Dairy products	0.4
<i>Shigella</i>	Dairy products, milk, sour cream	60.4
<i>Corynebacterium</i>	Raw cows' milk	0.1
<i>Streptococcus</i>	Cheese, raw cows' milk	0.1
<i>Campylobacter</i>	Raw milk	66.9
<i>Cryptosporidium</i>	Raw milk	0.5
<i>Escherichia coli</i>	Raw milk, raw milk cheese	15.0
<i>Listeria Monocytogenes</i>	Raw milk, raw milk cheese	2.6
<i>Yersinia</i>	Raw milk	0.2

Asia 2007–2010 (Safe Food International, 2011)

2.4.1. Contamination from udder infection

In healthy cows free from infection, milk emerging from the udder is essentially sterile, but it may contain commensal bacteria associated with the udder. A commensal organism derives food or other benefits from another organism without affecting it. These are usually members of the genera *Micrococcus* and *Streptococcus* as well as *Coryneform* bacteria (members of a particular family of bacteria named *Corynebacteriaceae*) and occasionally coliforms (a group of bacteria commonly found in the gastrointestinal tract of animals that ferment the sugar lactose) (White *et al.*, 2003). Significant numbers of

organisms are found in milk taken in a manner that prevents microbial contamination (i.e. aseptically) from the udders of apparently healthy cows.

Mastitis is defined as an inflammation of the mammary gland or udder; it can be subclinical in which there are no visible signs of infection, clinical in which there are signs of infection, or chronic when the symptoms persist over a long period of time. The most common agents of mastitis are *Staphylococcus aureus*, *Streptococcus agalactiae*, *Streptococcus uberis* and *Escherichia coli*. The organisms enter the udder by way of the duct at the teat tip and some, such as *Staph. aureus*, can colonize the duct. It is thought that machine milking plays a part in the propulsion of the organisms into the teat duct but this is by no means the only route of contamination (Bramley and Mckinnon, 2004). The results from one study have suggested subclinical mastitis is a greater problem in organic than in conventional production systems, but the differences were not marked (Roesch *et al.*, 2007). Whereas the organisms that cause mastitis do not generally grow in refrigerated milk, they are able to survive under these conditions and may be a concern from a public health aspect. For example, it has been demonstrated that staphylococcal enterotoxins and toxic shock syndrome toxin-1 can be preformed in the udder and secreted into milk in cows suffering from *Staph. aureus* mastitis (Valle *et al.*, 2004). Ingestion of the toxin in the milk may result in illness.

Apart from mastitis-causing organisms, other bacteria that are pathogenic to humans may infect the udder; these include *Mycobacterium bovis*, which can cause tuberculosis in humans (Griffths, 2007), *Brucella abortus* (the causative agent of brucellosis or undulant fever), *Listeria monocytogenes*, *Coxiella burnetii* and *Salmonella* spp.(Pal, 2007). For example, *C. burnetii* does not cause clinical disease in cattle, but it gives rise to Q fever in humans. It has been detected, using a polymerase chain reaction (PCR)-based assay, in 94% of pooled milk collected on farms in the US (Kim *et al.*, 2005). Recent attention has focused on *Mycobacterium paratuberculosis*, the causal agent of Johne's disease, a chronic, progressive gastroenteritis of ruminants, which has also been linked to Crohn's disease in humans. It has been estimated that at least 68% of all US dairy herds are infected with this organism (Kim *et al.*, 2005).

2.4.2. Contamination from the external surface of the udder

The external surface of the udder is also a prime source of microbial contamination of milk. Bedding materials, mud, feces, soil and other matter all readily stick to skin and are a rich source of microorganisms. Even after washing with water, the microbial count on teat surfaces can be high and the count in milk from washed udders may only be about 1 log cycle lower than from those that were unwashed (Thomas and Druce, 2001). Similar low-level reductions in total microbial count and coliform counts on both the udder surface and in milk were observed even after the use of disinfectants to treat teats (Gibson *et al.*, 2008). However, the importance of proper washing and drying of the udder before milking for the elimination of *Listeria* spp. has been demonstrated. In a study on the risk factors associated with contamination of raw milk by *Listeria monocytogenes* on dairy farms, showed that poor cleanliness of cows, inadequate lighting of milking parlors and barns (which may be an indication of neglect of milking hygiene) and incorrect disinfection of towels used to dry the udder significantly increased the likelihood of contamination (Sanaa *et al.*, 2003). Silage is also an important source of contamination by *Listeria* spp., including *L. monocytogenes*, and other potential human pathogens such as *Yersinia enterocolitica* and *Aeromonas hydrophila* (Sanaa *et al.*, 2003).

2.4.3. Environmental sources of contamination

The environment is also a major source of microorganisms on the dairy farm (Pangloli *et al.*, 2008). It was found that milking parlor air (62% positive samples) and bird droppings (63%) were major contamination sources during winter, while feeds (50-58%), water (53-67%), calf bedding (63%), soils (60-63%), milking parlor air (60%) and bird droppings (50%) were the main culprits in the spring. All animal and environmental samples (40-92%) except milking parlor air (25%) and bulk tank milk (29%) were found to contribute significantly to the presence of bacteria in the summer; whereas the major sources of contamination were feeds (60-71%), cow bedding (59%), cow soils (50%), air (46-71%) and insects (63%) during the fall (Pangloli *et al.*, 2008). Again this illustrates that there

are several potential sources of contamination by this pathogen that are difficult to control.

Personnel

It is unlikely that personnel contribute significantly as a source of microbial contamination of milk during machine milking, although workers suffering from certain zoonoses, such as Q fever, may pose a potential risk (Bramley and Mckinnon, 2004).

Aerial contamination

Air is thought to be an insignificant contributor to microbial contamination of raw milk. It has been calculated that airborne bacteria account for <5 cfu/ml of the bacterial load of milk; of these *Bacillus* spores would constitute <1 cfu/ml. However, a recent study by (Pangloli *et al.*, 2008) suggests that milking parlor air is a major source of *Salmonella* on the dairy farm.

Water

Water used in the production of milk should be of potable quality. Storage tanks should be protected to prevent access by insects, rodents, birds and other sources of contamination and equipment used to deliver water should be properly cleaned. Problems may arise when untreated water supplies are used to rinse and wash equipment. Such water may contain a diverse array of microorganisms including *Pseudomonas* spp., coliforms, *Bacillus* spp. and numerous other types of bacteria (Bramley and Mckinnon, 2004). Indeed, Perkins *et al.* (2007) have demonstrated the potential for contamination of milk with *E. coli* through wash water. The number of cells contaminating the milk may be small but there is the potential for growth in any residual water remaining on the equipment. Chlorination of the water used in the production of milk is recommended. Concerns about the use of untreated water, and even of mains supplies, have been heightened in recent years by the increased incidence of *Cryptosporidium parvum*. This is

a parasite that causes cryptosporidiosis, a disease of the mammalian intestinal tract, which results in acute, watery and non-bloody diarrhea. Cryptosporidiosis is of particular concern in immunocompromised patients (such as AIDS patients), in whom diarrhea can result in the loss of 10- 15 liters of water per day. It is known that oocysts of this parasite can resist chlorination and have been detected in raw milk, albeit at low incidence rates (<1%), but their source is undetermined.

2.4.4. Contamination from milking and storage equipment

Significant contamination of milk can arise from inadequately sanitized surfaces of milking and milk storage equipment. Organisms can proliferate in milk residues present in crevices, joints, rubber gaskets and dead-end of badly cleaned milking plant. A diversity of bacterial types can be introduced into milk from milk mineral deposits present in milking equipment and arguably the most important of these are the Gram-negative psychrotrophs, which predominate among the microflora that adhere to stainless-steel pipelines used for milk transfer (Griffiths, 2004). Differences in cleaning regimes and, hence, the level of contamination from farm to farm ensure that considerable variation occurs in the microflora of milking equipment. The only real protection against the introduction of bacteria into the milk supply from equipment during milking is adequate sanitation. Variations in temperature and cleaning procedures affect the attachment of bacteria to stainless steel surfaces and the effectiveness of sanitation depends to a large extent on the design of the plant and on other factors such as the hardness of the water supply, which itself can give rise to deposits on milking equipment (Palmer, 2001).

The opportunities for contamination of milking equipment at 31 dairy farms were studied. They found that milk quality was affected by the temperature of the rinsing water, with temperatures of less than 42°C increasing the likelihood of contamination with *Pseudomonas* spp. and coliforms. In addition, milking clusters kept out of the cluster pick-up between milking had a higher risk of microbial contamination. Contamination of the milking machine and the bulk tank milk with environmental bacterial contaminants

was not reduced by various methods of teat cleaning before milking or by post-milking teat disinfection. The type of bedding material influenced bacterial contamination of milking clusters and bulk tank milk. They concluded that microbial contamination of the milking machine was influenced not only by the sanitation procedure but by many other factors, such as milking procedures and the environment of the milking parlor (Feldmann *et al.*, 2006).

2.5. Public Health Impact

The economic and nutritional value of milk and dairy products in developing countries is evident. However, as the industry grows and becomes more market oriented, focus needs to be placed on the potential risks associated with dairy production and consumption. In developed countries, up to 30% of the population is affected by a food-borne illness per year causing great strain on public health and the economy. The American food supply system is among the safest in the world, but there are still an estimated 76 million cases of food-borne illness a year causing 5,000 deaths and 325,000 hospitalizations (WHO, 2007). The major pathogens alone are responsible for \$35 billion a year in medical costs and loss of productivity. Information on the impact of food-borne illness in developing countries is limited due to lack of reporting systems and poor health care infrastructure. Even so, the burden of food-borne illness in developing regions is estimated to be great based on the high number of diarrheal diseases. In 2005, 1.8 million deaths of children under 5 worldwide were attributed to diarrheal disease, and a large portion was due to contaminated food and drinking water. Of the 1.8 million deaths, 78% (1.46 million) occurred in Africa and Southeast Asia (Boschi-Pinto *et al.*, 2008). Diarrheal diseases in developing countries are a great public health concern due not only to their direct cause in illness and morbidity, but also their role in malnutrition in infants and young children (WHO, 2007). If a child is sick with a diarrheal disease, the inability to absorb nutrients undermines the nutritional benefits of a diet sufficient in quantity and quality and can exacerbate malnutrition.

Milk and dairy products are a potential source of transmission for many food-borne pathogens due to a neutral pH and rich nutrient composition (LeJeune and Rajala-Schultz, 2009; Pal and Jahdavi, 2013). Milk-borne outbreaks in the U.S. and other industrialized countries have been drastically reduced over the years due to the great amount of focus that is placed on quality control, to include the widespread use of pasteurization, the guidelines set forth in HACCP procedures. In 1938, milk-borne outbreaks accounted for 25% of all outbreaks in the U.S. due to contaminated food and water. Currently, milk-borne outbreaks account for less than 1% of all food-borne outbreaks in the United States. The majority of documented milk-borne outbreaks have been the result of unpasteurized dairy products. Between 2000 and 2006 in the United States, 40 outbreaks were traced back to raw milk compared to only 4 from pasteurized milk (Oliver *et al.*, 2009). Throughout the developing world, over 80% of the milk consumed is unregulated, and in Ethiopia less than 1% of the milk consumed is pasteurized (FAO, 2009). Again, there is limited information on the impact of milk-borne disease in these regions, but based on the large amount of unregulated milk consumed and the risks of consuming unpasteurized dairy products, the impact is likely to be great.

Milk can be contaminated with bacteria of both human and animal origin at any stage in the production to consumption process. Pathogenic organisms can be excreted in the milk from an infected animal (preharvest), or the contamination can occur at the time of collection, processing, distribution, and storage (postharvest) (LeJeune and Rajala-Schultz, 2009). As the dairy industry in developing countries moves towards a more market-oriented system, food safety becomes exceedingly important. When there is contamination with mass distribution, outbreaks affect more people and cause a greater economic impact. Focus needs to be placed on food safety standards and procedures for both preharvest and postharvest activities (LeJeune and Rajala-Schultz, 2009).

2.6. Antimicrobial Agents and Bacterial Resistance

Antimicrobial agents particularly antibiotics are veterinary drugs used in dairy cattle for treatment and prevention of various diseases. Also they are used to improve feed

efficiency, increase milk production or as growth promoters (Syit, 2008; Sharma *et al.*, 2011). Antibiotic use sometimes occur in response to several challenges that face the livestock industry that include high level of stress, diseases, poor animal genetic potential, poor management, poor nutrition and drought (Mellau *et al.*, 2010). Furthermore, misuse and incorrect applications of antimicrobials and antibiotics deposit noticeable residue in tissues of animals, particularly when the milk is harvested and marketed within the withdrawal period of the drug. The rampant and indiscriminate uses of antibiotics among the small-scale livestock keepers increase possibility of antibiotic resistant bacteria that may be transferred from animals to humans and leads to various chronic diseases to the users of milk and milk products.

Because of limited extension services and poor animal health delivery systems, the farmers buy veterinary drugs from veterinary shops and treat by themselves. Katakweba *et al.* (2012) reported that a lot of drugs such as oxytetracycline are used abusively to treat and protect cattle against various diseases. When such drugs are administered by non-professionals, correct dosages are unlikely to be observed that may lead to drug resistance.

2.7. The milk chain

The efficient production of milk under good hygienic conditions is the key to successful dairying. The principal constraint in particularly smallholder systems is a high level of bacterial contamination in the milk. This might lead to its spoilage before it reaches the market. The first step for a farmer is to produce good quality milk from healthy (non-mastitis) cows. This is the basis which enables successful collection and marketing of the milk. In the first place attention should be paid to the equipment used. This has to be suitable for effective cleaning and sanitization. In the second place emphasis should be given to good hygienic practices during milking. Finally, attention has to be paid to the transport and collection of the surplus milk to the point of sale or processing. Collection and transport of the milk should not take very long to minimize post harvest spoilage (Ruegg, 2003).

3. MATERIALS AND METHODS

3.1. Description of the Study Area

The study was carried out in the highland of *Sidama* zone, in Southern Nations and Nationalities People Region (SNNPR), Ethiopia. The specific districts of the study site were *Arbegona*, *Bona zuria* and *Bensa*, which have been clustered for implementation of the Livestock and Irrigation Value Chain for Ethiopian Small-holders (LIVES) project. *Sidama* zone is located at 6° 40' 60" North and 38° 43' 0" East of the equator. The altitude of the study area ranges from 2,117 – 2,653 meters above sea level. The mean annual rainfall of the study area is 1,251-1,464 mm. The annual minimum and maximum temperature is 11°C and 15°C, respectively. According to Central Statistics Agency, the livestock populations of the *Sidama* zone are 2,096,120 Cattles; 430,490 Sheep; 236,416 Goats; 68,388 Horses; 54,093 Donkeys; 1,189 Mules; 1,532,589 Poultry and 88,728 Bee hives (CSA, 2013).

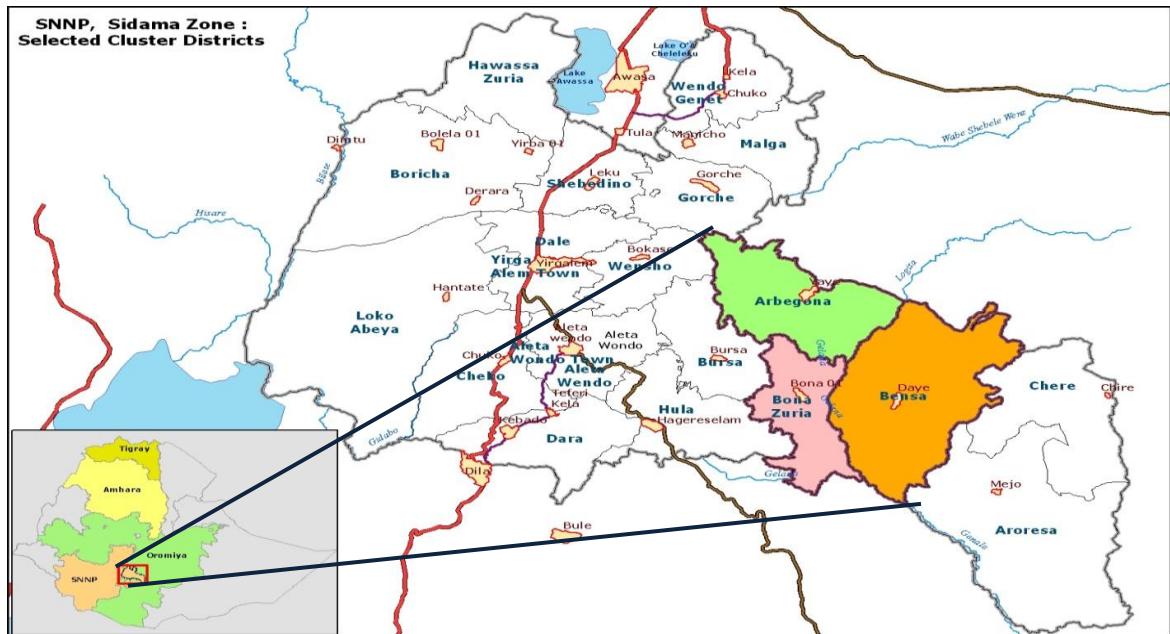


Figure 2. Geographical map of *Sidama* zone and the study area

Source: (LIVES, 2012)

The farming system practiced in the study area is mixed crop-livestock farming system. According to the information from districts Agricultural office, the livestock keeping and production systems are of small-holder dairying and mainly keep indigenous cattle and some of them have exotic (Holstein-Friesian and Jersey). Other animals kept by these livestock keepers are goats, sheep and poultry. They both practice a free grazing system and share water points but in most cases cattle and small ruminants are grazed separately. These animals are kept as source of income, meat, milk, draught power for farmers.

3.2. Study Design

A cross-sectional study was conducted from November 2014 to May 2015 to assess the hygienic practices, magnitude of bacterial contaminants in raw cow milk and public health importance of cow milk produced and supplied along the dairy value chain (producers to consumers) in the study area. Farmers involved in the study were small-holder dairy. The study unit was small-holder farmer with lactating cows where questionnaires were administered and raw cow milk was collected.

3.3. Study Animals and Population

The study animals were lactating cross breed cows (Holstein Frisian X indigenous Zebu and Jersey X indigenous Zebu) from small-holder dairy farmers in the *Arbegona*, *Bensa* and *Bona* districts.

3.4. Selection of Study Districts, Peasant Association's and Households

The three districts (*Arbegona*, *Bona zuria* and *Bensa*) were selected purposely based on the potential of livestock resources by, the Livestock and Irrigation Value Chain for Ethiopian Small-holders (LIVES) project. LIVES is a project aims at supporting governmental organizations' efforts to transform the small-holder subsistence agricultural sector to a more market-oriented small-holder sector to contribute to the new growth and transformation plan. The project uses the current value chain framework and the problem faced along the chain to improve targeted commodities, including milk.

Accordingly, from each district, 5 PA's were purposely selected based on the number of livestock keepers, milk production and accessibility. With the help of livestock extension officers, households with crossbred cattle were identified for the study. A total of 120 households, 8 from each PA and 40 from each district were selected based on inclusion and exclusion criteria stated in section 3.5.

3.5. Inclusion and Exclusion Criteria

The study inclusion criteria for the household were the following: the household must be a small-holder farmer (both men and women) with at least 3 cross breed lactating cows during the study time, willing to participate in the study, ready to give the required information through questionnaires and availability of milk during the survey. The exclusion criteria were: those households who were not around during the study, unwilling to participate in the study, unable to give the required information and absence of milk during the survey. Also those who had no time for questionnaires interviews were excluded.

3.6. Sample Size Determination

A formula by Kothari (2004), for unknown population (i.e. $n = Z^2SD^2/e^2$) was used to calculate the sample size for this study. Where Z, is the estimated standard variation at 95% confidence interval (CI) which was considered the point of the normal distribution corresponding to the level of significance (Z=1.96). Standard deviation (SD) was estimated at 0.15 or 15% and e, is the estimated error and was considered at 0.05 or 5%. Therefore, the sample size 'n' was calculated as:

$$n = \frac{(1.96)^2 \times (0.15)^2}{(0.05)^2} = 34.6 \text{ approximately } n = 35 \text{ samples per each district.}$$

Based on the above formula 105 milk samples were supposed to be collected, but to increase precision, 166 milk samples were collected.

3.7. Data Collection

This study had two parts: Questionnaire-based survey and bacteriological quality analysis.

3.7.1. Questionnaire survey

Structured questionnaire (Appendix 1) was used to collect information from small-dairy holders with lactating cows. The questionnaire was made with pre-coded response choices (closed-ended questions) with a few open-ended questions. Also, the questionnaire was used to collect information on possible risk factors for bacterial contaminations in milk. Risk factors considered in the current study were sanitary conditions of the barn/milking environment, hygiene of milking cows' udder and milk handlers, hygiene of milking equipment with special emphasis to hygiene of milking procedures and milk handling practices, utensils used for milking, milk storage and uses of milk (for selling or domestic purposes). Furthermore, milk consumption behaviors and their awareness on the risk of zoonotic diseases that are associated with the consumption of raw milk was also assessed.

The questionnaire was administered through face to face interview. While administering questionnaires, direct observation on general cleanliness and hygienic conditions and practices with regard to milk were also done and noted.

3.7.2. Milk sample collection

3.7.2.1. Milk sampling technique

Raw milk samples were collected from critical control points along the value chain in the three districts of 15 PA's that are considered to be associated with the hazard, when a measurement can be conducted and when control measures can be taken in order to reduce the hazard to an acceptable level.

The presence of bacteriological agents was assessed and aerobic mesophilic bacterial count and coliform count was performed on pooled milk samples collected at the following critical control points along the milk value chain; directly from the cows' udder at farm level, from the milking bucket at farm level, and from marketed milk containers up on arrival (from café, restaurants and home consumers).

Among those households previously considered for questionnaire survey study, 95 pooled milk samples from the udder and 55 milk samples from milking bucket were collected. Additionally, 16 milk samples were collected from marketed milk containers using the information from the producers. Thus a total of 166 samples of raw milk were used for the bacteriological analysis (Table 3).

Table 3. Sampling of raw milk from udder, milking bucket and marketed milk container in the study area

Districts	PA's	Udder	Bucket	Container	Total
		n=95	n=55	n=16	
<i>Bensa</i>	<i>Daye</i>	7	4	4	15
	<i>She/golba</i>	7	4	1	12
	<i>She/wene</i>	6	4	1	11
	<i>Huro tibiyo</i>	6	4	0	10
	<i>Sedeware</i>	6	3	0	9
	Sub total	32	19	6	57
<i>Arbegona</i>	<i>Yaye</i>	7	4	6	19
	<i>Hafursa</i>	7	4	0	11
	<i>Chucho</i>	6	4	0	10
	<i>Toga</i>	6	3	0	9
	<i>Roko</i>	6	3	0	9
	Sub total	32	18	6	56
<i>Bona</i>	<i>Bona 01</i>	7	4	4	15
	<i>Becha</i>	6	4	0	10
	<i>Merede</i>	6	4	0	10
	<i>Bona kike</i>	6	3	0	9
	<i>Beshero da</i>	6	3	0	9
	Sub total	31	18	4	53
Total		95	55	16	166

3.7.2.2. Milk sample handling

During sampling of raw milk from the udder, the surface of the teat end was cleaned by wiping it with clean cotton dipped in 70 % alcohol. Before sampling from milking bucket and marketed milk container upon arrival, the milk was thoroughly mixed after, which 25 ml of milk was transferred into sterile sampling bottles (Richardson, 1985). At all levels

of sampling, the sampling bottles were capped, labeled with a permanent marker and stored in an ice packed cool box and transported to Addis Ababa regional laboratory and kept in refrigerator until the time of analysis. The bacterial count and isolation of species was performed within 24 hours after sampling (Alganesh *et al.*, 2007).

3.8. Laboratory Analysis of Milk Samples

Analyses were carried out in the microbiology laboratory in the Addis Ababa regional laboratory. Two kinds of laboratory analyses of milk samples were performed. The first was analysis for bacterial quantity of raw milk which involved establishing the aerobic mesophilic bacterial count (AMBC) and coliform count (CC) and isolation of major milk-borne bacteria. The second was determination of antimicrobial susceptibility of major isolate.

3.8.1. Quantitative analysis of raw milk for bacteria

3.8.1.1. Aerobic mesophilic bacterial count (AMBC)

Milk samples were serially diluted by adding 1mL of the test portion into 9 mL of 0.1% sterile peptone water. Dilutions were made so that plate counts range between 30 and 300 colonies (Richardson, 1985). Appropriate dilutions were placed on Petri dishes and pour plated with 10 to 15 mL molten plate count agar (about 45°C) (Oxoid, UK) and allowed to solidify for 15 minutes and incubated for 48 hours at 37°C. Finally, counts were made using a colony counter. The plate counts were calculated by multiplying the count on the dish by 10^n , in which n stands for the number of consecutive dilutions of the original sample (Van den Berg, 1988).

3.8.1.2. Coliform count (CC)

After mixing the sample portion, appropriate decimal dilutions were made by transferring 1 mL of the sample into 9 mL of 0.1% peptone water for initial dilution and by transferring 1 mL of the previous dilution into 9 mL of peptone water. After surface

plating the appropriate dilution in duplicates on VRBA, Petri dishes were incubated at 32°C for 48 hours and counts made on typical dark red colonies normally measuring at least 0.5 mm in diameter on uncrowned plates (Richardson, 1985). While transferring raw milk samples from one test tube to another, separate sterile pipettes were used for the different dilutions for both counts.

3.8.1.3. Reading and interpretation results

Aerobic mesophilic bacterial count (AMBC): After incubation at 37°C for 48 hours, all colonies including those of pin point size is counted on selected plates using colony counter. Results from plates, which contained 30 to 300 colonies per plate were recorded. Plates with more than 300 colonies could not be counted and were designate as TMTC. Plates with fewer than 30 colonies were designate as TFTC. After counting and recording bacterial colonies in each petridish the number of bacteria in milliliter was calculated by the following formula given by APHA (1992).

$$N = \frac{\Sigma C}{V [(n_1 \times 2) + (0.1 \times n_2)] \times d}$$

Where: N = number of colonies per milliliter of milk,

ΣC = sum of colonies on plates counted,

V= volume of inoculum on each dish/plate, in ml

n_1 = number of plates on lower dilution counted,

n_2 = number of plates in next higher dilution counted and

d = dilution from which the first counts are obtained.

Coliform count (CC): after incubation of the plates for 48 hours, typical purplish red colonies with bile precipitations around them were counted as coliforms. Results from only those plates, which contained between 15 and 150 colonies were recorded. Interpretations were similar with that of AMBC.

3.8.2. Qualitative analysis of raw milk for bacteria

Cultural examinations were used to isolate and identify the pathogenic and spoiling bacterial species found in the raw milk. Isolation and identification of bacterial species was carried out based on conventional culture technique and biochemical assays. After thorough mixing of each milk samples, a loopful of the milk sample was streaked on the blood agar base enriched with 7% sheep blood agar and MacConkey agar. The plates were incubated at 37°C and examined for bacterial growth after 48 hours. From culture positive plates, typical colonies were subjected to Gram's stain to study the staining properties and cellular morphology. Pure cultures of a single colony type from the blood agar were transferred in to nutrient agar 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 to the species level was carried out based on their colony characteristics, Gram's staining and morphological characteristics, growth on MacConkey agar, catalase, urease, coagulase and oxidase tests, hydrogen sulfide production, indole, methyl red, Voges-Proskauer reaction, citrate utilization, oxidation-fermentation test, lysine and different carbohydrate tests.

Staphylococcus species were identified based on hemolysis pattern, catalase production and coagulase test, pigment production, O-F test and fermentation of manitol, and maltose (Appendix 3).

Streptococcus species were identified based on Gram's stain reaction, catalase production, hemolysis pattern, CAMP test, fermentation pattern of manitol, sorbitol, raffinose and salicine and aesculin hydrolysis (Appendix 4).

Corynebacterium species were identified based on staining morphology, hemolysis pattern, catalase production and acid production from glucose, lactose and maltose (Appendix 5).

Bacillus species were identified based on hemolytic pattern, staining morphology, fermentation pattern of arabinose and manitol, and citrate and VP tests (Appendix 6).

Coliform organisms were identified based on Gram's stain reaction, growth characteristics on MacConkey agar, oxidase test, reaction patterns on IMViC test, H₂S production, fermentation patterns from lactose and urease and lysine production (Appendix 7).

Other Gram-negative organisms were identified based on staining morphology, growth characteristics on MacConkey agar, oxidase test, urease production, indole production, and acid production from sucrose and glucose (Appendix 7).

3.8.3. Antimicrobial sensitivity testing

The common isolates were subjected to the commonly used antimicrobials using Kirby-Bauer method (Quinn *et al.*, 2002). About eight antimicrobials such as Chloroamphenicol, Gentamycine, Penicillin, Sulphamethizole, Streptomycin, Kanamycine, Tetracycline and Vancomycine (Oxiod) were selected from main class of antimicrobials and that were commonly used by the veterinary clinician found in those districts, and investigated for sensitivity testing. The antibiotic discs were placed on the surface of MullerHinton agar plate previously seeded with appropriate amount of the organism to be tested. Each disk was pressed down to ensure complete contact with the agar surface. The plates were incubated at 37°C for 18-24 hours. Subsequently, the plates were examined for the development of zone of inhibition around the discs. After measuring the zone of inhibition, it was classified as sensitive, intermediate and resistant according to National Committee for Clinical Laboratory Standard (NCCLS) break point to interpret the inhibition zone (Quinn *et al.*, 2002).

3.9. Data Management and Analysis

The data generated from the survey and laboratory were entered into MS excel spread sheets and analyzed using SPSS version 20. The survey data was described using descriptive and inferential statistics such as means, frequency distribution and percentage. Microbiological counts were transformed into logarithmic scale ($\log_{10}$ cfu/ mL) and analyzed using the General Linear Model (GLM) procedure of SPSS. Means were compared and declared significant at $\alpha=0.05$.

4. RESULTS

4.1. Dairy Cattle Housing and Cleaning Practices

According to the current study low proportion of farm owners (16.7%) constructed their farm with concrete materials (cement) in which it facilitates easy cleaning, while the floor of about (83.3%) was earthen and covered with manure since they do not remove the manure completely during cleaning and found in poor hygienic state. As observed in the present study (26.7%) of the respondents used grass and cereal straw as bedding material for their animals and have good conditioned barn (Table 4). The remaining households (73.3%) did not use any bedding material at all and milking cows lies on muddy bedding (Table 8). Teats and udders of cows inevitably become soiled while they are lying in such stalls. In the study about (21.7%) of the respondents clean the barn three times a week while (78.3%) reported that they clean daily. Cleaning of the barn with water was done on average every two weeks. The study also shown that (56.7%) of the respondents did not keep calves in a good hygiene, while about (43%) did very well. Further, most of the interviewed producers (94.2%) allow their calves to suckle dams.

Table 4. Types of housing, cleaning practice and calf management in three districts of Sidama zone

Variable		Districts		
		<i>Bensa</i> (n=40)	<i>Bonazuria</i> (n=40)	<i>Arbegona</i> (n=40)
Barn type	Concrete floor	27.5%	15%	7.5%
	Earthen	72.5%	85%	92.5%
Barn condition	Grass bedding	12.5%	22.5%	45%
	Muddy bedding	87.5%	77.5%	55%
Barn Cleaning frequency	Daily	87.5%	75%	72.5%
	3 times a week	12.5%	25%	27.5%
Calf management practices	Clean body	15%	55%	60%
	Soiled body	85%	45%	40%

4.2. Feeding and Watering Practices

Almost all respondents reported that they allow their cattle freely graze the natural grazing lands (Table 5). However, about 25% of the interviewed producers supplement their cows with locally available feed resources such as ensen. There were different source of water used for cattle in the study area. The majority of the respondents (94.2%) had access to river water followed by dug well water (5.8%) while none of them used pipe water.

Table 5 . Feeding and watering practices of dairy cattle in three selected districts of Sidama zone

Variable	Districts		
	<i>Bensa</i>	<i>Bona</i>	<i>Arbegona</i>
Feeding regime	(n=40)	(n=40)	(n=40)
• Grazing natural pasture	100%	100%	100%
• Supplemented with local feed	12.5%	22.5%	40%
Source of water for farm activities			
• Pipe	0%	0%	0%
• River	82.5%	95%	95%
• Well	17.5%	5%	5%
• Both river and well	52.5%	42.5%	47.5%

4.3. Milking and Hygienic Practices

Milking is done manually (stripping) mostly by women's. Cows were usually milked twice a day except with few farmers (about 8.3%) who milk three times a day (Table 6). Plastic containers (buckets) and pots are used during milking. Among the interviewed households (82.5%) use plastic jars while (17.5%) use clay pot as milking utensil. The majority of the respondents (85%) practiced washing of their milk utensils daily, while (15%) clean three times a week before milking. However, the cleaning is not efficient and

utensils are not properly dried. It was observed that milkers dip their fingers in the milking vessel to moisten teats of the cows with the intention of facilitating milking. The respondents in the urban market in which they collect the milk from producers were interviewed in order to assess the potential risk factors for milk contamination along the final value chain which comprises the duration from the final collection up to consumption. Accordingly, 100% of them replied that only plastic type of container is used to transport milk from producers.

In the present study, majority (64.2%) of the respondents across the 3 districts did not wash their hands before milking, while the remaining (35.8%) did wash their hands with water. However, none of the interviewees wash their hands between milkings of different cows. Among interviewed, about (65%) do not wash udder before milking while the remaining (35%) did wash. Similarly, (54.8)% of all the interviewees do not use towel to dry udder after washing rather they massage the udder with bare hands while, about (45.2%) reported that they use local material, *kacha* (*Agave sisalena*) for teat and hand drying purposes. Generally, it was observed that the person involved in milking was not clean, and the milking environments and utensils were also unhygienic indicating the possibilities for microbial contaminations of milk.

Table 6 . Hygienic practices followed by producers in three selected districts of Sidama zone

Hygienic practices followed by producers	Districts		
	<i>Bensa</i>	<i>Bonazuria</i>	<i>Arbegona</i>
Milking frequency	(n=40)	(n=40)	(n=40)
Twice a day	85%	95%	95%
Three times a day	15%	5%	5%
Milking utensils used for milking			
Plastic	97.5%	77.5%	72.5%
Pot	2.5%	22.5%	27.5%
Cleaning frequency of milking utensils used for milking			
Daily	97.5%	77.5%	80%
Three times a week	2.5%	22.5%	20%
Milking utensils used for transport			
Plastic	100%	100%	100%
Wash hands before milking?			
• Yes	42.5%	32.5%	32.5%
• No	57.5%	67.5%	67.5%
Wash udder and teats before milking?			
• Yes	40%	30%	35%
• No	60%	70%	65%
Milking after drying teats?			
• Yes	37.5%	31.3%	57%
• No	62.5%	68.7%	43%

4.4. Public Health Aspect

In the study area, about 49.2% of the interviewed producers consume raw milk while the remaining 50.8% boil raw milk before consumption. Although about half of the respondents (56.7%) were aware about the risk of public health hazards associated with consumption of raw cow milk, some of them did not boil milk for consumption and most of the respondents reported they were suffered from food borne infections of unknown origin. All dairy cow owners milk their cows by hand and did not cool the milk after milking. It is common that fresh milk is mixed with milk left over from previous milking, and milk of different cows of the same farm is mixed together before consumption.

Table 7. Public health aspects associated with consumption of raw milk

Variable	Districts		
	<i>Bensa</i>	<i>Bona</i>	<i>Arbegona</i>
	(n=40)	(n=40)	(n=40)
Habit of milk consumption			
• Raw	57.5%	57.5%	32.5%
• Boiled	47.5%	47.5%	67.5%
Knowledge of risk associated with consumption of raw milk			
• Yes	67.5%	57.5%	45%
• No	32.5%	47.5%	55%
Suffered from food borne infection			
• Yes	70%	57.5%	72.5%
• No	30%	47.5%	27.5%

4.5. Bacterial Count

4.5.1. Aerobic mesophilic bacterial count (AMBC)

The mean for aerobic mesophilic bacterial count (expressed in $\log_{10}$ cfu/ml) of raw milk sampled at three critical points are shown in Table 8. The overall mean AMBC was 5.86, 8.23 and 9.31 $\log_{10}$ cfu/ml for milk samples collected directly from the udder, milking bucket and marketed milk containers upon arrival, respectively. There was an increasing trend of aerobic mesophilic bacterial count as the milk passed through udder, milking bucket and marketed milk containers upon arrival. Accordingly, the count increased by 2.37 $\log_{10}$ cfu/ ml from point of production (milk sampled directly from the udder) to milk samples taken from milking bucket at the farm. Likewise, AMBC increased by 1.08 $\log_{10}$ cfu/ ml from milking bucket at the farm level to marketed milk container up on arrival. The increase from point of production to marketed milk containers upon arrival was 3.45 $\log_{10}$ cfu/ ml. Results of analysis of variance indicated that there were very significant differences in aerobic mesophilic bacterial count ($P= 0.000$) between the critical points and there was no significant variation between districts ($P = 0.976$).

Table 8. Mean ($\pm$ s.e.) aerobic mesophilic bacterial count of pooled milk sample ($\log 10$ cfu/ml) in the study area

Districts	Critical control points of sampling		
	Udder	Bucket	Containers up on arrival
<i>Bensa</i>	5.82 $\pm$ 0.09	8.20 $\pm$ 0.02	9.36 $\pm$ 0.05
<i>Arbegona</i>	5.86 $\pm$ 0.08	8.22 $\pm$ 0.06	9.34 $\pm$ 0.03
<i>Bona</i>	5.92 $\pm$ 0.08	8.26 $\pm$ 0.03	9.18 $\pm$ 0.04

4.5.2. Coliform count (CC)

The mean coliform counts (expressed in $\log_{10}$ cfu/ml) of raw milk sampled at three critical points are shown in Table 9. The overall mean CC was 3.61, 5.48 and 7.47 $\log_{10}$ cfu/ml for milk samples collected directly from the udder, milking bucket and marketed milk containers upon arrival, respectively. There was an increasing trend of coliform counts as the milk passed through udder, milking bucket and marketed milk containers upon arrival. Accordingly, the count increased by 1.87 $\log_{10}$ cfu/ ml from point of production (milk sampled directly from the udder) to milking bucket at the farm level. In a similar manner, it increased by 1.99 $\log_{10}$ cfu/ ml between sampling from milking bucket at the farm level to marketed milk containers at market. The overall increment from point of production to arrival at market was 3.86 $\log_{10}$ cfu/ ml. Results of analysis of variance indicated that there were very significant differences in coliform counts ($P=0.000$) among the critical points but no difference between districts ($P = 0.795$).

Table 9. Mean ($\pm$ s.e.) coliform counts of pooled milk samples ($\log 10$ cfu/ml)

Districts	Critical control points of sampling		
	Udder	Bucket	Containers up on arrival
<i>Bensa</i>	3.54 $\pm$ 0.05	5.44 $\pm$ 0.06	7.43 $\pm$ 0.15
<i>Arbegona</i>	3.67 $\pm$ 0.04	5.54 $\pm$ 0.07	7.50 $\pm$ 0.04
<i>Bona</i>	3.62 $\pm$ 0.03	5.46 $\pm$ 0.04	7.35 $\pm$ 0.07

4.6. Bacteriological Quality of Raw Cow Milk

4.6.1. Bacterial isolates of milk samples from the cows' udder

The present result revealed that all of the samples (n=95) considered for the study showed bacteria growth on the inoculated media. The major bacteria isolated out of 95 positive samples were *S. aureus*, *S. intermidus*, *S. hyicus*, *S. epidermidus*, *S. chromogenes*, *Strep. agalactae*, *Strep. dysgalactae*, *Strep. uberis*, *Strep. bovis*, *Strep. zooepidermicus*, *Enterococcus fecalis*, *Corynebacterium* spp, *Bacillus* spp and coliform (Table 10).

4.6.2. Bacterial isolates of milk samples from the milking bucket

Out of the total (n=55) milk samples taken, none proved to be negative. In addition to those bacteria isolated from the cow's udder, the following were detected; these are *S. saprophyticus*, *S. simulans*, *Ent. aerogenes*, *Ent.aglomerans* and *Serratia* spp. (Table 10).

4.6.3. Bacterial isolates of milk samples from the marketed milk containers

Out of the total 16 milk samples taken, none proved to be negative. In all of the positive samples, the types of isolated bacteria were similar to bacteria isolate in the udder and bucket except the increment of isolation rates of each bacterium (Table 10).

Table 10. Bacterial isolates from raw milk samples of different sources.

Species isolated	Number of isolate by source			
	Udder	Milking bucket	Milk containers up on arrival	Total
<i>S.aureus</i>	8	13	4	25
<i>S.intermidus</i>	12	15	6	33
<i>S.epidermidus</i>	9	7	3	19
<i>S.chromogenes</i>	6	9	3	18
<i>S.hyicus</i>	1	2	1	4
<i>S.simulans</i>	0	2	1	3
<i>S.saprophyticus</i>	0	2	1	3
<i>Strep.agalactae</i>	5	7	2	14
<i>Strep.dysgalactae</i>	2	2	1	5
<i>Strep.uberis</i>	8	12	4	24
<i>Strep.bovis</i>	3	5	2	10
<i>Strep.zooepidermicus</i>	1	1	1	3
<i>Enter.fecalis</i>	0	2	2	4
<i>C.ulcerans</i>	5	7	2	14
<i>C.bovis</i>	5	8	2	15
<i>C.haemolyticum</i>	1	3	1	5
<i>Bac.steaohemophil.</i>	3	5	1	9
<i>Bac.cerus</i>	1	2	1	4
<i>Bac.brevis</i>	2	3	1	6
<i>E.coli</i>	3	8	4	15
<i>Pseud.aeroginossa</i>	5	3	0	8
<i>Kleb.pneumoniae</i>	4	2	2	8
<i>Citrobacter freundii</i>	0	3	1	4
<i>Ent.aglomerans</i>	0	1	1	2
<i>Ent.aerogenes</i>	0	1	1	2
<i>Serratia</i>	0	1	1	2
Total	84	126	49	259

4.7. Antimicrobial Susceptibility Profile

The comparative efficacy of antimicrobials used indicates that Gentamycine, Chloroamphenicol, Kanamycine and Vancomycine were the most effective antibiotics where by 93.1%, 75.8%, 58.6% and 72.4% sensitivity, respectively. Tetracycline, Sulfamethizole, Penicillin and Streptomycin were showed very poor efficacies on many isolates, where by only 20.6%, 34.4%, 51.7% and 44.8% respectively. Vancomycine was sensitive for all gram positive and resistance for all gram negative.

In the current study *S. aureus*, *S. intermedius*, *S. chromogenes*, *S. hyicus*, *S. epidermidus*, *S. simulans* and *S. saprophyticus* isolates were more sensitive to Gentamycine (100%), Chloroamphenicol (100%), and Vancomycine (100%), whereas *S. aureus* was highly resistance to Pencillin (100%), Streptomycin (100%), Sulphamethizole (100%) and Tetracycline (100%). Similarly *Bacillus* species isolates were more sensitive to Vancomycine (100%) and Penicillin (100%) and resistance to Tetracycline and fairly to Gentamycine with the isolates having 0% and 60% sensitivity respectively. *Strep. agalactae*, *Strep. dysgalactae*, *Strep. uberis*, *Strep. bovis* and *Strep. zooepidermicus* were sensitive to Penicillin (100%), Vancomycin (100%), and Chloramphenicol (80%), and highly resistance to Streptomycin, Sulphamethizole and Tetracycline with 0% sensitivity to three of them. *P.aeruginosa* isolates were more sensitive to Penicillin (100%), Gentamicin (100%) and Streptomycin (100%) and resistance to Tetracycline, Sulphamethizole and Kanamycine with the isolates having 100% 0% and 50 % resistance, respectively. Whereas *Ent. aglomerans* isolates were more sensitive to Tetracycline (100 %), Gentamycine (100%) and Streptomycin (100%) and resistance to Vancomycine and Kanamycine with the isolates having sensitivity of 0% and 50%, respectively. *E .coli* isolates were more sensitive to Gentamycine (100%) and Streptomycin (100%) and resistance to Penicillin and Tetracycline with the isolates having sensitivity of 0% and 25%, respectively.

Table 11. Drug sensitivity test

Anti biotics	Unit	<i>S.aureus</i>			<i>S.intermedius</i>			C N S			<i>Streptococcus</i>			<i>Bacillus spp</i>			<i>E .coli</i>			N (%)
		S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	
G	10 µg	100	-	0	100	-	0	100	-	0	75	-	25	100	-	50	100	-	0	93.1
C	30 µg	100	-	0	100	-	0	100	-	0	75	-	25	50	-	100	50	-	50	75.8
VA	30 µg	100	-	0	100	-	0	100	-	0	100	-	0	100	-	50	0	-	100	72.4
K	30 µg	100	-	0	100	-	0	100	-	0	25	-	75	50	-	100	50	-	50	58.6
P	10 µg	0	-	100	0	-	100	25	-	75	100	-	0	100	-	50	0	-	100	51.7
S	10 µg	0	-	100	25	-	75	0	-	100	0	-	100	50	-	100	100	-	0	44.8
SU	10 µg	0	-	100	0	-	100	25	-	75	0	-	100	100	-	50	50	-	50	34.4
T	30 µg	0	-	100	25	-	75	0	-	100	0	-	100	50	-	100	25	-	75	20.6

Keys: CNS = Coagulase negative *Staphylococcus* N = Number of observation, S = Susceptible, I = Intermediate, R = Resistance, C = Chloramphenicol, G = Gentamycin, T = Tetracycline, P = Penicillin, VA = Vancomycin, S = Streptomycin, SU = Sulphamethizole, K = Kanamycin

5. DISCUSSION

The overall purpose of this study was to assess the hygienic condition, bacterial quality of raw cow milk and to determine antimicrobial susceptibility of the major bacterial isolate in Arbegona, Bensa and Bona districts in Sidama zone. This was due to the fact that milk produced in Ethiopia by the informal sector is not regulated by any agency and such milk may pose a health hazard due to contamination with pathogens. Generally, the present findings showed that, there are several practices undertaken at farm level such as type of animal house floor, not washing hands and udder/teats before milking, water used for cleanliness (hands and milk equipments), type of storage containers used and milk storage duration under room temperature predispose raw milk to microbial contaminations. Apart from that, it was observed that there are traditional ways practiced by small-holder dairy farmers which includes consumption of raw milk and milk products.

5.1. Questionnaire Survey

Result of questionnaire survey and observation in the study site showed that milk was generally produced by dairy producers under unhygienic environmental condition. This study further revealed that most smallholder dairy farmers managed their cattle in poor cattle houses that are 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 in Tanga (Shija, 2013) and in Arusha, Tanzania (Bukuku, 2013). Under traditional livestock keeping system, it becomes a challenge to have clean animal houses. 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 household level.

It was further found that factors that were likely sources of microbial contamination in milk include hand milking in a dirty animal house, not washing udder and/or teats before

milking, reckless milking personnel, not washing hands before milking, use of poor quality water for cleanliness (hands and milk equipments), not covering milk after milking and prolonged milk storage under room temperature. It was observed that majority of farmers do not comply with good milking practices and general sanitation.

However, except for a few urban cattle keepers, the barns were not constructed following recommended structure. Yoseph *et al.* (2003) and Yitaye *et al.* (2009), reported similar results in the northwestern Ethiopian highlands dairy production. As observed during the field visit, the barns were not constructed to facilitate drainage of the farm wastes, which leads to soiling of dairy cows and contamination of milk. Most of the respondents clean the barn on daily basis only by removing the feces, while few of them clean three times a week. This is in agreement with Zelalem (2010), reported that about 87% of the respondents cleaned their barn on daily basis, while few (9%) of them cleaned only three times a week in the Ethiopian highlands. Teats and udders of cows inevitably become soiled while they are laying in stalls or when they are allowed to stay in muddy barnyard. Used bedding has been shown to harbor large numbers of microorganisms (Murphy and Boor, 2000). Milking was also performed in the same place after cleaning. Although, the high proportion of dairy cow owners earthen their barn floors, clean, dry and comfortable bedding condition is important to minimize the growth of pathogenic microorganisms. Practices that expose the teat end to these organic bedding sources, wet and muddy pens increase the risk of occurrence of mastitis and milk contamination (Ruegg, 2006).

In general, providing proper shelter for animals has not been given the required attention. Housing conditions in many of households were dirty and unclean. This may have a negative impact on the quality of milk and milk products produced and processed. Proper and clean housing environment is a prerequisite to produce milk and milk products of acceptable quality (Asaminew, 2007). The general hygiene at milking time is known to affect the numbers of microorganisms in the milk. It is recommended that before milking, the animal house should be cleaned; the udder and/or teats should be washed and dried.

In the study area all of the interviewed households practiced hand milking. Indeed, the hand milking practiced by animal attendants could result in microbial contamination of the milk. These practices could have contributed to the observed high microbial load in

the milk. Previous studies had similar observations (Swai and Schoonman, 2011; Shija, 2013). The cows in the study area are usually milked twice a day except few households in the urban area who reported milking three times a day. The practices of milking found in this study were similar with other reports (Asrat, 2009). However, milking operation is only limited to once or twice per day during the last stage of lactation around Wolayta zone (Ayantu, 2006; Rahel, 2008). Milkers dip their fingers in the milking vessel to moisten teats of the cows with the intention of facilitating milking. However, such practice may cause microbial contamination of the milk from the milker's hand.

Most dairy farm owners in the present study do not clean their cow's udder and teat with water. But also, the owner's who washed the udder and teat did not perform the cleaning sufficiently and do not dry it properly. It was reported by Depiazzi and Bell (2002), that pre-milking udder preparation and teat sanitation play important part in the microbial load of milk, infection with mastitis, and environmental contamination of raw milk during milking. Cleaning the udder of cows before milking is important since it could have direct contact with the ground, urine, dung and feed refusals while resting. Lack of washing udder before milking can impart possible contaminants into the milk. The current study is in agreement with other reports (Derese, 2008). Contrary to this study, Haile *et al.* (2012) reported that 82.5% of the small size farms owning households in Hawassa city are practicing pre milking udder washing. Cleaning of the udder before milking is important to remove both visible dirt and bacteria from the outer surface of the udder. Unless properly handled, milk can be contaminated by microorganisms at any point from production to consumption. Producers should therefore make udder washing a regular practice in order to minimize contamination and produce good quality milk.

Most of the respondents who practiced udder and teat drying use either bare hands or local material "*Kacha*" in the current study and it results in insufficient drying of udder. Wet teats allow skin and environmental bacteria to have easy access into mammary gland (Ruegg, 2006). Since drying was not practiced sufficiently by the dairy cow owners in the study area, contamination level of the milk is expected to be high.

Production of milk of good hygienic quality for consumers requires good hygienic practices (clean milking utensils, washing milker's hands, washing the udder and use of individual towels) during milking and handling, before delivery to consumers or processors (Getachew, 2003). In the study area, the majority of the respondents practiced washing of their milk utensils. However, the cleaning is not efficient and utensils are not properly dried. Surfaces such as milking equipments and hands coming in contact with milk if not clean enough may cause milk contaminations. During the current study, pots and plastic containers were the major utensils for collection and storage of milk. Narrow necked plastic containers which were commonly used are not easily washed especially in the bottom and inner corners thus may lead to sticking of milk residues. In such a situation, microorganisms can rapidly build up in potentially nutritious milk residues of storage containers consequently contaminating the milk on subsequent uses. Similar observations were also reported by Bukuku (2013), and Shija (2013), who observed high microbial load in milk which was correlated with narrow necked plastic containers used in handling of milk.

The source of water available to farmers used for different purposes (to clean milking equipment and hands) is restricted to river and dug well water. However, the quality of both river and hand dug well waters used for cleaning may not be of the required standard thus can contribute to the poor quality of milk in the area. It is, therefore, important to heat treat water from river and hand dug wells intended for cleaning purpose.

It was further realized that a number of practices related to animal managements and consumption habits could predispose the livestock keepers to zoonotic infections. These practices included consumption of raw milk and milk products made from raw milk. The reasons could be lack of knowledge about the health risk posed by consumption of raw cow. In the current study, questionnaire survey results indicate that most of the dairy cow owners in the study area consumed milk in its raw state. The practices of milking found in this study were similar with other reports (Fanta, 2010). Thus, consumption of raw milk with no treatment may pose a public health hazard as a result of poor safety and quality. This habit therefore poses a lot of dangers to consumers in relation to milk-borne

diseases (Lues *et al.*, 2003). Elsewhere despite of livestock keepers being aware of the risk of contracting zoonotic infections and milk-borne diseases, the general public still consume raw milk (Shirima *et al.*, 2003; Mosalagae *et al.*, 2011). A study by Shirima *et al.* (2003) highlighted several zoonotic diseases that are common in small-holder dairy include, tuberculosis, brucellosis, anthrax and foot and mouth disease. Therefore, more public health education is needed at different levels along the food production chains (farmers, transporters and consumers) to safeguard the public from health problems emanating from animals. Furthermore, poor safety and quality of the milk as a result of poor hygiene may greatly discourage consumers demand. This in agreement with scientifically justified reports up on raw milk. Raw milk easily gets colonized by bacteria and thus can be spoiled and pathogenic microorganisms, which may have come from the teat canal, an infected udder or as environmental contaminants from unhygienic milking, handling and storage can affect the milk quality and safety (Farah *et al.*, 2007; Matofari *et al.*, 2007).

5.2. Bacteriological Count

The increment of bacterial load could be attributed to contamination of the milk throughout the value chain from production to market by different environmental factors through different exposures of contamination like pooling of milk from different sources together with unhygienic handling, and leaving the milk without cooling. This result was in agreement with the report of Farah *et al.* (2007) that there was an increase in bacterial counts through milk value chain showing the highest count from bulk milk of cow stored for 24 hrs without cooling in Somalia.

There was no significant difference in the mean AMBC among the districts. This may be due to the reason that the same practice which under goes in the three districts. In case of udder milk samples higher AMBC mean value (5.86 log₁₀ cfu/ml) was obtained as compared to the one reported by Alehegne (2004), for milk samples collected from udder (5.11 log₁₀ cfu/ml) in small-holder dairy farmers in Debre zeit, Ethiopia. Higher count was also obtained for samples collected from udder as compared to the reported

value by Mogessie and Fekadu (1993), for udder milk samples (3 to 4 log₁₀ cfu/ml) of Awassa College of Agriculture dairy farm, Ethiopia. The mean value of aerobic mesophilic bacterial counts for milk samples taken from the milking bucket at farm level (8.25 log₁₀ cfu/ml) found in this study is higher than that of previous reports for the same sample source, 6.0 log₁₀ cfu/ml for milk samples from selected dairy farms in Addis Ababa, Ethiopia (Bekele and Bayleyegn, 2000), 6.0 log₁₀ cfu/ml for samples taken from milk collection utensils of Awassa College of Agriculture dairy farm, Ethiopia (Mogessie and Fekadu, 1993), 5.84 log₁₀ cfu/ml for cow milk samples collected from different locations in India (Lingathurai *et al.*, 2009), Alganesh *et al.* (2007) for milk samples (7.6 log₁₀ cfu/ml) collected from East Wollega, Ethiopia and 6.57 log₁₀ cfu/ml for raw milk samples at the production point from India (Nanu *et al.*, 2007). The present AMBC value for milk samples collected from the marketed milk container (9.31 log₁₀ cfu/ml) was higher than the value reported by Yilma and Faye (2006), for milk samples (8.38 log₁₀ cfu/ml) collected from containers in central highlands of Ethiopia. The AMBC of the present study for milk samples collected from containers upon arrival was also higher than the count value reported by Abd Elrahman *et al.* (2009) for raw milk samples (6.63 log₁₀ cfu/ml) used for consumption, Sudan.

Such differences might be attributed to the differences in the hygienic conditions such as the quality of cleaning water, milk containers, personal hygiene followed by the various producers. The overall mean AMBC observed in the current study was higher than the maximum recommended level of 2.0×10^6 cfu/ml (EAS, 2007). As observed during sampling, higher AMBC obtained in the current study might be related to the overall sanitary conditions followed. Most of the farms in the present study housed and milked their animals under substandard hygiene, coupled with this they do not cool the milk. Murphy and Boor (2000), noted that ineffective use of cleaning water without heat treatment and the absence of sanitizers tend to fasten growth of less heat resistant organisms. A higher count also suggests that the milk has been contaminated by bacteria from different possible sources. This may be due the contribution of insufficient pre-milking udder preparation, the use of poor quality water for cleaning without heat treatment and the storage container and time. As reported by Van Kessel *et al.* (2004), the

use of insufficient and poor quality water for cleaning of milk handling equipments can result in milk residues on equipment surfaces that provide nutrients for the growth and multiplication of bacteria that can then contaminate the milk.

The overall coliform count observed in the current study ($5.52 \log_{10}$ cfu/ml) is higher than the value ($4.03 \log_{10}$ cfu/ml) reported for milk samples collected from cows kept under traditional condition in the Wolayta zone (Rahel, 2008). But lower values of $4.84 \log_{10}$ cfu/ml in milk samples collected in the Bahir Dar milkshed (Derese, 2008) and $4.49 \log_{10}$ cfu/ml in milk samples in the West Shewa zone of Oromia region (Asaminew, 2007) were reported. Coliform count of samples collected from udder milk samples ($3.61 \log_{10}$ cfu/ml) was higher than the reported by Mogessie and Fekadu (1993), for the udder milk samples ($1.0 \log_{10}$ cfu/ml) collected from Dairy Farm in Awassa, Ethiopia, also the present result is higher than the reported by Alehegne (2004), for milk samples collected in smallholder dairy farmers in Debre zeit, Ethiopia. The present CC value for samples collected from milking bucket samples ($5.47 \log_{10}$ cfu/ml) was higher than the result obtained by Bekele and Bayleyegn (2000), (4.11 to $4.85 \log_{10}$ cfu/ml) for milk samples collected from storage containers in Addis Ababa, Ethiopia, Alganesh *et al.* (2007) for whole milk samples ($4.46 \log_{10}$ cfu/ml) collected from storage in East Wallega Ethiopia, Nanu *et al.* (2007) for raw milk samples ($3.2 \log_{10}$ cfu/ml) at the production point. However, higher result was observed for raw milk samples collected from storage containers at farm level the reported by Rai and Dawvedi (1990), from India ($5.87 \log_{10}$ cfu/ml) and Zelalem *et al.* (2004), cow's milk samples ($6.57 \log_{10}$ cfu/ml) collected from different producers in central highlands of Ethiopia, but comparable with the value reported by Mogessie and Fekadu (1993), ($5.4 \log_{10}$ cfu/ml) for milk samples obtained from collecting utensils. The present mean value of CC of raw milk collected from marketed milk containers upon arrival ($7.47 \log_{10}$ cfu/ml) in this study was higher than the reported by Abd Elrahman *et al.* (2009) raw milk samples ($5.61 \log_{10}$ cfu/ml) used for processing in Sudan.

The overall values of coliform counts observed in the current study were much higher when compared with the recommended values given by the American Public Health

Service: < 100 cfu/ml for Grade A milk and 101- 200 cfu/ml for Grade B milk (WHO, 1997). Generally, the presence of high numbers of coliforms in milk indicates that the milk has been contaminated with fecal materials and it is an index of hygienic standard used in the production of milk, as unclean udder and teats can contribute to the presence of coliforms from a variety of sources such as poor farm hygiene, use of improperly washed milking equipment, unsanitary milking practices, contaminated water and cows with subclinical coliform mastitis can all lead to elevated coliform count in raw milk (Jayarao *et al.*, 2004).

These high counts in both aerobic mesophilic bacterial and coliform counts showed that the milk produced by dairy cow owners for consumption in the study area was of poor bacteriological quality. This extended difference in bacterial loads (both AMBC and CC) from the acceptability level for consumption level implies that milk is produced and handled under poor hygienic conditions in the study area. This indicates that there could possibility of contracting of infection or intoxication from milk-borne pathogens on consumption of the milk in the study area. The risk of infection and contaminations gets worse by consumption of the milk in its raw state which was observed to be common habit of most of the consumers in this study. This may be true with report by WHO (2006), in that zoonoses selectively affect families which live in rural areas.

From the results of this study, it was found that the majority of the milk samples had higher bacterial count than the maximum recommended level, suggesting unfitness for human consumption especially for those with habit of consuming raw milk and milk products made from raw milk. Presence of high total bacterial load in raw milk indicates contamination possibly from lactating cows, milking equipments, storage containers, unsatisfactory hygiene/sanitation practiced at farm level, unsuitable storage condition, unclean udder and/or teats, poor quality of water used for cleanliness and dirty hands of milkers. Generally, it further indicates the degree level of hygiene practices in the whole milk production process and reflects the time elapsed since milking at ambient temperature (Bukuku, 2013; Shija, 2013). From the observed practices involved in the

chain of milk production, handling, storage and consumption, during this study, the observed high bacterial count was expected. Therefore, based on these results and for the health safety of consumers, more food safety education should be given to producers, handlers and consumers.

It is not only the bacterial counts, which affect the hygienic quality of milk but also the type of bacteria. All samples collected from udder, bucket and marketed milk containers up on arrival contained bacterial agents, which were consistently in larger numbers and the isolates types, were more than those from the original (udder). In the course of this study, bacteria belonging to 10 genera from raw milk were isolated. The most predominant genera in raw milk were: *Staphylococcus* spp. (40.5%), *Streptococcus* spp. (23.2%), *Corynebacterium* spp. (13.2%), *Bacillus* spp. (7.3 %), *Escherchia coli* (5.8%), *Pseudomonas* spp. (3%), *Klebsiella* spp. (3%), *Citrobacter* spp. (1.6%), *Enterobactr* spp. (1.6%) and *Serratia* spp (0.8%). Among the different types of bacterial spp. identified in cow milk, *Staphylococcus* spp. followed by *Streptococcus* spp. were the most frequently isolated. This result shows that cow milk still represents a significant source of infection. The higher incidence rate of *Staphylococcus* spp. and *Streptococcus* spp. also has been reported in other studies (Haile, 2004). This is also evidenced from different reports in that cow milk contaminated with several pathogenic and spoilage bacterial spp. resulting in higher counts (Abera *et al.*, 2008). This is similar with report by Abeer *et al.* (2012) who isolated bacteria spp. including *E.coli* spp. with higher prevalence from cow milk in Egypt.

In this study isolation of *S. aureus*, *S. hyicus*, *S. intermidus*, *S. chromogenes*, *S. epidermidus*, *S. saprophyticus*, *S. simulans* , *Strep. agalactae*, *Strep. dysgalactae*, *Strep. uberis*, *Strep. bovis*, *Strep. zooepidemicus*, *Enterococcus fecalis*, *E. coli*, *Corynebacterium* spp, *Bacillus* spp, *Pseudomonas aeroginosa*, and *Enterobacter aerogenes* are incriminated as causes of subclinical and clinical mastitis in the cow (Harding, 1999). Microorganisms such as *Strep. agalactae*, *Strep. dysgalactae*, *Staphylococcus* spp., and *Coynebacterium bovis* are included in the contagious cause of mastitis, while *Strep. uberis*, *Strep. bovis*, *Enterococcus fecalis*, *E. coli*, *Corynebacterium*

spp, *Bacillus* spp, *Pseudomonas aeruginosa*, *Citrobacter freundii*, *Enterobacter agglomerans* and *Klebsiella pneumoniae* classified as causes of mastitis caused by environmental origin (Bonfoh *et al.*, 2003).

Generally, the organisms identified under the enterobacteriaceae group indicating probable environmental contamination, including fecal contamination of the milk as a result of poor hygienic condition. Similarly, Younan (2004), who studied in cow raw milk hygiene, reported that under rural production conditions, environmental contamination is likely to lay a bigger role in the hygiene of raw milk than mastitis bacteria. Many of the bacteria identified in the milk sampled are potential food-borne pathogens, and though some of the occurred in few samples, and the practice of pooling milk from different sources by producers generally observed could increase the risk posed by such organisms. These have been implicated in milk and other food related infections (Sivapalasingams *et al.*, 2004; Oliver *et al.*, 2009). *E.coli*, *Bacillus* spp. and *Staphylococcus* spp. which have been isolated in relatively higher frequency in this study, are associated with food borne intoxications through production of enterotoxins, mainly involved with *Bacillus cereus* and *S. aureus* (Donker *et al.*, 2007).

When the overall result of antimicrobials susceptibility test in the present study was compared on all isolates, Gentamycine, Chloramphenicol and Kanamycine were the most effective antibiotics as 80 to 100% of the total isolates were found to be susceptible. This could be because these drugs were the least frequently used in the study area in Veterinary services. Thus no more resistance was developed. Similar suggestion was given by Jaims *et al.* (2002), in that the development of antibiotic resistance is nearly always as a result of repeated therapeutic use and/or indiscriminate usage of them. Moreover, most of the isolates were Penicillin resistance in present study. In this study *S. aureus* isolates were most susceptible to Chloramphenicol, Gentamycin and Kanamycin while resistance to Tetracycline and Penicillin could be due to frequent usage of the latter two drugs in animal health. Similarly *E. coli* was highly resistant to Penicillin and Streptomycin but highly susceptible to Chloramphenicol due to its infrequent usage. These were comparable with the findings of Mekonnen *et al.* (2005).

6. CONCLUSIONS AND RECOMMENDATIONS

Milk intended for human consumption must be free from pathogens and must, if conditions permit, contain no or few bacteria. Clean milk could only be obtained if effective sanitary measures are taken starting from the point of milk withdrawn from the cow until it reaches the consumers.

From the findings of this study, it is concluded that:

1. Milk produced by small-holder dairy cow owners in Arbegona, Bensa and Bona districts of Sidama zone were of poor quality, risky for human consumption and can be a potential source of milk-borne infections.
2. Poor milking procedures, milk handling practices including the surrounding environment and treatment practices has greater influence on the bacterial contamination of raw milk and contributes to zoonotic pathogens.
3. This results showed that raw milk available to consumers has a high bacterial level of contamination. Measurable increased in AMBC and coliform counts throughout all sampling points was indicated. Based on the high level of counts found in the milk ready for consumption, one may suppose that this milk may pose a public health risk, and this suggests the need for more strict preventive measures.
4. Majority of raw milk samples from the udder, bucket and from marketed milk containers had higher AMBC and coliform counts, which was higher than the international acceptable limits. Hence, its keeping quality would be lower and some of the pathogens present in the milk have public health significance.
5. The study further concludes that there is a clear evidence of antimicrobial resistance to the most commonly used drugs by some of the isolates in the study area.

Based on the findings of the present study, the following recommendations are made:

- Awareness should be created among dairy cow producers on the importance of adequate udder preparation, hygienic milking technique, use of clean dairy equipment, washing of utensils and milkers hands using properly treated water in improve the milk hygienic quality and shelf life.
- If possible, potable water should be available for effective cleaning and sanitizing of milk equipment and udder preparations, otherwise boiled water should be used for such purposes.
- Routine assessment of milk quality produced by small-scale livestock keepers and consumed by the general public has to be mandatory in order to safeguard the public from milk-borne zoonotic infections, which may radiate through consumption of unsafe milk and milk products.
- The behavior of consuming raw milk and milk products made from raw milk should be discouraged. Milk stakeholders have to play their roles in educating the general public on likely public health consequences associated with such behavior.
- Veterinary and/or extension officers and associated stakeholders have to make periodic surveillance visit to small-scale livestock keepers, and create awareness, advice or conduct training on good animal health and management systems.
- Veterinarians should avoid indiscriminate use of antimicrobials and drugs showing resistance to pathogens in the study area.

7. REFERENCES

- Abeer, A.A., Gouda, S.A., Dardir, H.A. and Ibrahim, A.K. (2012): Prevalence of some milk bacterial pathogens threatening cow milk consumers in Egypt. *Global Vet.*, **8**: 76-82.
- Abera, B.H., Assefa, E.K. and Gebreselasse, H.K. (2008): Assessment of bacteriological quality of raw cows' milk in Ab-'Ala, north eastern Ethiopia. *Live. R. Dev.*, **3**: 1-4.
- Abd Elrahman, A.M., Ahmed, M.S., El Zubeir, M., Owni, O. and Ahmed, A. (2009): Microbiological and Physiochemical Properties of Raw Milk Used for Processing Pasteurized Milk in Blue Nile Dairy Company (Sudan). *Australian J. Basic Appl. Sci.*, **3**: 3433-3437.
- Addo, K.K., Mensah, G.I., Aning, K.G., Nartey, N., Nipah, G.K., Bonsu, C., Akyeh, M.L. and Smits, H.L. (2011): Microbiological quality and antibiotic residues in informally marketed raw cow milk within the coastal savannah zone of Ghana. *Trop.Med. Int. H.J.*, **16**: 227 – 232.
- Alehegne, W. (2004): Bacteriological quality of Bovine milk in small holder dairy farms in Debre Zeit, Ethiopia, Faculty of veterinary Medicine, Addis Ababa University, M.Sc thesis, Addis Ababa, Ethiopia, Pp 34.
- Alganesh, T., Ofodile, L.N. and Fekadu, B. (2007): Microbial quality and chemical composition of whole milk from Horro cattle in East Wallega Ethiopia. *Chemical Society of Nigeria*, **33** (1): 31-36.
- American Public Health Association (APHA). (1992): Standard method for the examination of dairy products. 16th ed., APHA, Washington, Pp 213-223.
- Asaminew, T. (2007): Production, handling, traditional processing practices and quality of milk in Bahir Dar milk shed, M.Sc.Thesis. Haramaya University, Ethiopia.
- Asrat, G. (2009): Evaluation of traditional practices and effects of lactoperoxidase system on keeping quality of cottage cheese, M.Sc.Thesis. Department of Animal and Range Sciences, Hawassa College of Agriculture, School of Graduate Studies, Hawassa University, Ethiopia.

- Atkins, P.J. (2005): Fattening children or fattening farmers. School milk in Britain, 1921-941. *Economic History Review*, **58** : 57-78.
- Ayantnu, M. (2006): Women's role on production, processing and marketing of milk and milk products in *delbo* watershed of *Wolayta*, Ethiopia. Department of Animal and Range Sciences, Hawassa College of agriculture, School of Graduate Studies, University of Hawassa, M.Sc.thesis.
- Bekele, G. and Bayileyegn, M. (2000): Bacteriological quality of raw cow's milk from four dairy farms and a milk collection center in and Around Addis Ababa. *Berliner and Mucnchener Tieraerztliche Wochenschrift*, **113**: 276-278.
- Bertu, W.J., Dapar, M., Gusi, A.M., Ngulukun, S.S., Leo, S. and Jwander, L.D. (2010): Prevalence of Brucella antibodies in marketed milk in Jos and environs. *Afr. F. Sc.J.*, **4**: 062 - 064.
- Bonfoh, B., Wasem, A., Traore, A.N., Fane, A., Spillmann, H. C., Simbe, C., Alfaroukh, I.O., Nicolet, J., Farah, Z. and Zinsstag, J. (2003): Microbiological quality of cow's milk taken at different intervals from the udder to the selling point in Bamako (Mali). *Food Control*, **14**: 495-500.
- Boschi-Pinto, C., Velebit, L. and Shibuya, K. (2008): Estimating child mortality due to diarrhoea in developing countries. *Bull World Health Organ.*, **86**: 59-67.
- Bramley, A. J. and Mckinnon, C. H. (2004): The microbiology of raw milk, in R. K. Robinson (ed.), Dairy Microbiology, 2nd edn. *Elsevier Applied Science, London*, **1**:63-208.
- Bukuku, J.N. (2013): Awareness of health risks as a result of consumption of raw milk in Arusha City and Meru District, Tanzania. Unpublished dissertation for award of MSc. degree at Sokoine University of Agriculture, Morogoro, Tanzania, Pp 1 - 89.
- Clare, D.A., Bang, W.S., Cartwright, G., Drake, M.A., Coronel, P. and Simunovic, J. (2005): Comparison of sensory, microbiological, and biochemical parameters of microwave versus indirect UHT fluid skim milk during storage. *D. Sci.J.*, **88**: 4172-4182.

- CSA. (2013): Agricultural Sample Survey. Livestock, Poultry and Beehives population (private peasant holdings). Federal Democratic Republic of Ethiopia Central Statistical Authority (CSA), Addis Ababa, Ethiopia.
- Depiazzi, L.J. and Bell, J.R. (2002): Effect of pre-milking teat sanitation on the quality of raw milk. Department of Agriculture, Government of Western Australia. South Western Highway Bunbury, W.A. 6230, Bulletin 4563.
- Derese, T. (2008): Present situation of urban and peri-urban milk production and quality of raw milk produced in *West Shewa Zone*, Oromia Region. M.Sc.Thesis. Haramaya University, Ethiopia.
- Donkor, E.S., Aning, K.G. and Quaye, J. (2007): Bacterial contaminations of informally marketed raw milk in Ghana. *Ghana Med.J.*, **41**: 58 - 61.
- EAS (2007): *Raw Cow Milk – Specification*. East African Community (EAC 67:2007) standard. Pp 1 - 19.
- Fanta, D. (2010): Quantitative risk assessment of consuming milk contaminated with *staphylococcus aureus* in Debre-zeit, Ethiopia, MSc. Degree. Department of Agriculture and Veterinary medicine, Addis Ababa University, Debre-zeit, Ethiopia, Pp. 44-46.
- FAO. (2009): Animal production and health division; milk and dairy products. Pp 21.
- Faraha, M. and Salik, S. (2007): Evaluation of bacteriological contamination in raw (unprocessed) milk sold in different regions of Lahore, Pakistan. *J. Agric. Soc. Sci.*, **3**(3): 104-106.
- Feldmann, M., Zimmermann, A. and Hoedemaker, M. (2006): Influence of milking technique, milking hygiene and environment on microbial contamination of milking machine. *Deutsche Tierärztliche Wochenschrift*, **113**: 274-281.
- Flint, J., Duynhoven, Y., Angulo, F., DeLong, S., Braun, P., Kirk, M., Scallan, E., Fitzgerald, M., Adak, G., Socket, P., Elias, A., Hall, G., Gargour, N., Wale, H. and Braam, P. (2005): Estimating the burden of acute gastroenteritis, food-borne diseases and pathogens commonly transmitted by food. *Cli. Inf. D.J.*, **41**: 698 - 704.

- Food protection services. (2011): Raw milk contaminants and pathogens. Available from: <http://www.bccdc.ca/NR/rdonlyres/3A2C5D87-5615-4B4B-9279-F28BE80E7764/0/> Raw milk pathogens.
- Frisvad, J.C., Skouboe, P. and Samson, R.A. (2005): Taxonomic comparison of three different groups of aflatoxin producers and a new efficient producer of aflatoxin B1, sterigmatocystin and 3-O- ethylsterigmatocystin, *Aspergillus rambellii*. nov. *Syst. Appl. Microbiol.*, **28**: 442–453.
- Getachew, F. (2003): A Review of the small scale dairy sector in Ethiopia. FAO prevention of food losses programme. milk and milk products, post-harvest losses and food safety in sub-saharan Africa and Near East.
- Giangiacomo, R. (2000): Milk testing, quality control, hygiene and safely. FAO e-mail conference on “Small-scale milk collection and processing in developing countries”. June 6– August 3, 2000, Pp. 108.
- Gibson, H., Sinclair, L. A., Brizuela, C. M., Worton, H. L. and Protheroe, R. G. (2008): Effectiveness of selected premilking teat-cleaning regimes in reducing teat microbial load on commercial dairy farms. *L. App. Microbiol.J.*, **46**: 295-300.
- Griffiths, M. W. (2004): Milk and unfermented milk products. Gaithersburg, MD, *Aspen Publishers*, **1**: 507–534.
- Griffiths, M. W. (2007): The safety of milk and dairy foods: Is there anything new we should be aware of? *Australian D. T.J.*, **62**: 67-75.
- Haile, T. (2004): Prevalence and factors associated with bovine mastitis in *South Wollo*, Ethiopia. *Bull. Anim. Hith. Prod. Afr.*, **52**: 1-6.
- Haile, W., Zelalem, Y. and Yosef, T.G. (2012): Hygienic practices and microbiological quality of raw milk produced under different farm size in Hawassa, Southern Ethiopia. *Agric. Res. and Rev.*, **1**: 132- 142.
- Harding, F. (1999): Milk Quality. 2nd.ed. Gaithers burg, Maryland, *Aspen*, Pp 25-38.
- Haughton, P., Garvey, M. and Rowan, N. (2010): Emergence of *Bacillus cereus* as a dominant organism in Irish retailed powdered infant formulae (PIF) when reconstituted and stored under abuse conditions. *Food Saf.J.*, **30**: 814– 831.

- Jaims, E.C., Montros, L.E. and Renata, D.C. (2002): Epidemiology of drug resistance; the case of *Staphylococcus aureus* and Coagulase negative staphylococci infections. *Epidemiol. Drug Res.*, **44**: 108-112.
- Jayarao, B.M., Pillai, S.R., Sawant, A.A., Wolfgang, D.R. and Hegde, N.V. (2004): Guidelines for monitoring bulk tank milk somatic cell and bacterial counts. *J. Dairy Sci.*, **87**: 3561-73.
- Katakweba, A.S., Mtambo, M.A., Olsen, J.E. and Muhairwa, A.P. (2012): Awareness of human health risks associated with the use of antibiotics among livestock keepers and factors that contribute to selection of antibiotic resistance bacteria within livestock in Tanzania. *Live. Res. R. D.J.*, **24**: 179-184.
- Kim, S. G., Kim, E. H., Lafferty, C. J. and Dubovi, E. (2005): *Coxiella burnettii* in bulk tank milk samples, United States. *Emerging Infectious Diseases*, **11**: 619-621.
- Kothari, C.R. (2004): Research Methodology, Method and Techniques. India. *New age inter. Pub.*, Pp 175 - 180.
- LeJeune, J.T. and Rajala-Schultz, P.J. (2009): Unpasteurized milk: A continued public health threat. *Clin. Infec. Dis.*, **48**: 93-100.
- Lingathurai, S., Vellathurail, P., Ezli Vendan, S. and Anand, A. (2009): A comparative study on the microbiological and chemical composition of cow milk from different locations in Madurai, Tamil Nadu. India *J. Sci.Technol.*, **2**: 51-54.
- Livestock and Irrigation Value chains for Ethiopian Smallholders (LIVES). (2009): Zonal diagnosis and intervention plan *Sidama* zone, SNNP Region.
- Lues, J.F., Venter, P. and Van der Westhuizen, H. (2003): Enumeration of potential microbiological hazards in milk from a marginal urban settlement in Central South Africa. *J. Food Microbiol.*, **20**: 321-326.
- Matofari, J.W., Shitandi, A., Shalo, P.L., Nanua, N.J. and Younan, M. (2007): A survey of *Salmonella* enteric contamination of cow milk in Kenya. *Afr. J. Microbiol. Res.*, **1**: 46-50.
- Mekonnen, H., Workineh, S., Bayleyegne, M., Moges, A. and Tadele, K. (2005): Antimicrobial susceptibility profile of mastitis isolates from the cows in three major Ethiopian dairies. *Med. Vet.*, **176**: 391 394.

- Mellau, L.B., Nonga, H.E. and Karimuribo, E.D. (2010): A slaughterhouse survey of liver lesions in slaughtered cattle, sheep and goats at Arusha, Tanzania. *Res. Vete. Sci.*, **3**: 179 – 188.
- Mogessie, A. and Fekadu, B. (1993): Effect of container smoking and udder cleaning on microflora and keeping quality of raw milk from a dairy farm in Awassa. *Tropical Sci.*, **33**: 368-378.
- Mosalagae, D., Pfukenyi, D.M. and Matope, G. (2011): Milk producer's awareness of milk-borne zoonoses in selected smallholder and commercial dairy farms of Zimbabwe. *Trop. A. H. Prod.*, **43**: 733 - 739.
- Murphy, S.C. and Boor, K.J. (2000): Trouble-shooting sources and causes of high bacteria counts in raw milk. *Dairy, F.Env. San.*, **20**: 606-611.
- Nanu, E., Latha, C., Sunil, B.P., Magna, T. and Vrinda, K.M. (2007): Quality assurance and public health safety of raw milk at the production point. *Am. J. Food Technol.*, **2**: 145-152.
- National Mastitis Council (NMC), WI, USA. (2005): Using Bulk Tank Milk Cultures in a Dairy Practice. <http://www.nmconline.org/bulktank.htm>.
- Oliver, S.P., Boor, K.J. and Murphy, S.C. (2009): Food safety hazards associated with consumption of raw milk. *Foodborne Path. and Dis.*, **6**:793-806.
- O'reilly, C. E., O'connor, P. M., Kelly, A. L., Beresford, T. P. and Murphy, P. M. (2006): Use of hydrostatic pressure for inactivation of microbial contaminants in cheese. *Appl. Environ. Microbiol.*, **66**: 4890–4896.
- Palmer, J. (2001): Contamination of milk from the milking environment. IDF Bulletin, Pp 16-21.
- Pal, M. (2007): Zoonoses. 2nd Ed, *Satayam Publishers*, Jaipur, India.
- Pal, M. (2012): Public health hazards due to consumption of raw milk. *The Ethiopian Herald* March 14, 2012.P 10.
- Pal, M. and Jahdav, V.J.(2013): Microbial contamination of various Indian milk products. *Bev. Food World*, **40**: 43-44.
- Pandey, G.S. and Voskuil, G.C. (2011): Manual on milk safety, quality and hygiene. Golden Valley Agricultural Research Trust, Zambia, P 52.

- Pangloli, P., Dje, Y., Ahmed, O., Doane, C. A., Oliver, S. P. and Draughon, F. A. (2008): Seasonal incidence and molecular characterization of *Salmonella* from dairy cows, calves, and farm environment. *Foodborne Path. and Dis.*, **5**: 87-96.
- Parekh, T.S. and Subhash, R. (2008): Molecular and bacteriological examination of milk from different milk animals with special reference to Coliforms. *Current Res. in Bacteriol.*, **1**: 56 - 63.
- Perkins, N. R., Kelton, D. F., Leslie, K. E., Hand, K. J., Macnaughton, G. and Berke, O. (2007): An analysis of the relationship between wash water quality and bulk tank milk quality on Ontario dairy farms. *Dairy Sci.J.*, **90**: 330.
- Quinn, P.J., Carter, M.E., Markey, B. and Carter, G.R. (1999): Clinical Veterinary Microbiology. *Mosby International Limited*, Spain, Pp. 11-13.
- Quinn, P. J., Markey, B. K., Carter, M. E., Donnelly, W. J. and Leonard, F. C. (2002): *Staphylococcus* species. In: Veterinary Microbiology and Microbial Disease species. In: *Veterinary Microbiology and Microbial Disease*. Blackwell Science Ltd. Oxford, Pp 43-48.
- Rahel, N. (2008): Traditional and improved milk and milk products handling practices and compositional and microbial quality of milk and buttermilk in *delbo* watershed of *Wolayta* Zone, M.Sc.Thesis. Department of Animal and Range Sciences, Hawassa College of Agriculture, School of Graduate Studies, Hawassa University, Ethiopia.
- Rai, A.C. and Dawvedi, F. (1990): Bacteriological quality of market milk. *Indian Dairyman*, **101**: 487-491.
- Richardson, G.H. (1985): Standard method for the examination of dairy products, 15th ed., American Public Health Association, Washington D.C.
- Rodrigues, A.C., Caraviello, D.Z. and Ruegg, P.L. (2005): Management of Wisconsin dairy herds enrolled in milk quality teams. *Dairy Sc.J.*, **88**: 2660– 2671.
- Roesch, M., Doherr, M. G., Scharen, W., Schallbaum, M. and Blum, J. W. (2007): Subclinical mastitis in dairy cows in Swiss organic and conventional production systems. *Dairy Res.J.*, **74**: 86-92.

- Ruegg, L. (2006): Role of hygienic in efficient milking. WCDS advances in Dairy Technology. Department of Dairy Science, University of Wisconsin, **18**: 285-293.
- Safe food international. (2011): Food/Water borne illness outbreaks. Available from: <http://regionalnews.safefoodinternational.org/page/Food%2FWater+Borne+Illness+Outbreaks>.
- Sanaa, M., Poutrel, B., Menard, J. L. and Serieys, F. (2003): Risk factors associated with contamination of raw milk by *Listeria monocytogenes* in dairy farms. *Dairy Sci.J.*, **76**: 2891-2898.
- Sharma, D., Sharma, P.K. and Malik, A. (2011): Prevalence and antimicrobial susceptibility of drug resistant *Staphylococcus aureus* in raw milk of dairy cattle. *International Res. Microbiol.J.*, **2**: 466 – 470.
- Shija, F. (2013): Assessment of milk handling practices and bacterial contaminations along the dairy value chain in Lushoto and Handeni districts in Tanga region. Unpublished dissertation for award of MSc. degree at Sokoine University of Agriculture, Morogoro, Tanzania, Pp 1 - 88.
- Shirima, G.M., Fitzpatrick, J., Cleaveland, S., Kambarage, D.M., Kazwala, R.R., Kunda, J. and French, N.P. (2003): Participatory survey on zoonotic diseases affecting livestock keeping communities in Tanzania. *Animal and Vet. Adv.J.*, **2**(4): 253 - 258.
- Sivapalasingams, S., Friedman, C.R., Cohen, L. and Tauxe, R.V. (2004): Fresh produce: a growing cause of outbreaks of foodborne illness in the United States. *Journal of Food Protection*, **67**: 2342 - 2353.
- Spreer, E. (1998): Milk and dairy product technology. Mixa, A (translator). Marcel Dekker, INC. ISBN: 0-8247-0094-5. New York, Pp. 39-58.
- SPSS (2011): Statistical Package for Social Science, Inc. SPSS for window (Version 20) Chicago, Illinois, USA.
- Swai, E.S. and Schoonman, L. (2011): Microbial quality and associated health risks of raw milk marketed in the Tanga region of Tanzania. *Asian Pacific Trop. Biomed.J.*, **1**: 217 - 222.

- Syit, D.A. (2008): Detection and determination of oxytetracycline and penicillin G antibiotic residue levels in Bovine bulk milk from Debrezeit and Nazareth dairy farms in Ethiopia. In: Proceedings of the 1st International Technology, Education and Environment Conference African Society for Scientific Research. May, 2008, Addis Ababa, Ethiopia, Pp 325 – 346.
- Thomas, S. B. and Druce, R. G. (2001): Bacteriological quality of alternate day collected farm bulk tanks milk. *Dairy Sc. Abstracts*, **33**: 339-342.
- Valle, J., Gomez-lucia, E., Piriz, S. and Vadillo, S. (2004): Staphylococcal enterotoxins and toxic shock syndrome toxin-1 production during induced cow mastitis. *Food Protec.J.*, **54**: 267-271.
- Van den Berg, J.C. (1988): Dairy technology in tropics and subtropics. Pudoc, Wageningen, the Netherlands, P 290.
- Van Kessel, S.J., Karns, S.J. and Gorski, L. (2004): Prevalence of *Salmonellae*, *Listeria monocytogeneus*, and fecal coliforms in bulk tank milk on US dairies. *J. Dairy Sci.*, **87**: 2822- 2830.
- Van Schaik, G., Green, L.E., Guzman, D., Esparza, H. and Tadich, N. (2005): Risk factors for bulk milk somatic cell counts and total bacterial counts in smallholder dairy farms in the 10th region of Chile. *Preventive Vet. Med.*, **67**: 1–17.
- White, D. G., Harmon, R. J., Matos, J. E. and Langlois, B. E. (2003): Isolation and identification of coagulase-negative *Staphylococcus* species from bovine body sites and streak canals of nulliparous heifers. *Dairy Sc.J.*, **72**: 1886-1892.
- WHO. (1997): Global estimation of food- borne disease. World Health Statistics in Raw Milk, International Dairy Federation Brussels, Pp 8-11.
- WHO. (2003): Diet, nutrition and prevention of chronic disease. Report of a joint FAO and WHO Expert Consultation, Geneva, 2002. WHO Technical Report Series 916. Geneva, World Health Organization.
- WHO. (2007): Food safety and foodborne illness: World Health Organization. P.27
- WHO. (2006): Safer food for better health. A Global WHO food strategy, P 2.
- Yilma, Z. (2003): Sanitary conditions and microbial qualities of dairy products in urban and peri-urban dairy shed of the central Ethiopia.

- Yilma, Z. (2010): Quality factors that affect ethiopian formal milk business; experiences from selected dairy potential areas. Addis Ababa, Ethiopia: Netherlands Development Organization (SNV).
- Yilma, Z. and Faye, B. (2006): Handling and microbial load of cow's milk and Irigo fermented milk collected from different shops and produced in central highlands of Ethiopia. *Eth. J. Anim. Prod.*, **6**: 67-82.
- Yitaye, A., Wurzinger, M., Azage, T. and Zollitsch, W. (2009): Handling, processing and marketing of milk in the North western Ethiopian highlands. *Livestock Res. for Rural Dev.*, **21**: 97.
- Yoseph, M., Azage, T. and Alemu, Y. (2003): Evaluation of the general farm characteristics and dairy herd structure in urban and peri-urban dairy production system in Addis-Ababa milkshed. Proceedings of the 10th annual conference of the Ethiopian Society of Animal Production (ESAP), held in Addis Ababa, Ethiopia, 22-24 August 2002. ESAP, Addis Ababa, Ethiopia, Pp. 139-144.
- Younan, M. (2004): Milk hygiene and udder health. In: Farah, Z. and Fischer, A. (eds): Milk and meat from the cow- Handbook on products and processing. *Vdf Hochschulverlag*, Zurich, Switzerland, Pp 67-76.
- Zelalem, Y. (2010): Microbial properties of ethiopian marketed milk and milk products and associated critical points of contamination: an epidemiological perspective, Addis Ababa, Ethiopia.
- Zelalem, Y., Yohannes, G., Gizachew, B., Alemu, G.W. and Sendros, D. (2004): Major milk chemical composition and feed dry matter intake of multiparous Boran cows as affected by level of milk production under bucket feeding and partial suckling calf-rearing methods. Proceedings of the 11th annual conference of the Ethiopian Society of Animal Production (ESAP) held in Addis Ababa, Ethiopia, August 28-30, 2003, Pp. 397-402.

8. APPENDICES

Appendix I. Questionnaire survey format

Number..... Date.....

1. Personal data

Name..... address..... kebele..... sex.....
age.....

2. Source of water:

a. pipeline water..... b. well..... c. river..... d.
other.....

3. Feeding regime:

a. grazing..... b. stall feeding..... c. supplemented.....

4. Barn hygiene

4.1. Barn cleaning frequency

a. once in 3 days..... b. once in a week..... c. not cleaned.....

4.2. Bedding condition:

a. dusty..... B. muddy.....

5. Pre-milking udder preparation and hygiene:

5.1. Are flanks, udder or teats washed before milking? Yes..... no.....

5.2. Are flanks, udder or teats dried after washing? Yes..... no.....

6. Milk utensils

6.1. What type of milking utensils do you use? a. plastic.... b.... pot.... c.
other....

6.2. Cleaning frequency:

a. after each usage using water from well.....

b. after each usage using water from river.....

c. Three times a week using water from river.....

d. Not cleaned at all.....

6.3. Milking frequency? a. twice a day..... b. three times a day.....

7. Milking technique, storage and transport

7.1. Do milker's wash their hands before milking? A. yes..... b. no.....

If yes, hand cleaning water: a. tap water..... b. river water..... c. water from
well.....

7.2. Are hands washed between milking? A. yes..... B. no.....

7.3. Purpose of milk production:

a. Household consumption..... b. commercially..... c. others.....

7.4. Type of container to store and transport milk to market:

a. Pots..... b. Plastic..... c. others.....

7.5. Does the milk cooled? a. Yes..... b. No.....

7.6. Does the milk processed? a. Yes..... b. No.....

8. Calf management

8.1. Calf hygiene. a. Good..... b. not good.....

8.2. Calf feeding a. suckling..... b. hand feeding.....

9. Public health and producer/consumer awareness level:

9.1. Habit of milk consumption: a. raw..... b. boiled..... c. other.....

9.2. Do you know any health risk associated with raw milk consumption?

a. yes..... b. no...

9.3. Do you mix fresh and left over milk for consumption? a. yes..... b. no...

9.4. Do you pool/bulk milk of different sources/cows? a. yes..... b. no.....

10. Did the animal treated before? a. yes.... (When?)..... b. no.....

11. Did you suffered from food borne infections? a. yes.. disease name?..... b. no...

12. Value chain actors awareness;

12.1. Extension workers

12.1.1. Do you give extension service for consumers about health risk associated with raw milk consumption? a. yes..... b. no.....

12.2. Animal health workers

12.2.1. Do you give a health service to cow's of milk producers? a. yes...b. no...

12.2.2. If yes, how frequently? a. monthly..... B. twice a year.....

Appendix II . Primary identification of bacterial pathogens

One loopful of milk streaked on blood agar and MacConky agar



Incubation at 37 °c for 24-48 hours



Morphology characterization



Gram stain

Gram positive		Gram negatives rods	
Cocci	Rods	Growth on MacConky agar	No growth on Mac Conky agar
Oxidative, cat+ = <i>Micrococcus</i>	Fermentative, cat+/oxidase-= <i>Coryn.</i> spp	Oxidative, cat+/oxidase+ = <i>Pseud.</i> spp	Oxidative, cat+/oxidase+ = <i>Flavobacterium</i>
Fermentative, cat+ = <i>Staphylococcus</i>	Fermentative, cat-/oxidase- = <i>Act. pyogenes</i>	Fermentative cat+, oxidase- = <i>Past. haemolytica</i>	Fermentative, cat+/oxidase+ = <i>Past. spp.</i>
Fermentative, cat- = <i>Streptococcus</i>	Uncreative, cat+/oxidase- = <i>Rhodococcus</i>	Fermentative cat+, oxidase- = <i>Entrobacteriaceae</i>	
Uncreative, cat+ = <i>Rhodococcus</i>		Unreactive, cat+/oxidase+ = <i>Alcaligenes</i> spp, <i>Past. cabali</i>	

Source: Quinn, *et al.*, 1999.

Appendix III . Differential tests used for *Staphylococcus* species

Ser. No.	<i>Staphylococcus</i> spp.	Hemolysis	Pigment production	Coagulase test	Sugar fermentation		
					Manitol	Maltose	Trehalose
1	<i>Staph. Aureus</i>	+	+	+	+	+	+
2	<i>Staph. intermidus</i>	+	-	+	d	(+)	+
3	<i>Staph. Hyicus</i>	-	-	+	-	-	-
4	<i>Staph. epidermidus</i>	d	-	-	-	+	-
5	<i>Staph. chromogenes</i>	-	+	-	d	d	NT
6	<i>Staph. simulans</i>	d	-	-	+	(+)	NT
7	<i>Staph. saprophyticus</i>	-	-	-	d	+	-

NT= not tested, d=11-89% strains are positive, (+) =76-89% are positive

Source: Quinn, *et al.*, 1999

Appendix IV . Differential tests used for *Streptococcus* species

Ser. No.	<i>Streptococcus</i> spp.	CAM P test	Asculin hydrolysis	Growth MacConk y agar	Sugar fermentation			
					Sorbitol	Manitol	Salcin	Raffinose
1	<i>Strep. agalactae</i>	+	-	-	-	-	(+)	-
2	<i>Strep. dysgalactae</i>	-	-	-	-	-	-	-
3	<i>Strep. bovis</i>	-	-	-	-	v	+	+
4	<i>Strep. uberis</i>	(+)	+	-	+	+	+	-
5	<i>Strep. zooepidermicus</i>	-	-	-	+	-	+	-
6	<i>Enter fecalis</i>	-	+	+	+	+	+	-

(+)= majority of strains are positive, v = variable reactions.

Source: Quinn, *et al.*, 1999

Appendix V .Differential tests used for *Corynebacterium* species

Ser. No.	<i>Corynebacterium</i> spp.	Catalase test	Hemolysis test	Sugar fermentation			
				Glucose	Lactose	Maltose	Trehalose
1	<i>C. ulcerans</i>	+	V	+	-	+	-
2	<i>C. bovis</i>	+	-	-	-	-	-
3	<i>C. haemolyticum</i>	+	-	+	+	+	-

v = variable reaction.

Source: Quinn, *et al.*, 1999

Appendix VI . Differential tests used for *Bacillus* species

Ser. No.	<i>Bacillus</i> spp.	Citrate test	Arabinose test	Manitol	VP test
1	<i>Bac. Stearothermophilus</i>	-	v	-	-
2	<i>Bac. Cerus</i>	+	-	-	+
3	<i>Bac. Brevis</i>	d	-	D	-

V = variable reaction, d = 11-89% strains are positive.

Source: Quinn, *et al.*, 1999

Appendix VII . Differential tests for Gram-negative rods

Ser. No.	Gram negative bacteria	1	2	3	4	5	6	7	8
1	<i>E. coli</i>	+	+	-	-	(+)	-	+	+
2	<i>Kleb. pneumoniae</i>	-	(-)	+	+	+	+	-	+
3	<i>Citrobacter freundii</i>	-	+	-	+	-	+	-	+
4	<i>Pseud.aeruginosa</i>	-	-	-	+	+	-	+	+
5	<i>Serratia spp.</i>	-	-	+	+	+	-	-	+
6	<i>Ent. agglomerans</i>	+	-	+	+	-	D	-	+
7	<i>Ent. aerogenes</i>	-	-	+	D	+	-	-	+

1 = Indole test, 2 = Methyl red test, 3 = Voges proskeur test, 4 = citrate utilization, 5 = Lysine decarboxylase test, 6 = Urease test, 7 = Oxidase test, 8= Growth on MacConkey Agar, D=26-75% of strains positive, (+)=76-89% of strains positive, (-)=11-25% strains are positive.

Source: Quinn, *et al.*, 1999

Appendix VIII . Media used for isolation and counts of bacteria

Blood agar base, 500 g:

Composition (g/l): Nutrient substrate (heart extract and peptones) 20.0; sodium chloride 5.0; agar-agar 15.0. pH 6.8 ± 0.2 at 25⁰c.

Preparations: Suspend 40 g in 1 litre of demineralized water by heating in a boiling water bath or in current of steam and autoclave at 121⁰C for 15 minutes. Cool to 45-50⁰c, add 5-8% sterile defibrinated sheep blood and mix taking care to avoid bubble formation. Pour into petridishes.

Mac Conkey, 500g:

Composition (g/l): Peptone from casein 17.0; peptone from meat 3.0; sodium chloride 5.0; lactose 10.0; bile salt mixture 1.5; neutral red 0.031; crystal violet 0.001; agar-agar 13.5. pH 7.1 ± 0.2 .

Preparation: Suspend 50g in 1 litre of demineralized water by heating in boiling water bath or in a current of steam; autoclave 15 minutes at 121°C .

Nutrient agar, 500g:

Composition (g/l): 'Lab-Lemco' powder 1.0; yeast extract 2.0; peptone 5.0; sodium chloride 5.0; agar 15.0. pH 7.4 ± 0.2 .

Preparation: Suspend 28 g in 1 litre of distilled water. Bring to boil to dissolve completely. Sterilize by autoclaving at 121°C for 15 minutes.

Trypton water, 500gm:

Composition (g/l): Peptone from casein 10.0; sodium chloride 5.0. PH 7.0 ± 0.2
Preparation: Dissolve 15 gm/l, autoclave in 15 min at 121°C .

Procedure and interpretation: One milliliter of ether was added to a 5ml portion of a 48 hrs culture grown at 37°C in a peptone water and shaken well and allowed to stand until the ether rises to the top. Gently Kovac's reagent was added down the side of the test tube and the formation of brilliant red ring between the medium and ether was indicative of an indole production.

MR-VP broth (Methyl Red- Voges-prouskauer) 500gm:

Composition (g/l): Peptone from meat 7.0; D(+) glucose 5.0; tampon phosphate 5.0. pH 6.9 ± 0.2 .

Preparation: Suspend 17 g in 1 litre of demineralized water; dispense 5 ml portions into tubes and autoclave 15 minutes at 121⁰C.

OF- basal medium (Oxidation- Fermentation), 500g:

Composition (g/l): Peptone from casein 2.0; yeast extract 1.0; sodium chloride 5.0; dipotassium hydrogen phosphate 0.2; bromothymol blue 0.08; agar-agar 2.5. pH 7.1± 0.2.

Preparation: Suspend 11 g in 1 litre of demineralized water by heating in a boiling water bath or in a current of steam; autoclave 15 minutes at 121⁰C; at approximately 50⁰C mix in 100 ml/l of a filter sterilized 10% solution of D (+) glucose, lactose or other carbohydrates; dispense into tubes to give depth of approximately 5cm, in half of the tubes immediately overlay the medium with a 1cm layer of sterile paraffin viscous.

Testes for Enzymes:

Catalase test: This demonstrates the presence of catalase, an enzyme that catalyses the release of oxygen from hydrogen peroxide. A drop of 3 % hydrogen peroxide poured on the glass slide and then small amount of the culture to be tested is picked from a nutrient agar with a clean sterile platinum loop or a clean, thin glass rod and this is added into hydrogen peroxide solution held on glass slide. The production of gas bubbles indicates a positive reaction. It occurs almost immediately. A false positive reaction may be obtained if the culture medium contains catalase (ex. blood agar) or if an iron wire loop is used (Collee, 1989).

Oxidase test: This test depends on the presence of oxidases in the bacteria that will catalyse the transport of electrons between electrons donors in the bacteria and a redox dye - tetramethyl-p-phenylene-diamine. The dye is reduced to a deep purple colour. The dye is used for screening species of *Alcaligenes*, *Pseudomonas*, *Flavobacterium* and *Pasteurella* spp, which give positive reactions and for excluding the Enterobacteriaceae, all species of which give negative reactions.

Urease test: 500g (Merck, Germany): Composition (g/l): Yeast extract 0.1; potassium dihydrogen phosphate 9.1; disodium hydrogen sulphate 9.5; urea 20.0; phenol red 0.01. Preparation: Dissolve 38.5 g/l and sterilize in 5 minutes in a current of steam under mild condition. Dispense approximately 3 ml into test tubes.

Violet red bile dextrose agar (VRBD agar), 500 g (for coliform counts), OXOID, England:

Composition (g/l): Peptone from meat 7.0; Yeast extract 3.0; sodium chloride 5.0; D (+) glucose 10.0; bile salt mixture 1.5; neutral red 0.03; crystal violet 0.002; agar-agar 13.0. Preparation: Dissolve 39.5 g/l, sterilize by boiling for 1 minute, and do not autoclave it. The prepared medium is clear and slightly red.

Plate count agar (for total aerobic plate count), 500g (OXOID, England):

Composition (g/l): peptone from casein 5.0; yeast extract 2.5; D (+) glucose 1.0; agar-agar 14.0.

Preparation: dissolve 22.5 g/l, sterilize by autoclaving at 115⁰C for 20 minutes.