INVESTIGATION OF *STAPHYLOCOCCUS AUREUS* ALONG THE MILK VALUE CHAIN AND ASSESSMENT OF ITS PUBLIC HEALTH SIGNIFICANCE IN ARSI NEGELLE TOWN, ETHIOPIA

MSc Thesis

By

Eyasu Tsige

Addis Ababa University, College of Veterinary Medicine and Agriculture, Department of Veterinary Microbiology, Immunology and Veterinary public health

JUNE, 2018

BISHOFTU, ETHIOPIA

INVESTIGATION OF *STAPHYLOCOCCUS AUREUS* ALONG THE MILK VALUE CHAIN AND ASSESSMENT OF ITS PUBLIC HEALTH SIGNIFICANCE IN ARSI NEGELLE TOWN, 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 Veterinary Public Health

By

Eyasu Tsige

JUNE, 2018

BISHOFTU, ETHIOPIA

INVESTIGATION OF *STAPHYLOCOCCUS AUREUS* ALONG THE MILK VALUE CHAIN AND ASSESSMENT OF ITS PUBLIC HEALTH SIGNIFICANCE IN ARSI NEGELLE TOWN, ETHIOPIA

Submitted by: Eyasu Tsige (DVM) _____ _____

Name of Student Signature Date

Approved *for submittal to* thesis assessment committee

1. Dr.Fanta Dessisa (DVM, MSc, Assoc professor) _____ _____

Major Advisor Signature Date

Addis Ababa University

College of Veterinary Medicine and Agriculture

Department of Microbiology, Immunology and Public Health

As member of the Examining Board of the final MSc open defense, we certify that we have read and evaluated the thesis prepared by: Eyasu Tsige 'Studies On Investigation of *staphylococcus aureus* along the milk value chain and assessment of its public heath significance in Arsi negelle town, Ethiopia' And recommended that it be accepted as fulfilling the thesis requirement for the degree of Masters of Veterinary public Health.

_____	_____	_____
Chairman	Signature	Date
_____	_____	_____
External Examiner	Signature	Date
_____	_____	_____
Internal Examine	Signature	Date
Dr. Fanta Dessisa	_____	_____
Major Advisor	Signature	Date
_____	_____	_____
Department chairperson	Signature	Date

TABLE OF CONTENTS **Page**

STATEMENT OF AUTHOR

I declare that this thesis is my genuine work and that all sources of materials used for this thesis been duly acknowledged. This thesis has been submitted in partial fulfillment of the requirements for 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. Brief quotations from this thesis are allowable without special permission provided that accurate acknowledgement of source is made. Requests for permission for extended quotation from or reproduction of this manuscript in whole or in part may be granted by the head of the major department or the Dean of the College when in his or her judgment the proposed use of the material is in the interests of scholarship. In all other instances, however permission must be obtained from the author.

Name: Eyasu Tsige Signature: _____

College of Veterinary Medicine and Agriculture, Bishoftu

Date of Submission: June 15, 2018

ACKNOWLEDGMENT

I am highly indebted to my academic advisors, .Fanta Dessisa(DVM,Msc,MPH,PhD Candidate,Associate Prof) for his technical advice and his help throughout the work, time devotion to review the manuscript and encouragement. Also I am highly grateful to my friend Dr. shimelis Mengistu , who helped me in edition of the material and provision of valuable ideas.

My special thanks also goes to my family my mother Etenesh Tesema, my sister Meseret Tsige and my brother in law Mesay kebede for their love, ceaseless support, esteem, unlimited moral and financial support.

LIST OF ABBREVIATIONS

a_w	Water Activity
CDC	Center of Disease Control
CLSI	Clinical and laboratory standard institute
CNS	Coagulase negative Staphylococci
CPS	Coagulase positive Staphylococci
DNA	Deoxy ribonuclotide
FBD	Food Borne Disease
FDA	Food and Drug Administration
H_2O_2	Hydrogen peroxide
Kms	Kilometers
m.a.s.i	Meters above sea level
MRSA	Methicillin-resistant *Staphylococcus aureus*
MSA	Manitol salt agar
MCC	Milk Collection Center
NaCl	Sodium Chloride
NAP	Nutrient Agar Plate
PAB	Purple agar base
PAHO	Pan American Health Organization
PH	Power of hydrogen
SE	Staphylococcal Enterotoxin
SFP	Staphylococcal Food Poisoning
TSB	Tryptone Soya Broth
µg	Microgram
x^2	Chi square

LIST OF TABLES **Page**

LIST OF TABLE IN THE APPENDIXES **Page**

LIST OF FIGURES **Page**

LIST OF FIGURES ON APPENDIX

LIST OF APPENDIXES **Page**

ABSTRACT

A cross sectional study was conducted from November 2017 to May 2018 in Arsi negelle town, west arsi zone, oromia, Ethiopia, to isolate and identify S. aureus and their resistance to different antimicrobials and also to assess the milk handling practices and consumption behavior among actors in the milk value chain. A total of 318 samples examined, 25.47 % (81) were positive for S.aureus. Of this, 17.85% and 63.15 % were positive for S. aureus at farm and milk collection centers level, respectively. The study has also showed relatively a higher contamination rate of S. aureus at MCCs than farm. There was statistically a significant difference (P=0.000) in isolation of S. aureus from Farm, MCC, swab of milk container and swab of milkers hand. All (n=81) isolates of S. aureus were tested for antimicrobial susceptibility with 11 selected antimicrobials. The isolates were highly susceptible to ciprofloxacin (76.54%), followed by sulphamethoxazole-trimethoprim (62.96%) and vancomycine (60.49%), however, they were highly resistant to Ampicilline(100%) , penicillin G (100%), Amoxacilline clavulanic acid (82.7%), Streptomycin (72.8%), tetracycline(60.49%) respectively. Lacks of stringent regulation and monitoring in the dispensing and use of antimicrobials in the area might contribute to the occurrence of high antimicrobial resistance to these drugs. An attempt was made to assess the milk handling practices and consumption behavior of actors in the milk value chain by using semi structured questionnaire survey that include farmers, consumers, hotel/café owners and milk collectors at MCC. It revealed poor milk handling practices, raw milk consumption behavior, and inadequate knowledge of milk borne disease. In general, the study has revealed the possibility of public health risk posed by S. aureus in Arsi negelle town. Creation of public awareness about good milk handling practices, pasteurization or boiling of milk prior to consumption, rational use of drugs and periodic assessment of the antimicrobial sensitivity of drugs prior to use are recommended.

***Key words**: cross sectional, S. aureus, milk, value chain, public health, resistant, antimicrobial*

1. INTRODUCTION

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 (Mansel, 2010). The demand of consumers for safe and high quality milk has placed a significant responsibility on dairy producers, retailers and manufacturers to produce and market safe milk and milk products (Adesiyun *et al*., 1997; Hahn, 1996; Mennane *et al*., 2007).

Microorganisms may contaminate milk at various stages of procurement, processing and distribution. The ill health of the cow and its environment, improperly cleaned and sanitized milk handling equipment, and unhygienic workers who milk the cow, come in contact with milk due to a number of reasons could serve as sources of contamination for the milk. These have inevitably increased the risk of infection of many people from common source. Lack of refrigeration facilities at farm and household level in developing countries of tropical regions, with high ambient temperature implies that raw milk will easily be spoiled during storage and transportation (Gilmour, 1999; Godefay and Molla, 2000).

In developed countries it is estimated that up to one-third of the population are affected by microbiological foodborne diseases each year (Schlundt *et al*., 2004).There have been around 250 different foodborne diseases described, and bacteria are the causative agent of two third of foodborne diseases outbreaks (Loir *et al*., 2003). Among the FBD, Staphylococcal food poisoning is one of the most common food-borne diseases worldwide (Hennekinne *et al*., 2012), resulting from the ingestion of staphylococcal enterotoxins preformed in food by coagulase-positive staphylococci, mainly *S. aureus*. As staphylococcal enterotoxins are heat stable, they may be present in food when *S. aureus* are absent (Balaban and Rasooly, 2000). Enterotoxins are proteins produced by some strains of staphylococci (Bergdoll and Lee, 2006) which, if allowed to grow in foods, may produce enough enterotoxin to cause illness when the contaminated food is consumed. These structurally related, toxicologically similar proteins are produced primarily by *S. aureus*, although *S. intermedius* and *S. hyicus* have also been shown to be enterotoxigenic (Adesiyun *et al*.,1984).

Prevention of staphylococcal food poisoning from the infected food handlers may be difficult as carriers are asymptomatic (Schmid *et al*., 2007). Staphylococcal food poisoning (SFP) is usually self-limiting and typically resolves within 24-48hr after onset. Symptoms like vomiting, abdominal pain and diarrhea usually occur approximately 2–6 h after the consumption of food containing enterotoxins. Although some cases have been reported, a fatal outcome is very rare. Occasionally it can be severe enough to warrant hospitalization, particularly when infants, elderly or debilitated people are concerned (Murray, 2005). It is a common disease whose real incidence is probably underestimated for a number of reasons, which include misdiagnosis, unreported minor outbreaks, improper sample collection and improper laboratory examination. The control of this disease is of social and economic importance. In fact, it represents a considerable burden in terms of loss of working days and productivity, hospital expenses, and economical losses in food industries, catering companies and restaurants (Anonymous, 2007; Chiang *et al*., 2008).

In addition to causing food poising, *S.aureus* causes clinical and subclinical mastitis in dairy cows and the reported prevalence of infected cows in affected herds ranges widely (Iman *et al.,* 2012). Intramammary infections caused by *S.aureus* in bovines are very difficult to cure with antibiotics because of their association with biofilms (Wallenberg *et al.,* 2002). The expansion of resistance against antimicrobials both in human and animal bacterial pathogens has been allied with the widespread remedial use of antimicrobials or with their administration as growth promoters in animals. Further transfer of antimicrobial resistant bacteria such as *S. aureus* to humans via the food chain has been reported (Angulo *et al.,* 2004).

Livestock farming in general and milk production in particular still play an important socioeconomic role in developing countries. Dairy products, including milk, cheese, dry milk powder, cream, butter and yoghurt are widely manufactured and consumed by the people of Ethiopia (Ashenafi, 1990). Therefore, it is important that foods and raw ingredients, including milk, should be subject to microbiological controls. However, these products have not been subjected to hygiene or sanitary control, because they are made at home (Ashenafi and Beyene, 1994; Yilma *et al*., 2007). Currently, in developing countries like Ethiopia, where high consumption of raw milk is common (Makita *et al*.2012), and also antimicrobial resistant strain of the bacteria is emerging in the country. Therefore, The aim of current research work is to

provide basic data on the situation of *S.aures* along the milk value chain in Arsi negelle town and if possible to partly contribute towards the development of national food safety strategies.

General objective

The general objective of this research project is to investigate and determine antimicrobial susceptibility of *staphylococcus aureus* in the milk value chain and assess its public health importance in Arsi negelle town.

Specific Objectives

- To estimate the prevalence of *Staphylococcus aureus* along the milk value chain..
- To determine antimicrobial susceptibility pattern of *Staphylococcus aureus* isolates.
- To assess the milk handling practices and consumption behavior among actors in the milk value chain

2. LITERATURE REVIEW

2.1 General description of *Staphylococcus aureus*

2.1.1 Morphology and general characteristics

The staphylococci were first described by the Scottish surgeon, Sir Alexander Ogston as the cause of a number of pyogenic (pus forming) infections in humans. In 1882, he gave them the name staphylococcus (Greek: staphyle, bunch of grapes; coccus, a grain or berry), after their appearance under the microscope (Martin and Maurice, 2008).

Staphylococci are Gram-positive cocci, approximately 1 μm in diameter that tends to occur in irregular clusters (Roberts and Greenwood, 2003) resembling bunches of grapes. Most Staphylococci are facultative anaerobes and catalase-positive. They are non-motile, oxidase-negative and do not form spores(Quinn *et al*., 2002). *S. aureus* produce golden yellow colonies (Bhunia, 2008) on blood agar; they appear as glistening, smooth, entire, raised, translucent colonies that often have a golden pigment. The colonies are 2-3mm in diameter after 24hr incubation and most strains show β-haemolysis surrounding the colonies (SU, 2014).

The cell wall of *S. aureus* contains three main components: the peptidoglycan comprising repeating units of N-acetyl glucosamine β-1, 4 linked to N-acetyl muramic acid; a ribitol teichoic acid bound via N-acetyl mannosaminyl-β-1, 4- N-acetyl glucosamine to a muramyl-6-phosphate; and Protein A, which is covalently linked to the peptidoglycan and particularly is characterized by its ability to bind to Fc component of the immunoglobulin in plasma causing auto agglutination. Most of the other species of Staphylococci lack protein A in their cell wall (Bhunia, 2008).

They are quite resistant to desiccation and high osmotic conditions. These properties facilitate their survival in the environment and growth in food products. Staphylococci organisms are usually readily killed at cooking, pasteurization temperatures and survives frozen storage. On the other hand, Staphylococcal enterotoxins (SEs) are extremely heat stable (Silva *et al*., 2000; Quinn *et al*., 2002).

2.1.2 Taxonomy and classification

Clinically, the most important genus of *Staphylococcaceae* family is *Staphylococcus* (Rho and Schaffner, 2007). To date, more than 50 species and subspecies of Staphylococci have been described according to their potential to produce coagulase. Their classification thus distinguishes between coagulase-producing strains, designated as Coagulase-Positive Staphylococci (CPS) and non coagu lase-producing strains, called Coagulase-Negative Staphylococci (CNS). Among CNS, some species are known to play an important role in the fermentation of meat and milk-based products and are therefore considered as food grade (Hennekinne *et al*., 2012).The scientific classification of *Staphylococcus* is as follows: Kingdom Bacteria, Phylum Firmicutes, Class Bacilli, Order Bacillales, Family *Staphylococcaceae,* and Genus Staphylococcus. The pathogenic staphylococci, *S. aureus*, (most strains) are coagulase-positive (Morrison, 2008).

2.1.3 Growth and survival characteristics

S. aureus is a facultative anaerobe so can grow under both aerobic and anaerobic conditions. However, growth occurs at a much slower rate under anaerobic conditions (Stewart, 2003). The growth and survival of *S. aureus* is dependent on a number of environmental factors such as temperature, water activity (aw), pH, the presence of oxygen and composition of the food. These physical growth parameters vary for different *S. aureus* strains (Stewart, 2003). The temperature range for growth of *S. aureus* is 7–48°C, with an optimum of 37°C. *S. aureus* is resistant to freezing and survives well in food stored below -20°C; however, viability is reduced at temperatures of -10 to 0°C. *S. aureus* is readily killed during pasteurization or cooking. Growth of *S. aureus* occurs over the pH range of 4.0–10.0, with an optimum of 6–7 (ICMSF, 1996; Stewart, 2003).

Table 1. Factors permitting growth and enterotoxin production by *Staphylococcus aureus*(stewart, 2003).

Factor	**Growth**		**Enterotoxin Production**	
	Optimum	Range	Optimum	Range
Temperature, ^{o}C	35–37	7–48	35–40	10–45
pH	6.0–7.0	4.0–9.8	Ent. A. 5.3–6.8 others 6–7	4.8–9.0
NaCl	0.5–4.0%	0–20%	0.5%	0–20%
Water activity	0.98–40.99	0.83–0.99	>0.99	0.86–40.99
Atmosphere	Aerobic	Aerobic-Anaerobic	5–20%DO_2	Aerobic-Anaerobic

S. aureus is uniquely resistant to adverse conditions such as low aw, high salt content and osmotic stress. In response to low aw, several compounds accumulate in the bacterial cell, which lowers the intracellular aw to match the external aw (Montville and Matthews, 2008). As such, most *S. aureus* strains can grow at low water(aw) activity over a range of 0.83 to >0.99 corresponding with a salt content of about 14 % (FDA, 2012; Stewart, 2003). *S. aureus* is a poor competitor, but its ability to grow under osmotic and pH stress means that it is capable of thriving in a wide variety of foods, including cured meats that do not support the growth of other foodborne pathogens. Several chemical preservatives, including sorbates and benzoates, inhibit the growth of *S. aureus*. The effectiveness of these preservatives increases as the pH is reduced. Methyl and propyl parabens are also effective. *S. aureus* can grow both aerobically and anaerobically in various foods. Production of various enzymes and toxins, some of which cause damage to mammary tissue and allow tissue invasion, an ability to survive in the keratin of the teat canal of healthy cows, a capability to resist phagocytosis, for example, 'protein A' in the bacterial cell wall of some *S. aureus* strains binds to the portion of antibody molecules making the bacteria unrecognizable to neutrophils, even if phagocytosed, *S. aureus* may survive and even multiply inside phagocytes. As well as these intrinsic properties, many *S. aureus* strains have the ability to resist antibiotic therapy. They produce beta-lactamase, an enzyme that inactivates penicillin and closely related antibiotics. Probably around 50% of mastitis *S. aureus* strains produce beta-lactamase and there is evidence that these strains are more difficult to cure with antibiotics, Establishment of abscesses and

fibrosis within the mammary gland that reduces penetration of antibiotics and movement of *S. aureus* to an intracellular site where, there will generally be reduced concentrations of antibacterial agents (Gyles *et al*., 2004).

2.1.4 Toxins produced by *Staphylococcus aureus*

The SEs are short proteins belonging to a large family of pyrogenic toxin super antigens with a disulphide bridge secreted in the medium and soluble in water and saline solutions (Loir *et al*., 2003; Salandra *et al*., 2008). They are highly stable, resist most proteolytic enzymes, such as pepsin, or trypsin, and thus keep their activity in the digestive tract after ingestion. They are highly heat resistant as well, which can resist 100^0C for at least 30 minutes and probably longer (Martın *et al*., 2004; Walderhaug, 2007). Although pasteurization and cooking kill staphylococci cells which are heat labile, thermostable SEs generally retain their biological activity. Thus, cases of illness might occur although no viable bacteria can be isolated from the suspected foodstuff and since SEs are more heat stable than the staphylococci bacteria, it is possible to test a food product and obtain negative staphylococci culture results and positive SEs tests (Atanassova *et al*., 2001; Soejima *et al*., 2007).

The amount of enterotoxins produced is determined by factors such as the composition of the food, competition from other microorganisms (the presence of other bacteria affects the production of enterotoxin apparently by limiting the multiplication of the staphylococci), temperature and time (Hagstad and Hubbert 1986; Salyers and Whitt, 2002). To date, a family of 14 different SE types have been identified, which share structure and sequence similarities, of which the six antigenic types (named SE-A, B, C, D, E and G) are most commonly encountered in SFP. In general, SE-A is recovered from food poisoning outbreaks more often than any of the others, with SE-D being second most frequent and the fewest number of outbreaks are associated with SE-E (Jay, 2000; Shah, 2003; Salandra *et al*., 2008).

2.1.5 Antimicrobial resistance

Antimicrobial resistance is the most puzzling question of public health in the earlier decade of 21 century. Among bacteria this question seems to be more alarming due to its short generation time and efficient gene recombination mechanisms (Soares *et al*., 2012). Drug resistance is an almost inevitable consequence of the use of antimicrobial drugs in food-producing animals, and specifically in the developing countries by use of medicines in humans (Threlfall *et al.*, 2000, Bogaard and Stobberingh, 1996). A recent estimate in the United States suggests that 24.6 million pounds of antibiotics are given to animals each year as growth promoters at sub-therapeutic amounts in their feed compared to 3 million pounds consumed by humans (White *et al*., 2001).

The epidemiological and clinical importance of staphylococcus species is not only because of its distribution and pathogenicity but especially due to its ability to overcome antimicrobial effects (Soares *et al*., 2012). More than 90% of *S. aureus* strains contain plasmids that encode β-lactamase, the enzyme that degrades many, but not all, penicillins. Some strains of *S. aureus* are resistant to the β-lactamase resistant penicillins, such as methicillin, by virtue of changes in the penicillin-binding protein in their cell membrane. These strains are commonly known as methicillin-resistant *S. aureus* (MRSA). Rare strains called vancomycin-intermediate *S. aureus* (VISA), with reduced sensitivity to vancomycin have also emerged (Levinson, 2008; Waters *et al*., 2011).Methicillin resistant *S aures* (MRSA) is the major cause of nosocomal infection in human. In addition, community acquired MRSA has now become a major concern (Otter and French, 2008). New evidence also suggests that domestic animals, including food animals, are capable of serving as reservoir and shedders of MRSA, and the transmission between hosts species may be possible (Loo *et al*., 2007).

2.2 EPIDEMIOLOGY

2.2.1. Source of contamination and reservoir

In general, staphylococci are facultative pathogenic organisms that are part of the normal skin flora of most animal species. Epidemiological studies have revealed several bacterial sources and carrier

sites varying according to the animal species. The staphylococci are intimately associated with animals and cannot be regarded as environmental bacteria. Ruminants are carriers of staphylococcal strains on their skin, which includes the teat skin. The species distribution, however, differs in different body regions, and the teat skin and teat apex flora differ from the flora associated with the hairy skin. The development of mastitis is related to the entrance in the teat duct of staphylococci colonizing the teat apex (Gyles *et al*., 2004).

The principal reservoir of *S. aureus* is the human carrier. A high proportion of healthy people have staphylococci in the nasopharynx and on the skin. The organism has been isolated from the head, body, legs and nose of cows, from the hands and nose of people, and from the environment such as the milking equipment, bedding materials and water courses (Ludmilla *et al.*, 2007).

2.2.2. Food products commonly implicated in staphylococcal food poisoning

The presence of small numbers of *S. aureus* on foods is not uncommon. It will occur naturally in poultry and raw meat as a frequent component of the skin microflora. Similarly, it can be isolated from raw milk where levels may sometimes be elevated as a result of *S. aureus* mastitis in the producing herd. As a poor competitor, it normally poses no problem in these situations since it does not grow and is eliminated by cooking or pasteurization. There have however been outbreaks caused by milk products such as dried milk and chocolate milk where growth and enterotoxin production occurred in the raw milk and the enterotoxin survived subsequent pasteurization. A good example of this is a large outbreak that occurred in Japan in 2000, affecting more than 13,000 people. A power cut during production of dried skimmed milk led to delays in processing that allowed *Staph. aureus* to multiply and produce enterotoxin. The contaminated powder was then used in a number of dairy products. Though not in itself a health threat, the presence of *Staph. aureus* on raw meats does pose the risk of cross-contamination of processed food (Martin and Maurice, 2008).

Contamination by food handlers is also probably a frequent occurrence in view of the high rate of human carriage. Colonization of the nose and throat with the organism will automatically imply its presence on the skin and food may also be contaminated from infected skin lesions or by coughing and sneezing. Since large numbers, typically $>10^6$ g $^{-1}$, are required for the production of enough

toxin to cause illness, contamination is necessary but is not alone sufficient for an outbreak to occur. In particular, temperature and time conditions must also be provided that allow the organism to grow (Martin and Maurice, 2008).Studies in the United States and the UK have found that poultry products and cold, cooked meats are the most common vehicles. Salted meats such as ham and corned beef are particularly vulnerable since the *S. aureus* is unaffected by levels of salt that will inhibit a large proportion of the competitive flora. Buffet meals where such meats are served are a common scenario for outbreaks as the food is necessarily prepared some time in advance and too often stored at ambient temperature or inadequately chilled. Canned foods also offer *S. aureus* a congenial, competitor-free environment and post-process leakage contamination of cans has been an occasional cause of outbreaks. Other outbreaks have been caused by hard cheeses, cold sweets, custards and cream-filled bakery products. In Japan, rice balls that are moulded by hand are the commonest vehicle while in Hungary, it is ice cream (Martin and Maurice, 2008).

2.2.3 Vehicle of transmition

Staphylococci exist in air, dust, sewage, water, milk, food, or on food equipment, environmental surfaces, humans, and animals (Bennett and Monday, 2003). Staphylococci are present in the nasal passages and throats and on the hair and skin of 50% or more of healthy individuals. This incidence is even higher for those who associate with or who come in contact with sick individuals and hospital environments. Although food handlers are usually the main source of food contamination in food-poisoning outbreaks, equipment and environmental surfaces can also be sources of contamination with *S. aureus*. Human intoxication is caused by ingesting enterotoxins produced in food by some strains of *S. aureus*, usually because the food has not been kept hot enough (60°C, 140°F, or above) or cold enough (7.2°C, 45°F, or below) (Acco *et al*., 2003; Bennett and Monday, 2003).

2.2.4 Distribution of *staphylococcus aureus* in raw milk in Ethiopia

Due to changing management conditions and using of different diagnostic tests, wide variation in the prevalence of *S. aureus* have been reported (Rodistitis, 2000).

Table 2: The Prevalence of Staphylococcus *aureus* isolated from raw milk in different areas of Ethiopia.

Study Area	Prevalence (%)	References
Sebeta	19.6	(Ayele *et al*., 2017)
Bahir dar	15.02	(Bitewa, 2015)
Addis Ababa	16.2	(Mekuria *et al.*, 2013)
Debre-Zeit	8	(Mokennen *et al*,. 2011)
Dire Dawa	25	(Tesfay *et al.*, 2013)
Jimma town	13.9	(Getahun and GebreSelassie, 2003)
Abaya, Borana pastoral area	6.8	(Worku *et al.*, 2012)
Ambo annd Guder town	12.6	(Megersa, 2015)
Hawassa	17.9	(Daka *et al.*,2012)
Wolaita sodo	15.1	(Tadesse,2014)

2.3. Pathogenesis and clinical feature

2.3.1 Staphylococcus infection in cattle

It is universally accepted that the route of infection in staphylococcal mastitis is via the teat. Staphylococci colonize the tip of the teat, especially when it is damaged or eroded. The organisms pass through the teat duct into the cistern and may subsequently establish in an area of secretory tissue. The pathogenesis of *S. aureus* in the mammary gland most likely involves the generally accepted concept of specific colonization. In vitro adhesion of *S. aureus* to ductular and alveolar mammary gland epithelial cells indicates that colonization might be an important step in the development of mastitis (Gyles *et al*., 2004). Furthermore, *S. aureus* bacteria are able to bind to extracellular matrix molecules. It is suggested that staphylococci might use matrix proteins exposed by micro lesions or appearing in blood clots as substrates for adhesion as a step in

colonization and the development of mastitic infections. Staphylococci isolated from bovine mastitis have the ability to bind to fibronectin, fibrinogen, laminin, and different types of collagen (Barkema *et al*., 2006).

Milk is an adequate medium for multiplication of staphylococci. During the course of staphylococcal multiplication, cytotoxigenic substances are produced, which causes an infiltration of the mammary gland by neutrophils. Aggregation of neutrophils results in clots in the milk and interalveolar edema. The presence of *staphylococci* and neutrophils obstructs the lobules, which start to involute. Accumulation of fibroblasts, macrophages, and lymphocytes results in expansion of the interalveolar connective tissue. The bacteria remain in the alveoli and ducts from where they are intermittently excreted. Local intense multiplication of *S. aureus* bacteria may result in abscesses or granulomata (Gyles *et al*., 2004).

S. aureus mastitis in cattle may be clinical or subclinical, and in its clinical form, the disease may vary from a severe peracute form to a very mild form without general signs of infection. The peracute or gangrenous form results in a severe general illness. Unless treatment of acute infections is successful, the reaction becomes chronic. This chronic reaction may also occur subclinically (Barkema *et al*., 2006). Treatment failures are particularly high in multiparous with more than one infected quarter and recurrences are frequent and culling is commonly the only solution (Michelle and Jeffrey, 2011).

2.3.2 Staphylococcal food born disease and enterotoxines.

The first description of food poisoning caused by staphylococci is thought to be that of Vaughan and Sternberg who investigated a large outbreak of illness in Michigan believed to have been caused by cheese contaminated with staphylococci (Martin and Maurice ,2008). Clear association of the organisms with foodborne illness had to wait until Barber (1914) demonstrated that staphylococci were able to cause poisoning by consuming milk from a cow with staphylococcal mastitis. In 1930, Dack showed that staphylococcal food poisoning was caused by a filterable enterotoxin (Martin and Maurice ,2008). There are currently 27 species and 7 subspecies of the genus Staphylococcus; enterotoxin production is principally associated with the species *S. aureus*, although it has also been reported in others including *S. intermedius* and *S. hyicus*. As a relatively mild, short-lived type of illness, staphylococcal food poisoning is perhaps more likely to be under-

reported than others. Most reported cases are associated with outbreaks and only a few sporadic cases are detected. In the United States between 1983 and 1987, staphylococci accounted for 7.8% (47) of the 600 bacterial food poisoning outbreaks that were recorded. Equivalent figures for England and Wales over the same period were 1.9% (54) out of a total of 2815 outbreaks. Outbreaks of staphylococcal food poisoning in the UK peaked during the 1950s at 150 outbreaks per year but have since declined to an annual level of 5–10 outbreaks in the period 1990 to 1996 and an average of one per year in the period 2000 to 2005(PAHO, 2001; Martin and Maurice ,2008).

Staphylococcal Food poisoning is characterized by a short incubation period, typically 2–4 h. Nausea, vomiting, stomach cramps, retching and prostration are the predominant symptoms, although diarrhea is also often reported, and recovery is normally complete within 1–2 days. In severe cases dehydration, marked pallor and collapse may require treatment by intravenous infusion (Atanassova *et al*., 2001; Lamprell *et al*., 2004). The short incubation period is characteristic of intoxication where illness is the result of ingestion of a pre-formed toxin in the food. *S. aureus* produces at least 11 enterotoxins designated SEA to SEJ. To add a touch of Byzantine complexity and confuse the unwary, there is no SEF and there are three variants of SEC. Toxin types A and D, either singly or in combination, are most frequently implicated in outbreaks of food poisoning. In the UK, type A is responsible for 52% of outbreaks, type D for 6%, types A and D combined for 19%, and types C and D combined for 9%. Susceptibility varies between individuals but it has been estimated that in outbreaks less than 1 mg of pure toxin has been required to elicit symptoms. The toxins are small single chain polypeptides which share considerable amino acid homology. With the exception of SEI each contains a single disulfide loop near the molecule's centre. As a result of their compact structure they are resistant to gut proteases and heat stable, being inactivated only by prolonged boiling. Such procedures would of course eliminate viable *Staph. aureus* from a food so it is possible for someone to become ill from eating a food which contains no viable *S. aureus* (Martin and Muarice, 2008).

Staphylococcal enterotoxins are exoproteins which, when produced in food that is then ingested by humans, give rise to symptoms of acute gastroenteritis. The toxins have been shown to be proteins of low molecular weight, approximately 27–31 kilodaltons, consisting only of amino acids and are usually produced by CPS species (Omoe *et al*., 2005; Ash, 2008; Chiang *et al*., 2008). Though

frequently described as enterotoxins the *S. aureus* toxins are strictly neurotoxins. They elicit the emetic response by acting on receptors in the gut, which stimulate the vomiting centre in the brain via the vagus and sympathetic nerves. If these nerves are severed then vomiting does not occur. It is not known how the toxin induces diarrhoea but it has been shown not to stimulate adenylate cyclase activity.The *S. aureus* enterotoxins are now also known to be superantigens, molecules that are able to stimulate a much higher percentage of T cells than conventional antigens. What role this may play in gastrointestinal illness, if any, is not known (PAHO, 2001;Martin and Muarice, 2008).

According to recent studies, a high proportion of strains isolated from staphylococcal mastitis produce 'enterotoxin A', which causes many human outbreaks. Several studies were successful in isolating the *S. aureus* from skin lesions and cow milk, which is related to epidemic infections in man. One of the studies proved that produced interstitial mastitis in cows. The same phage type was found among animal caretakers, which indicates that the bacterium can be transmitted between man and animals and that the latter may re-infect man. An important causal factor in poisoning is keeping food at room temperature or inadequate refrigeration, practices which allow staphylococci to multiply. Lack of hygiene in food handling is another notable factor. Outbreaks of food poisoning may often be traced to a single dish. Pasteurization of milk does not guarantee safety if toxins were produced prior to heat treatment, as the toxins is heat-resistant. Outbreaks have also been caused by reconstituted powdered milk, even when the dried powder contained few or no staphylococci (Walderhaug, 2007).

2.4 Public health and economic importance

Staphylococcal Food Poisoning (SFP) is one of the most common Food borne diseases (FBD) and is of major concern in public health programs globally (Hennekinne *et al*., 2012). In developing countries, the surveillance system of FBD hardly exists and it is therefore, difficult to estimate the real magnitude of the problem . Even in countries where surveillance services are very efficient, the precise incidence of food poisoning is not known, as outbreaks are often not reported to public health authorities.

Hence, the incidence of FBD caused by Staphylococci is thought to be much higher than reported since many cases remain undeclared (Argaw, 2015). Food borne diseases are a serious and

growing problem in the world. *S. aureus* is a significant cause of FBD, causing an estimated 241,000 illnesses per year in the United States (Kadariya *et al*., 2014) and at least before the 1980s, it was implicated in many outbreaks. However, in recent years, the number of staphylococcal food poisoning outbreaks has declined. CDC reports indicate that during 1972 to 1976, it was associated with 21.4% of the foodborne disease outbreaks affecting 29.7% of the total cases; in contrast, between 1983 and 1987, there were 5.2% staphylococcal foodborne outbreaks with no deaths. This decline is probably a reflection of the better use of refrigerated temperatures to store food and improved sanitary practices that can control contamination and growth of *Staphylococcus aureus.* Even then, the number of outbreaks and number of cases of staphylococcal gastroenteritis is much higher than several other microbial foodborne disease outbreaks. In Japan, the annual average of food poisoning outbreaks from 1976 to 1980 was 827 of a total of 8,742 cases, 28.2% were caused by staphylococcal poisoning. (PAHO, 2001).

Among FBD, SFP is of major concern in global public health programmes. Staphylococcal organisms alone have found to cause hospitalization rates as high as 14%. Although not considered especially lethal, death can ensue if large amounts of SE are ingested: fatality rates range from 0.03% in the general population to as high as 4.4% for highly sensitive persons such as immune compromised persons, elderly persons and children (Kerouanton, 2007).

2.5 Diagnosis of Staphylococcal Food Poisoning

Symptoms of FBD associated with staphylococci are not suggestive and have little importance to warrant diagnosis (Loir *et al*., 2003). In the diagnosis of SFP, proper interviews with the victims and gathering and analyzing epidemiologic data are essential (Hobbs and Gilbert, 1981). Incriminated foods should be collected and examined for staphylococci or the SEs produced. The latter is especially important when foods that have been heated before consumption are implicated in the outbreak. Food handlers are also tested to ensure whether they are carriers of the strain responsible (Bautista *et al*., 1988; Rho and Schaffner, 2007).

Incorrect identification of an isolate can impact on the implementation of effective treatment and/or control measures (Rowland *et al*., 1994; Salyers and Whitt, 2002; Smith, 2007). In the diagnosis of SFP, detailed history, including the duration of the disease, characteristics and

frequency of bowel movements and associated abdominal and systemic symptoms, may provide a clue to the underlying cause. The presence of a common source, types of specific food, travel history, and use of antibiotics always should be investigated. Diagnosis is confirmed by isolation of the organism or SE from relevant specimens (Jay, 2000; Walderhaug, 2007).

The presence of relatively large numbers of enterotoxigenic staphylococci is a good circumstantial evidence that the food contains SEs. The most conclusive test is the linking of an illness with a specific food or in cases where multiple vehicles exist, the detection of the toxin in the food samples (Martın *et al*., 2004; Hein *et al*., 2005; Chiang *et al*., 2008). In cases where the food may have been treated to kill the staphylococci, as in pasteurization or heating, direct microscopic observation of the food may be an aid in the diagnosis (Hagstad and Hubbert. 1986; Bania *et al*., 2006; Walderhaug, 2007).

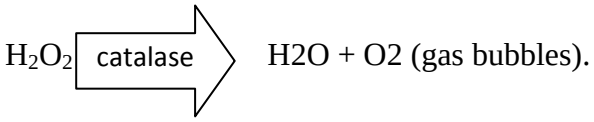
2.6 Isolation and identification of *staphylococcus aureus*

The isolation and identification of Staphylococcus species is conducted on the basis of colony morphology, haemolytic properties, Gram-stain, catalase production, coagulase production and biochemical profile or sugar fermentation (Quinn *et al*., 2002; Aycicek *et al*., 2005). Samples were inoculated aseptically on the surface of the blood agar medium by spreading with a sterile loop in such a way that bacteria are ultimately deposited singly because when the bacteria are at a sufficient distance from each other, the whole progeny of each accumulates locally during growth to form a discrete mass or colony which is readily visible to the naked eye. Each colony was presumed to be a pure culture, consisting exclusively of the descendants of a single cell (Loir *et al*., 2003; Shah, 2003).

On agar plates, staphylococcal colonies appear opaque to golden yellow in colour, glistening, smooth and in circular form. Blood agar is the medium of choice for isolation of the organism from specimens, and on 24 hours incubation staphylococci give good growth of creamy, often deeply pigmented colonies that is surrounded by the narrow zones of clear haemolysis, a broader zone of incomplete haemolysis or none depending on the species (Quinn *et al*., 2002; Bendahou *et al*., 2008). Some species of Staphylococcus synthesize the enzyme haemolysin. Haemolysin is an exoenzyme that lyses red blood cells. If a colony of bacterial cells is producing haemolysin and secreting it into the medium, there will be a round, clear zone surrounding the colony because the

red blood cells in that area have been lysed. The presence or absence of haemolytic properties, therefore, cannot be used as a definitive identification of Staphylococcus species as some species and strains of Staphylococcus species may not cause haemolysis (Quinn *et al*., 2002; Salandra *et al*., 2008).

Preparation and examination of Gram stained smears from typical colonies shows Gram positive spherical bacterium (coccus), which on microscopic examination appears in pairs, short chains, or bunched, grape like clusters (Aycicek *et al*., 2005). Catalase test is important to distinguish streptococci (catalase-negative) from staphylococci, which are catalase-positive. The catalase test determines if the organism produces the enzyme catalase that breaks down hydrogen peroxide (H_2O_2) to water and oxygen (Rowland *et al*., 1994; Shah, 2003). When mixed with 3% H_2O_2, catalase-positive organisms will generate bubbles of oxygen, which are visible to the naked eye while catalase negative organisms do not. This enzyme allows organisms to breakdown harmful metabolites of aerobic respiration and may be seen in aerobic and facultatively anaerobic organisms. It is preferable to test colonies for catalase production from media without blood since erythrocytes possess catalase activities (Quinn *et al*., 2002).



Pathogenic organisms require mechanisms to help them overcome host defense mechanisms. One mechanism involves coating the bacterial cells in a body substance, such as fibrin, to fool the immune system. The coating of a natural body substance will not trigger an immune response and this is accomplished through the production of coagulase. Coagulase is an exoenzyme that causes fibrin of blood plasma to be deposited on bacterial cells resulting in clot formation. Pathogenic staphylococci produce coagulase, while non-pathogenic strains are coagulase negative (Shah, 2003; Morrison, 2008).

A range of selective and diagnostic media have been developed to assist in the detection and enumeration of staphylococci in routine food surveillance programmes and food poisoning investigations (Baird and Lee, 1995). Selective bacteriological media containing one or more

agents that are inhibitory to microorganisms other than the target pathogen (staphylococci) can be applied. The microorganism of interest is not inhibited by the presence of these components in the medium, and will therefore, form visible colonies during incubation (Quinn *et al*., 2002). The two selective agents most commonly used for these pathogens are sodium chloride and potassium tellurite (Baird and Lee, 1995; Pal, 2007).

A common medium used for the isolation of pathogenic staphylococci is the mannitol salt agar (MSA). Some organisms cannot tolerate high osmotic pressure. Media containing higher than normal salt concentrations that inhibit the growth of these non-tolerant organisms other than the salt tolerant staphylococci (Baird and Lee, 1995). Mannitol salt agar contains a high salt concentration so only salt tolerant staphylococci will grow on high salt concentration of this medium that inhibits the growth of most other organisms. Additionally, MSA contains the sugar mannitol. Staphylococcal organisms can utilize mannitol as a fermentable carbohydrate (food source) and will produce acid end products from this metabolism. Since this process is invisible an indicator is added to the media to detect changes in pH. Phenol red is the indicator used in MSA. It is red at a neutral pH but turns yellow if conditions in the media become acidic. Pathogenic staphylococci not only grow on the medium, but they also produce acid from it. This acid production turns the pH indicator from red to yellow. Non-pathogenic staphylococci can grow on the medium but produce no acid from it and the medium remains pink (Jay, 2000; Quinn *et al*., 2002).

2.7. Management strategies

2.7.1 Prevention and control of infection and intoxication

It is certainly possible to maintain a low herd prevalence with <2% cows infected (in one or more quarters) with *S. aureus*. Since in the majority of herds, the most important reservoirs of the organism are infected cows, most control procedures are based on reducing the probability of spread between cows. These can be achieved through carrying out treatment; culling, drying off infected quarters and ensuring new heifers/cows are uninfected (Green and Bradley, 2004). However, there are other potential sources of *S. aureus* associated with the environment and these may be of special significance in particular herds in which the condition is difficult to control with

the traditional methods. Sources of *S. aureus* outside the mammary gland are also the reason why, it is virtually impossible to eradicate *S. aureus* mastitis from a commercial dairy unit.

The normal occurrence of *S. aureus* in raw food materials, among food handlers, and many food environments makes it impossible to produce sterile foods that are free of this bacterium. Thus, a zero tolerance is not economically possible to achieve. Many foods can contain *S. aureus*, and consumption of a food containing 100 or 500 cells/g (or /ml) will, in all probability, not make a person sick unless the food has large amounts of preformed toxin. To reduce the incidence of staphylococcal food poisoning, the aim is to reduce initial load of *S. aureus* in a food by proper selection of the quality of the raw materials and ingredients, sanitation of the food environments, and proper personal hygiene among food handlers. People with respiratory diseases, acute types of facial acne, skin rash, and cuts in hands should not handle the food. Where possible, products should be heat-treated to ensure killing of live cells. Following heating, recontamination of the products should be avoided. The most important aim is to chill the processed products and ready-to-eat foods quickly. Suitable preservatives can also be used to kill or arrest growth.The inside of the food, and not only the surface, should reach the chilled temperature, preferably within 1 h. Finally, the food should not be subjected to temperature abuse and stored for a long period of time at growth temperature before eating. Once heat stable toxins are formed, heating before eating does not ensure safety (Ray, 2004).

Control measures include education of those who prepare food at home and other food handlers, so that they will take proper personal hygiene measures; prohibiting individuals with abscesses or other skin lesions from handling food; refrigeration at 4°C or lower of all foods in order to prevent bacterial multiplication and the formation of toxins. Foods must be kept at room temperature for as little time as possible. The veterinary milk inspection service should supervise dairy installations, the correct operation of refrigeration units and their use immediately after milking, and refrigerated transport of the milk to pasteurization plants (PAHO, 2001).

2.7.2 Treatment

The objective of treatment in human patients is to replace fluids, salt, and minerals that are lost by vomiting or diarrhoea (Sandel and McKillip, 2004). Some strains of Staphylococcus have acquired genes making them resistant to multiple antimicrobial agents. These organisms are uniformly resistant to penicillins and cephalosporins. Penicillinase resistant penicillins such as oxacillin and flucloxacillin are used for serious infections. First or second generation cephalosporins such as cephalothin, cephalexin and cefuroxime are usually safe in patients who are hypersensitive to penicillins. Vancomycin is usually effective for methicillin-resistant staphylococci. Erythromycin and its newer relatives are used in milder infections. The infections can also be treated with combination therapy using sulfa drugs and minocycline or rifampin (Kloos and Bannerman, 1994; Rho and Schaffner, 2007).

3. MATERIALS AND METHODS

3.1. Description of the Study Area

Arsi Negelle town is found in the West Arsi zone of the Oromia regional state at a distance of 225km from Addis Ababa. The town is situated about 2043 m above sea level at latitude of 7^0 21' N and longitude of 38^0 42' E. The average annual temperature of the area varies from 10 to 25^{o}C while rainfall varies between 500 and 1000 mm (Abebe *et al*, 2016). The town is the administrative centers of Arsi-Negelle woreda and has 3 administrative kebeles. The total human population of the town is estimated to be 103,785 of which 52,105 were males and 51,680 were females. The annual population increase is by 2.9%, where the average family size per household is about 6 ranging from 1-12 persons. There are a lot of smallholder dairy farmers in Arsi Negele town. "ALMI Tikus" Milk Company collects more than 1500 liters of milk per day (Wytze *et al*,. 2013). The main economic activities in the study area are cattle fattening, dairy production, katikala production, petty trade and farming of crops. Livestock rising is their primary economic activity in the area. Farmers in Arsi-Negelle produce milk for home consumption and sale. About 75% of the population distills local alcohol as a means of livelihood and an average 200 liters of alcohol produced per day per household and its distillation residues (cakes) is widely used as conventional feed for dairy cattle (Tesfaye, 2009).

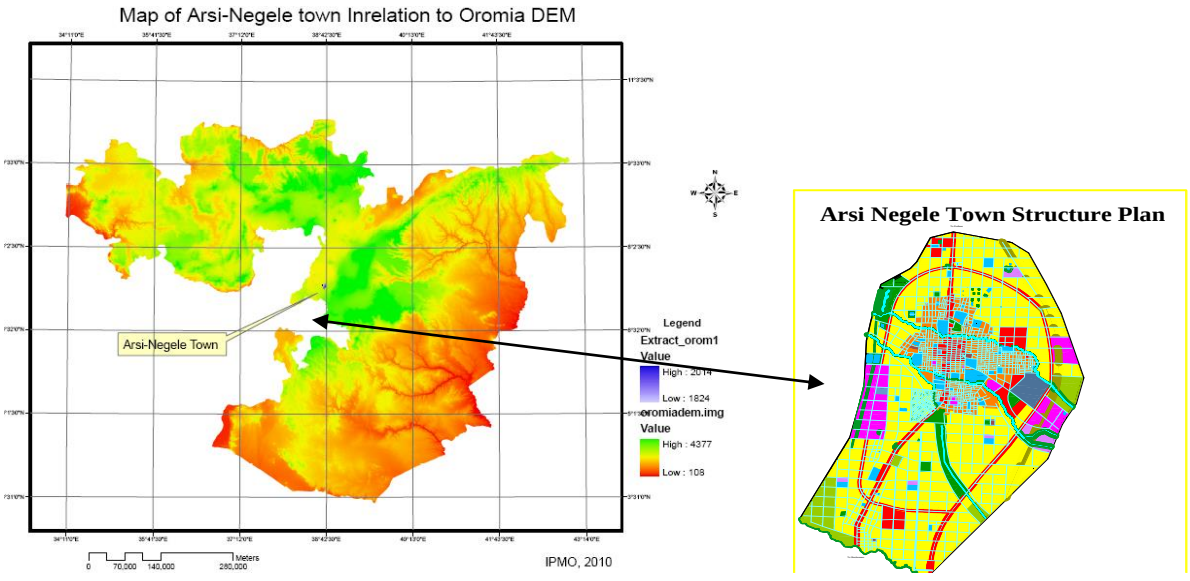


Figure 1. Map of the area which show the relative location of arsi negelle town.

Source: Oromia Finance and Economy Development Bureau (BoFED)

3.2 Study design and sample type

A cross-sectional study design was employed to generate the required data from November 2017-May 2018. Types of sample included were milk from lactating dairy cows, bulk milk from containers in MCCs, swabs of milkers' hands, swab of milk container at MCC.

3.3 Study population

The study population was lactating dairy cows from Arsi Negelle town dairy farms. All lactating cows were kept under intensive management system.

3.4. Sampling Methods and Determination of Sample Size

224 dairy cows were randomly selected from 31 dairy farms. Seven MCCs were also randomly selected and labeled from the total of 25 MCCs identified, from which 38 bulk milk samples were collected randomly from milk containers and 21 Swab samples were taken from milking containers at MCCs and 35 swab samples from milker's hands. So a total of 318 samples were tested for the presence of *staphylococcus aureus*. All the samples were assumed to represent the critical control points along the milk value chain. Besides, milk handling practices and milk consumption behavior among actors in the milk value chain were assessed to determine the possibility of public health significance of *Staphylococcus aureus*. All respondents of questionnaire survey were selected purposively based on their voluntariness; thus, a total of 120 respondents were interviewed.

The sample sizes were determined using the formula given in Thrusfield (2005) for random sampling. To calculate the sample size the following parameters were used: 95% level of confidence (CL), 5% desired level of precision and with the expected prevalence of *S. aureus* 15.1% (Tadesse, 2014) at Wolayta sodo area, which is close and has similar features with the current study area.

$$n = \frac{1.96^2 \times Pexp\ (1-P)}{d^2}$$

Where, n= the total sample size

P= expected prevalence

d= desired accuracy level at 95% interval

Accordingly, 197 where calculated but to increase accuracy 224 lactating dairy cows were sampled.

3.5. Collection, transportation and handling of samples

A 25 ml volume of raw Milk sample was collected according to the procedure recommended by Quinn *et al.,* (1999). Strict aseptic procedures were followed when collecting milk samples in order to prevent contamination with microorganisms present on the skin udder and teats, on the hands of samplers and on the barn environment. Teat ends were cleaned and disinfected with ethanol (70%) before sampling Quinn *et al.,* (1999). Sterile bottle with tight fitting cups were used. The bottles were labeled with permanent marker before sampling. To reduce contamination of teat ends during sample collection, the near teats were sampled first and then followed by the far ones, after milking out and discarding the first two drops. Swabs from hands of the milking personnel and milking containers were collected using sterile, cotton-tipped swabs. After agitating the bulk tank milk, sample was taken from the top of bulk milk using a sanitized dipper from MCCs. Using Ice box, the collected samples was transported to Hawassa University veterinary microbiology laboratory within 24 hrs and the samples was kept at 4ºC overnight.

3.6. Isolation and identification of *Staphylococcus aureus*

3.6.1. Cultural and gram's stains test

The techniques recommended by the International Organization for Standardization, ISO 6888-3: 2003 was employed for the isolation and identification of *Staphylococcus* species from raw bovine milk samples. Samples which were kept for overnight in a refrigerator at 4^0C were thawed for 3-5 hours at room temperature. The bacteriological media used for the study was prepared following the instructions of the manufacturers. In order to get discrete separate colonies, the surface of the agar media used in the study was made dry by keeping the medium in the incubator for overnight. 25 ml of each raw milk sample was stirred into 225 ml of sterile buffered peptone water (BPW) in a sterile stomacher bag (Quinn *et al.,* 2002).

The pre-enriched samples were homogenized in a stomacher (Lab-Blender 400) for 2 minutes and incubated aerobically at 37^0C for 24 hours. Following this, 0.1 ml or a loopful of the pre-enriched broth of the various dilutions were streaked (seeded) aseptically onto sterile blood agar plates (BAP)

enriched with 7% heparinized sheep blood and incubated at 37^0C for 24-48 hours under aerobic culture conditions. The plates were examined for the presence of Staphylococcus colonies. Isolates supposed to belong to *Staphylococcus* species on the basis of their morphological aspects (creamy, grayish, white or yellow colonies) and haemolytic pattern on the surface of BAP were collected. Presumed staphylococcal colonies were then sub-cultured on nutrient agar plates (NAP) and incubated at 37^0C for 24-48 hours to get a pure culture (clone of cells derived from a single cell). The pure isolates in the nutrient slant were preserved and maintained at 4^0C for further need (Quinn *et al.*, 2002). The slants were preserved and maintained for characterizing the isolates.

All suspected cultures of Staphylococcus species were subjected to Gram's stain and observed under a light microscope for Gram's reaction, size, and shape and cell arrangements. The Grams stained smears from typical colonies that showed Gram-positive cocci occurring in bunched, grapelike irregular clusters were taken as presumptive Staphylococcus species.

3.6.2. Biochemical tests

Final identification of *Staphylococcus aureus* assignment were done based on biochemical tests such as catalase test, oxidase test, Mannitol sugar fermentation, Coagulase test and 1% maltose fermentation.

Catalase test

Pure culture of the isolates to be tested for catalase were picked up by bacteriological loop from the agar plate and mixed with a drop of 3% hydrogen peroxide on a clean slide. When the organism was positive, bubbles of oxygen was liberated within a few seconds. Those positive cocci were considered as Staphylococci (Quinn *et al*., 2002).

Oxidase test

A piece of filter paper was moistened in a petridish with 1 percent aqueous solution of tetramethyl –p-phenylenediamianedihydrochloride. The test colony was streaked firmly across the filter paper with a glass rod. The disappearance of dark purple color along the streak on the filter

paper was considered as Staphylococcus. Oxidase test usually used as differentiation for Staphylococci (oxidase negative) from Micrococci (oxidase positive) (Quinn *et al*., 2002).

Mannitol salt Agar (Mannitol fermentation)

The colonies that were confirmed by gram's staining reaction, haemolysis on the blood agar, colony characterization ,catalase positive and oxidase negative were selected and streaked on Mannitol salt agar plate and incubated at 37^0C and examined after 24-48 h for growth. The presence of growth and change of PH in the medium (red to yellow) was regarded as presumptive identification of *Staphylococcus aurous* or coagulase positive *Staphylococcus aurous*. Phenol red pH indicator detected the acidic metabolic product of mannitol. Fermentation of mannitol by *S. aureus* causes yellow discolouration of the medium with in 24 hrs of incubation (Quinn *et al*., 2002)

Coagulase test

The coagulase tests used were both slide coaglase and tube coagulase tests. The presumptively identified *Staphylococcus aurous* from mannitol salt Agar were sub-cultured to nutrient agar plate and after 24 hours culture colonies of *Staphylococcus aurous* was picked by bacteriological loop and placed on clean slide with a small drop of distilled water and emulsified. The test suspension was treated with a drop of rabbit plasma and mixed well with a needle for 5-10 seconds. Those forming Clumping of cocci were taken as positive (Quinn *et al.*, 2002).

For those slide coagulase negative isolates, the tube coagulase test was performed in sterile tubes by adding 0.5 ml of selected isolates of Staphylococcus grown on trypton soya broth at 37^0C for 24 hours to 0.5 ml of rabbit plasma (Quinn *et al*., 2002).

The reaction was considered positive, if any degree of clotting from a loose clot to a solid clot that is immovable when the tube is inverted (tilted) was visible within the tube and no degree of clotting would be taken as negative.

Purple agar Base (1% maltose fermentation)

Purple agar base (PAB) with the addition of 1 percent maltose was used to differentiate the pathogenic staphylococci, particularly the coagulase-positive isolates. The suspected culture was inoculated on PAB media plate with 1% of maltose and incubated at 37^0C for 24-48 hours. The identification was based on the fact that *S. aureus* rapidly ferment maltose with in 24 hrs and the acid

metabolic products cause the pH indicator (bromocresol purple) to change the medium and colonies to yellow. The rapid fermentation (24hrs) was considered as *Staphylococcus aurous* isolates (Quinn *et al.*, 2002).

3.7. Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed for *S. aureus* isolates according to the criteria of the Clinical and Laboratory Standards Institute (CLSI, 2012). For susceptibility testing, direct colony suspension of the isolates were adjusted to a turbidity equivalent to a 0.5 McFarland standard Susceptibility to antimicrobial agents were determined for isolated strains by the disk diffusion method on Mueller-Hinton agar following the Clinical and Laboratory Standards Institute guideline. For susceptibility test antimicrobials from each subclass and antimicrobials which were commonly used for treatment of bovine mastitis were selected. Thus a total of eleven antimicrobials were used in this study(CLSI, 2012). The antibiotic discs used were, Amoxicillin-clavulinic acid (AMC/30 µg),Ampicillin(AMP10µg),penicillinG(10IU),Vancomicin(VA/30µg),Gentamicin(CN/10µg),Streptomcin(S/10µg),Ciprofloxacin(CIP/5µg),sulphamethoxazole-trimethoprim(SXT/25µg),Erythromycin(E/15 µg), Nalidixic acid(NA 30 µg),and tetracycline (TE 30 µg)(Himedia, India).

Finally, the diameters of the zone of inhibition around the disks were measured to the nearest millimeter using caliper, and the isolates were classified as susceptible, intermediate and resistant according to the interpretative standards of Clinical and Laboratory Standards Institute (Quinn *et al.*, 2002; CLSI, 2012). Moreover, isolates showing resistance to two or more antimicrobial subclass were considered as multidrug resistant (Intrakamhaeng and Komutarin, 2012).

3.7. Questionnaire survey

A pre-tested structured questionnaire was used to assess the knowledge, attitude and practices of study participants or the target population which are dairy farm owners, consumers, hotel/café owners and milk collectors at MCC towards handling and consumption of milk in the study area. The questions were originally written in English and translated into Amharic Language when administered. The answers were than translated to English and entered into the original form.

Sample size determination for questioner will be based on Arsham (2002).

$N = \frac{0.25}{SE2}$ where, N-sample size SE-standard error

SE is at a maximum (5%) when p = 0.5 and the calculated sample size was a minimum of 100.A total of 120 actors in the milk value chains were interviewed.

4. Data management and analysis

Data was coded and entered to MS Excel spreadsheet and checked for accuracy. It was transferred and processed using computer software such as STATA statistical software version 14 for analysis. Descriptive statistics was used to analyze the data. The significance level was set at $\alpha = 0.05$.

5. Results

5.1. Prevalence of *S. aureus*

Among 318 samples examined, 25.47 % (81) were positive for *S.aureus*. Of this, 17.85 % (95% CI: 13.07-23.51, 40/224) and 63.15 % (95%CI: 45.99-78.18, 24/38) were positive for *S. aureus* at dairy farms and bulk milk at milk collection centers, respectively. The study has showed relatively a higher prevalence rate of *S. aureus* at MCCs than farms. There was statistically a significant difference (P=0.000) in isolation of *S.aureus* from farms and MCCs. *S. aureus* was found in 34.28% (n= 12) of the total 35 swab samples taken from the hands of milkers. In addition, among the 21 milking containers swab samples, 23.8% (n= 5) yielded *S. aureus*.

Table 3: Isolate of *Staphylococcus aureus* derived from dairy farms , bulk milk at MCCs, swabs of milk container at MCCs and milkers hand swab.

No.	Sample type	No. of isolates	Total no. sample	Prevalence	95% CI
1	Milk from lactating cow at Farm	40	224	17.85	13.07-23.57
2	Bulk milk from milk containers at MCC	24	38	63.15	45.99-78.18
3	Swab of milk containers at MCC	5	21	23.8	8.21-47.16
4	Swab of milkers hand	12	35	34.28	19.13-52.21

$X^2 = 36.734$ P=0.000

5.2. Antimicrobial susceptibility test

All the 81 isolates from dairy farm, MCCs, milkers hand swab, milk container swabs of *S. aureus* were tested for antimicrobial susceptibility by 11 selected antibiotics. Antibiotics of veterinary and human health relevance were considered in this study has demonstrated, the existence of alarming levels of resistance of *S. aureus* to commonly used antimicrobial agents.

The isolates were highly resistant to Ampicilline(100%) , penicillin G (100%), Amoxacilline clavulanic acid (82.7%), Streptomycin (72.8%), tetracycline(60.49%) respectively and moderately resistant to Nalidixic acid (52.4%). However, they were also susceptible to ciprofloxacin (76.54%), followed by sulphamethoxazole-trimethoprim (62.96%) and vancomycine (60.49%).

Table 4. Antimicrobial susceptibility profile of *S. aureus* isolates according to the types of sample

Antimicrobials	**unit**	Type of samples susceptible to different antimicrobial agent											
		Farm milk (n=40) No. %			MCC (n=24) No. %			Milkers hand swab (n=12) No. %			Milk container swab(n=5) no.%		
		R	I	S	R	I	S	R	I	S	R	I	S
Amoxacillin clavulanic	30 µg	35(85.5)	0(0.0)	5(14.5)	21(87.5)	0(0.0)	3(12.5)	8(66.6)	0(0.0)	4(33.3)	3(60)	0(0.0)	2(40)
Ampicillin	10µg	40(100)	0(0.0)	0(0.0)	24(100)	0(0.0)	0(0.0)	12(100)	0(0.0)	0(0.0)	5(100)	0(0.0)	0(0.0)
Ciprofloxacine	5µg	6(15)	9(22.5)	25(62.5)	3(12.5)	0(0.0)	21(87.5)	1(8.3)	0(0.0)	11(91.6)	0(0.0)	0(0.0)	5(100)
Erythromycin	15µg	4(10)	24(60)	12(30)	2(8.3)	13(54.16)	9(37.5)	3(25)	6(50)	3(25)	2(40)	2(40)	1(20)
Gentamycin	10µg	8(20)	13(32.5)	19(47.5)	3(12.5)	8(33.3)	13(54.16)	0(0.0)	6(50)	6(50)	0(0.0)	3(60)	2(40)
Nalidixic acid	30µg	18(45)	15(35.5)	7(19.5)	13(54.16)	9(37.5)	2(8.33)	7(58.3)	4(33.3)	1(8.3)	4(80)	1(20)	0(0.0)
penicillin G	10 IU	40(100)	0(0.0)	0(0.0)	24(100)	0(0.0)	0(0.0)	12(100)	0(0.0)	0(0.0)	5(100)	0(100)	0(0.0)
streptomycin (S)	10µg	28(55)	4(22.5)	8(22.5)	18(75)	6(25)	0(0.0)	9(75)	3(25)	0(0.0)	4(80)	1(200	0(0.0)
Sulphamethoxazole-trimetoprim	25µg	0(0.0)	17(42.5)	23(57.5)	3(12.5)	6(25)	15(62.5)	0(0.0)	4(33.3)	8(66.3)	0(0.0)	0(0.0)	5(100)
tetracycline	30µg	20(45)	6(15)	14(40)	21(87.5)	0(0.0)	3(12.5)	7(58.3)	4(33.3)	1(8.3)	1(20)	2(40)	2(40)
vancomycine	30 µg	18(45)	00(0.0)	22(55)	4(16.6)	3(12.5)	17(70.83)	2(16.6)	3(25)	7(58.3)	2(40)	0(0.0)	3(60)

5.3. Result of questioner survey

In addition to the laboratory survey, issues of public health implication arising from *staphylococcus aureus* and possible sources of milk contamination with *staphylococcus aureus* were assessed using structured questionnaire survey on 27 farm owners, 23 milk collectors, 50 consumers and 20 hotel and café owners, specifically design for each type of respondent accordingly and a total of 120 respondents were participated.

In the current study risk factors for public health such as milk consumption, form of milk consumption, illness of consuming milk, clinical signs and symptom of milk born disease, awareness on milk born disease and frequency of milk consumption were assessed. Among the total of 27 interviewed dairy farmers, 21.7 % of them consume raw milk. The consumption of raw milk is relatively higher among uneducated dairy farmers (60%, 3/5) than those who at least read and write (20%, 1/5). Only 37.03% of the dairy farmers were aware of the occurrence of foodborne diseases due to raw milk consumption, but none of them have aware of staphylococcal food poisoning associated with consumption of raw milk and milk products. Of the 27 farmers, 33.3%, 48.14% and 18.51% of them practiced cleaning of barn twice per day, one times per day and three times per day , respectively, however, 59.25% (n = 16) didn't wash udder and teat and none of them did disinfect their hands before milking using antiseptic solutions. On the other hand, all of them practiced washing of dairy equipment with detergents before milking. 77.7 % (n=21) of the farmers used plastic containers for milking and none of them use refrigerator.

Table 5: Results of questionnaire survey at farm level on public health significance of *staphylococcus aureus* at Arsi negelle town.

Public health implication issues raised for farm owners/workers	**Value**	**Frequency**	**Percentage**
Milk consumption	Yes	23	85.18%
	No	4	14.82%
Form of milk consumption	Raw	5	21.7%
	yoghurt	8	34.78%
	Cottage cheese	3	13.04%
	boiled	7	30.34%
Acquiring illness	Yes	8	34.78%
	No	15	65.21%

Table 5. Cont……

Public health implication issues raised for farm owners/workers	Value	Frequency	Percentage
Knowledge on Signs of illness	diarrhea	3	37.5%
	vomiting	2	25%
	Abdominal cramp	2	25%
	Both diarrhea and vomiting	1	12.5%
Awareness on milk borne illness	Yes	10	37.03%
	No	17	62.9%
Awareness about Staphylococcal food poisoning	Yes	0	0.0 %
	No	27	100%
Feeding habit	Rarely	6	26.08%
	Frequently	7	30.4%
	As a common diet	5	21.73%
	As required	5	21.73%
Use of refrigeration	Yes	0	0.0%
	No	27	100%
Time gap between taking and using milk	Yes	16	59.25%
	No	11	40.74%
Average time gap between taking and using milk	1hr	13	48.14%
	1-4hr	6	22.2%
	4-10hr	3	11.1%
	>10hr	3	11.1%
Level of education those who consume raw milk	Read and write	1	20%
	High school	1	20%
	college	0	0.0%
	No education	3	60%

Table 5. Cont…..

Public health implication issues raised for farm owners/workers	Value	Frequency	Percentage
Floor type living area	concrete ground	15	55.5%
	natural floor	3	11.1%
	stone and sand	4	14.8%
	Made from wood	5	18.5%
Floor type milking area	concrete ground	15	55.5%
	natural floor	3	11.1%
	stone and sand	4	14.8%
	Made from wood	5	18.5%
Time length of cleaning dairy house	Three times a day	5	18.51%
	two times a day	9	33.3%
	one times a day	13	48.14%
Time length of cleaning milking cows udder	No cleaning	16	59.25%
	Only before milking	11	40.7%
	Only after milking,	0	0.0%
	Before and after milking	0	0.0%
Equipment used for milking	Aluminum cans	6	22.2%
	Plastic can	21	77.7%
	clay pot	0	0.0%
	other traditional	0	0.0%
Hand washing	Yes	14	51.85%
	No	13	48.14%
Use of antiseptic solution	Yes	0	0.0%
	No	27	100%
Use of detergent to clean bucket	yes	27	100%
	No	0	0.0%

Among the 50 consumers, 46% (n= 23) of the interviewed consumers drink boiled milk while 26% (n=13) consume raw milk and 20 %(n=10) consume raw milk products like yogurt. 48% of them (n= 24) had no aware of milk borne disease associated with drinking raw milk and none of the respondents had knowledge about staphylococcal food poisoning. Of the consumers, 46% (n= 23), 40% (n= 20) and 14% (n= 7) of them purchased milk from farms, cafe and from MCCs, respectively. Fifty eight percent of them (n= 29) used plastic containers while the rest (n= 21) used metallic

containers to transport milk to their homes. Twenty percent (n=10) of them kept milk in a refrigerator while 80% (n= 40) of them kept milk at room temperature.

Table 6 : Results of questionnaire survey of (n=50) milk consumers on public health significance of *staphylococcus aureus* at Arsi negelle town

Public health implication issues raised for milk consumers	**Value**	**Frequency**	**Percentage**
Form of milk consumption	Boiled milk	23	46%
	Yoghurt	10	20%
	Cheese	5	10%
	Raw milk	13	26%
Awareness about milk born disease	Yes	26	52%
	No	24	48%
Awareness about Staphylococcus food born disease	Yes	0	0.0%
	No	50	100%
Acquiring illness after consuming milk and milk product	Yes	18	36%
	No	32	64%
Knowledge on Signs of illness	Diarrhea	4	22.2%
	Vomiting	2	11.1%
	Stomach cramp	4	22.2%
	Diarrhea and vomiting	5	27.7%
	Stomach crap and vomiting	3	16.6%
Where do you purchase milk	Farm	23	46%
	MCC/retailers	7	14%
	Hotel /café	20	40%
Type of container usually	Plastic	29	58%
	Metallic	21	42%
Duration of milk stay at home prior consumption	<1hr	21	42%
	1-2hr	13	26%
	>2hr	16	32%
temperature of milk storage	< 4 ^{o}c /refrigerator	10	20%
	Room temperature	40	80%

At the MCCs, milk from dairy farmers was checked either with lactometer reading or an alcohol test for its freshness. Only those milk samples passed the tests were collected from the farmers. Among 23 milk collection centers, 56.52% (n= 13) used plastic cans to collect milk.

Table 7: Results of questionnaire survey of milk collectors at MCC on public health significance of *staphylococcus aureus* at Arsi negelle town

Public health implication issues raised for milk collectors at MCC	**Value**	**Frequency**	**Percentage**
quality assessment usually conducted at the center	Lactometer reading	14	60.86%
	Alcohol test	9	39.13%
	Organoleptic test (smell, visualize and test)	0	0.0%
Length of the time milk stay at Milk collection centre	<1hr	23	100%
	1-2hr	0	0.0%
	>2hr	0	0.0%
temperature of milk kept at the center	<4^{o}c	0	0.0%
	Room temperature	23	100%
Awareness about milk born disease	Yes	12	52.17%
	No	11	47.82%
Awareness about Staphylococcus food born disease	Yes	0	0.0%
	No	23	100%
Type of container usually used to collect milk	Plastic	13	56.52%
	Metallic	10	43.47%

60 % (n=12) of the interviewed hotels/ cafes purchased raw milk from farms. Among them, 45% (n= 9) used metallic container while 55% (n= 11) used plastic containers for milk transportation. On the other hand, the respondents (hotels/cafes owners) indicated that they used different methods of

quality assessments like boiling (35%) and visualizing and smelling (65%) before purchasing milk. Milk was found to be kept in a refrigerator by all hotels/cafes until consumption. Thirty percent of them had no aware of the occurrence of milk borne diseases associated with drinking raw milk and none of the respondents had aware of staphylococcal food poisoning.

Table 8: Results of questionnaire survey of hotel and café owners on public health significance of *staphylococcus aureus* at Arsi negelle town

Public health implication issues raised for hotel/café owners	**Value**	**Frequency**	**Percentage**
Where do you purchase raw milk	Farm	12	60%
	Milk collection centers	8	40%
	Others source	0	0.0%
Type of container usually used to collect milk	Plastic	11	55%
	Metallic	9	45%
Type quality assessment usually used before purchasing the milk	Alcohol test	0	0.0%
	Lactometer reading	0	0.0%
	Organoleptic test (smell, test, visualization)	13	65%
	Boiling	7	35%
milk product do you serve for customer	Boiled milk	11	55%
	Yoghurt	7	35%
	Cheese	2	10%
	Raw milk	0	0.0%
awareness about food born disease associated with milk	Yes	14	70%
	No	6	30%
Awareness about staphylococcal food born disease	Yes	0	0.0%
	No	20	100%
Time gap between purchasing and serving the milk	yes	14	70%
	No	6	30%
how much is the average time gap	1-4hrs	3	30%
	4-10hrs	2	20%
	10-16hrs	1	10%
	more than 16hr	0	0.0%
Temperature of kept milk	<4 ^{o}c(refrigerator)	20	100%
	Room temperature	0	0.0%

6. Discussion

The consumption of raw milk and its derivatives is common in Ethiopia, which is not safe from consumer's health point of view as it may lead to transmission of various diseases. It may be contaminated at the site of production and during processing, the cow itself, from air/ dust, unclean milk containers and the milk handlers. Pathogens and other organisms can gain access to milk as a result of the milk handlers' activities such as coughing, sneezing, scratching and from body surfaces in contact with milk, particularly the fingers (Getahun and Gebre-Selassie, 2003).

In this study, from 224 lactating cow's milk samples subjected to bacteriological examination, 17.85% (40/224) were found to be positive for *S. aureus*. This finding is nearly in agreement with the findings observed in Wolaita sodo area, 15.1% (Tadesse, 2014), Addis Ababa, 17.2% (Gizaw, 2014); 15.5% (Mekuria *et al*, .2013) and 19.6% (Ayele *et al*,.2017) in Sebeta. However, the result of the present study showed a slight lower contamination rate compared to other works, 28.1% (Abera *et al*, .2011) in Shashemene, 42.1% (Abera *et al*, .2010) in Adama and 26.6% (Tasew *et al*,.2015) in kombolcha. The result is also slightly higher than (Mokennen *et al*,. 2011) with 8% prevalence of *S.aureus* in raw milk in Debreziet. Wide variation in the prevalence of *S. aureus* has also been reported (Rodistitis, 2000). This variation is largely attributed to the changing management conditions and using of different diagnostic tests.

In the present study, 63.15% (24/34) of the bulk milk samples from the MCCs were found to be contaminated with *S. aureus*. The results showed a higher contamination rate at MCCs than dairy farms. This might be attributed to cross contamination of milk while bulking and poor handling during transportation from farm to collection centers and at milk collection centers ((Getahun and Gebre-Selassie, 2003; Addis *et al*, .2010). The contamination of *S. aureus* at MCC centers was slightly lower than the previous work (Desissa *et al*, 2012; Wubete, 2004) where *S. aureus* was isolated at recovery rate of 75% and 72%, respectively from bulk tank milk. But, this finding was relatively higher when compared with the report by Addis *et al*. (2010) from which they recovered 46% of *S. aureus* from bulk milk at MCCs. One can easily observed a significant increase of milk contamination with *S. aureus* from dairy farms 17.85 % than milk collection centers 63.15%; this might be due to mixing of milk from mastitic cows and/or poor handling.

The isolation of *S. aureus* from hands of milker's and milk containers were 34.28% and 23.8%, respectively. These clearly indicated that milk handlers and milk containers could be the potential sources of contamination of milk with *S. aureus*. The isolation rate from milker's hand was relatively in agreement with the prevalence rate reported by Deandrade (1989) and Tondo *et al*,. (2000) whose results were 35.7% and 35.2% respectively. Much higher isolation from milkers hand was reported by Gidwa and El Gohary, (2013), 60% in Egypt. This may be attributed to the fact that staphylococci are ubiquitous organisms and at least 50% of individuals carry the organism in their nasal passages, throat and can contaminate through coughing or sneezing. In this study there is significance difference (P=0.000) among the number of isolation rate of *S.aureus* in dairy farm and MCC were observed.

The antimicrobial susceptibility tests carried out in this study indicated the occurrence of resistance of *S. aureus* to some of the commonly used antimicrobials. This study presents the sensitivity of the *S. aureus* isolates towards ciprofloxacin (76.54%), Sulphamethoxazol trimethoprim (62.96%) and Vancomycine (60.49%). However, the isolates were found to be highly resistant to Ampicillin (100%), penicillin G (100%) and amoxicillin-clavulanic acid (82.7), streptomycine(72.8%) and tetracycline(60.49%). The high resistance pattern of the isolates to penicillin G was relatively high similar to the findings reported by; 100% (Thaker *et al*,. 2013) in India and 100% (Megerssa , 2015), 96.7% (Mekuria *et al*,.2013), in Ethiopia. This is probably due to resistance to β-lactams and frequent use of the antibiotics. Absolute resistance to Penicillin G must be of a great concern; since this antibiotic represents, the main antibiotic group recommended for Staphylococcal mastitis treatment and regular use of antibiotics for the treatment of cows may result in the spread of resistant strains. Antibiotic resistance is carried on plasmids and transposons which can pass from one Staphylococcal species to another (Hulya *et al.,* 2006). The prevalence of antibiotic resistance usually varies between isolates from the different sampled stations and even between isolates from different herds on the same farm (Waage *et al.,* 2002). Moreover, the present study showed high resistance pattern of *S. aureus* to streptomycin (72.8%), tetracycline (60.49%). The findings are nearly similar with the report of Tadesse, (2014) with a resistant pattern of streptomycin (66.7%) and tetracycline (69.2%) in and around wolaita sodo, south of Ethiopia. This is due to the fact that these drugs specifically tetracycline and streptomycin are commonly used in the treatment of infections in the study area. Lacks of stringent regulation and monitoring in the dispensing and use of antimicrobials in the country have also might contribute to the occurrence of

high antimicrobial resistance to these drugs. Antibiotic therapy is an important tool in the treatment of *S. aureus* related infections. However, the misuse or intensive use of antibiotics can lead to the development of resistance among different bacterial strains (White *et al*., 2001).

The hygienic condition or quality of milk has serous implication on public health safety. The questionnaire result mainly gave broad understanding of the milking and hygienic practice in the study area. Maintaining the hygienic conditions of dairy house, milking area, milking equipment and milker's hand is important for production of good quality milk (Magnusson *et al*.,2007;Yilma,2007). Cleaning the udder of cow before milking is important since it could have direct contact with the ground, urine dung, and feed refusals while resting. Not washing udder before milking can import possible contaminants into the milk. The current study revealed that, 59.25% of the farmers didn't wash udder and teat of the cows before milking and all of them didn't wash their hands using antiseptics but all washed equipment with detergent before milking. This is slightly lower than he report of (Bejano, 2014) 75.8% of farmers did not clean udder before milking. These practices combined with the observation of the occurrence of *S. aureus* on hands of milkers' indicated that farmers lack knowledge on the importance of good milking practices in minimizing microbial contamination of milk raising the public health issues. Yet pre-milking udder preparation and employing good milk handling practices play an important role in minimizing contamination at the farm with *S. aureus* (Michelle and Jeffrey, 2011).

In the present study, the use of plastic containers for milking, storing, collecting and transporting at farm(77.7%), milk collection centers(56.56%), hotels and café(55%) was observed. Plastic containers have inherent characteristics that make them unsuitable for milk handling. Plastic containers scratch easily and provide hiding places for bacteria during cleaning and sanitization and poor conductor heat and hence will hinder effective sanitization by heat leading to bacterial contamination of the milk (Omoe *et al*, .2005; GK, 2011).

In this study among the farmers, 21.7% had a habit of drinking raw milk and 62.9% of them didn't have awareness about food borne diseases associated with consumption of raw milk. This result is close to a study done by Bejano, (2014) around Assossa district, which is 29.1% of raw milk consumption and 65.8% of them have no awareness about milk borne disease among farmers. In United States 35–60% of farm families and farm employees consume raw milk probably because, it is a traditional practice and it is less expensive to take milk from the bulk tank than buying pasteurized

retail milk (Stephen *et al*, 2009). Though the results showed relatively a lower percentage of raw milk consumption, still these individuals are at a greater risk of contracting food born intoxication than those who do not consume raw milk. Cross-contamination with pathogenic micro organism can gain access to milk either in contamination or direct excretion from the udder into milk (El-Ziney and Al-Turki, 2007).Thus consumption of raw milk with no treatment may pose public to staphylococcus food born poisoning as a result of contamination. Similarly numerous epidemiological reports have implicated non-heat treated milk and raw milk products as the major factors responsible for illness caused by food borne pathogens (El-Ziney and Al-Turki, 2007). Similar to the farmers, 26% of milk consumer's drink raw milk and raw milk products like yogurt (20%) and 48% of them has no awareness of milk borne diseases. This is in line with (Ayele *et al*,.2017) who reported 54% of consumers have no awareness with milk born disease. Consumers are the last group of the food chain and therefore they are at risk of any malpractice occurring in the chain. Also 80% of the consumers kept milk at room temperature. Lack of refrigeration facilities at farm and household level in developing countries of tropical regions, with high ambient temperature implies that raw milk will easily be spoiled during storage and transportation (Gilmour, 1999; Godefay and Molla, 2000).

7. Conclusions and recommendations

In conclusion, the present study revealed the occurrence of contamination of milk with *S. aureus* along the milk value chain at farm and milk collection centers. The recovery of the bacteria from hands of milkers' and milk containers were found to be the potential sources of milk contamination with this pathogen. The high prevalence of *S. aureus* at MCCs might indicate cross contamination of milk while bulking and poor handling during transportation from farm to collection centers and at milk collection centers. The study also revealed poor milk handling practices, raw milk consumption behavior, inadequate knowledge of milk borne disease and occurrence of antimicrobials resistant *S. aureus*. In general, the study has revealed the possibility of the public health risk posed by *S. aureus* in Arsi negelle town. Creation of public awareness about good milk handling practices, milk borne diseases and their prevention is important.

In light of the present study, the following recommendations are forwarded;

- Raw milk intended for human consumption must be subjected to pasteurization or heat treatment at least equivalent to pasteurization temperature.
- Milk should be maintained in a cold chain starting from production until consumption.
- Awareness should be created among the public for the implementation of better control and subsequent reduction of SFP.
- Future studies should consider investigation and designing of cost effective preventive and control options that would enable to reduce milk contamination by *S. aureus* and there by the associated public health risks.
- In addition monitoring, rational use of drugs and periodic assessment of the antimicrobial sensitivity of drugs prior to use are recommended.

8. References

Abebe,R., Hatiya,H., Abera,M., Megersa,B. and Asmare,k. (2016): Bovine mastitis: prevalence, risk factors and isolation of *Staphylococcus aureus* in dairy herds at Hawassa milk shed, South Ethiopia. Published online 2016 Dec 3.PMCID: PMC5135792

Abera M, Demie B, Aragaw K, Ragassa F, Ragassa A(2010). Isolation and identification of *Staphylococcus aureus* from bovine mastitic milk and their drug resistance pattern in Adama town, Ethiopia. *J Vet Med Animal Health*. 2010; 2:29–34.

Abera,M. Elias,B. Aragaw,K. Denberga,Y. Amenu, K. and Sheferaw,D.(2011). Major causes of mastitis and associated risk factors in smallholder dairy cows in Shashemene, southern Ethiopia, African Journal of Agricultural Research Vol. 7(24), pp. 3513-3518.

Acco, M., Ferreira, F., Henriques, J. and Tondo, E. (2003): Identification of multiple strains of *Staphylococcus aureus* colonizing nasal mucosa of food handlers. Food Microbiol., 20:489-493.

Addis M, Pal M, Kyule M. (2011). Isolation and identification of Staphylococcus species from raw bovine milk in Debre zeit. Ethiop Vet Rese. 2011;4:45–9

Adesiyun AA, Webb LA, Romain H and Kaminjolo JS. (1997). Prevalence and characteristics of strains of Escherichia coli isolated from milk and feces of cows on dairy farms in Trinidad. J. Food Protection, 60: 1174-1181.

Adesiyun, A. *et al*.(1984): Production of enterotoxin(s) by *Staphylococcus hyicus*. Vet. Microbiol., 9:487–495.

Angulo F., Nargund V.N., Chiller T.C. (2004): Evidence of an association between use of antimicrobial agents in food animals and antimicrobial resistance among bacteria isolated from humans and human health consequences of such resistance. *J. Vet. Med*. 51: 374-379.

Anonymous (2007): The community summary report on trends and sources of zoonoses, zoonotic agents, antimicrobial resistance and foodborne outbreaks in the European Union in 2006. EFSA J., 130, 1-310.

Ash, M. (2008): *Staphylococcus aureus* and Staphylococcal Enterotoxins. In: Food borne microorganisms of public health importance, 5th Edition, AIFST (NSW Branch), Sydney, Australia, Pp: 313-332.

Ashenafi, M. (1990): Microbiological quality of ayib, a traditional Ethiopian cottage cheese. International Journal of Food Microbiology, 10: 263-268.

Ashenafi, M. and Beyene, F. (1994): Microbial load, microflora, and keeping quality of raw and pasteurized milk from a dairy farm. Bulletin of Animal Health and Production for Africa, 42:55-59.

ASHRAM, (2002): Arsenic Health Risk Assessment And Molecular Epidemiology Final Report 1 JAN 2002 – 31 DEC 2004 Version 25 August 2005 QLRT-2001-00264 - Quality of Life and Management of Living Resources

Atanassova, V., Meindl, A. and Ring, C. (2001): Prevalence of *Staphylococcus aureus* and staphylococcal enterotoxins in raw pork and uncooked smoked ham-a comparison of classical culturing detection and RFLP-PCR. *International Journal of Food Microbiology*, **68**:105–113.

Aycicek, H., Cakiroglu, S. and Stevenson, T. (2005): Incidence of *Staphylococcus aureus* in ready-to-eat meal from military cafeteria in Ankara, Turkey. Food control, Pp: 531-534.

Ayele *et al*., (2017). Assessment of *Staphylococcus aureus* along milk value chain and its public health importance in Sebeta, central Oromia, Ethiopia, BMC Microbiology (2017) 17:141 DOI 10.1186/s12866-017-1048-9

Baird, R. and Lee, W. (1995): Media used in the detection and enumeration of Staphylococcus aureus. International Journal of Food Microbiology, 26:15-24.

Balaban, N. and Rasooly, A. (2000): *Staphylococcal* enterotoxins. Int J Food Microbiol, 61(1):1-10.

Bania, J., Dabrowska, A., Bystron, J., Korzekwa, K., Chrzanowska, J. and Molenda, J. (2006): Distribution of newly described enterotoxin-like genes in *Staphylococcus aureus* from food. *International Journal of Food Microbiology*, **108**: 36 – 41.

Barkema, W., Schukken, H.and Zadoks, N. (2006): Invited Review: The role of cow, pathogen, and treatment regimen in the therapeutic success of bovine Staphylococcus *aureus* mastitis. *Journal of Dairy Science.* **89**:1877-1895.

Bautista, L., Gaya, P., Medina, M. and Nunez, M. (1988): A quantitative study of enterotoxin production by sheep milk staphylococci. Applied and Environmental Microbiology, 54: 566-569.

Bejano,S. (2014). A study on prevalence, public health significance and Asssociated risk factors of bacillus cereus on bovine raw milk in and around assosa district, in household dairy farms of the benishangul gumuz regional state, western Ethiopia. Addis Ababa University ,Thesis.

Bendahou, A., Lebbadi, M., Ennanei, L., Essadqui, F. and Abid, M. (2008): Characterization of Staphylococcus species isolated from raw milk and milk products (lben and jben) in North Morocco. Journal of Infectious Diseases for Developing Countries, 2: 218-225.

Bennett, R. and Monday, S. (2003): *Staphylococcus aureus*. In: Marianne D. Miliotis and Jeffrey W. Bier (eds.), International handbook of foodborne pathogens, Pp. 53-69. Marcel Dekker, Inc. New York, NY

Bergdoll, M. and Lee Wong, A. (2006): Staphylococcal intoxications. In: Foodborne Infections and Intoxications; Reimann, P. and Cliver. D. 3rd Ed.; Academic Press, Elsevier: New York, NY, USA, Pp. 523–525.

Bhunia, A. K. (2008): Foodborne Microbial Pathogens: Mechanisms and Pathogenesis. Springer Science, USA, Pp 125-134.

Bitewa A.,(2015). Isolation and identification of methicilin resistant *s. aureus* from Bovine mastitic milk in dairy farms of Bahir dar and its Surrounding North West 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 Microbiology, Pp-33.

Bogaard, V. D. and Stobberingh, E. E. (1996): Is it time to ban all antibiotics as animal growth promoting agents? Lancet; 348: 1454-1456.

Chiang, Y., Liao, W., Fan, C., Pai, W., Chiou, C. and Tsen, H. (2008): PCR detection of staphylococcal enterotoxins (SE) and survey of SE types in *Staphylococcus aureus* isolates from food poisoning cases in Taiwan. *International Journal of Food Microbiology*, **121**: 66–73.

Clinical and Laboratory Standards Institute (CLSI) (2012): Performance Standards for Antimicrobial susceptibility testing; Twenty-second informational supplement: CLSI document M100-S22. Clinical and Laboratory Standards Institute, Wayne, PA, USA, 32(3):70-90.

Daka ,D. G. Silassie, S. Yihdego, D. 2012, Antibiotic-resistance *Staphylococcus aureus* isolated from cow's milk in Hawassa area, South Ethiopia, *African Journal of Microbiology Research Vol.* 6(27), pp.5618-5624.

Deandrade F. Z. (1989). Simultaneous occurrence of enterotoxigenic *Staphylococcus aureus* on the hands, mouth and faeces of asymptomatic carriers. Rev Saude Publica. 1989;23:277:84.

Deresse, D., Solomon, G. and Dawit, Y. (2012): Antibiotic-resistance *Staphylococcus aureus* isolated from cow's milk in the Hawassa area,South Ethiopia, *Mannals of Clinical Microbiology and Antimicrobials* ,**11**:26-30.

Desissa F, Makita K, Teklu A, Grace D. Contamination of informally marketed bovine milk with *Staphylococcus aureus* in urban and peri urban areas of Debre-Zeit, Ethiopia. African J Dairy Farming Milk Prod. 2012;1(1):8–1

El-Ziney, M. G. and Al-Turki, A. I. (2007): Microbilogical quality and safety assessment of camel milk in Saudi-Ararabia (Quassim region). Ecol.Enviro.Res., 5115-5122.

FDA (2012): Foodborne pathogenic microorganisms and natural toxins handbook, 2nd ed. US Food and Drug Administration, Silver Spring, Pp: 87–92.

Getahun, T. and Gebre-Selassie, S. (2003): Assessment of the Bacteriological Quality of Milk at Dairy Farms and Individual Breeders in Jimma Town, South West Ethiopia. *Ethiop J Health Sci*., 13: 21-29.

Gilmour D. (1999). Milking. In Falvey, L., Chantalakhana, C. (Eds.) Smallholder Dairy in the Tropics, ILRI, Nairobi, Kenya. 289-298.

Gizaw, F. (2014): Staphylococcus: Epidemiology and Its Drug Resistence in Cattle, Food Chains and Human in Central Ethiopia. Thesis. Addis Ababa University, Ethiopia.

GK,(2011). Food safety in milk markets of smallholder farmers in Tanzania: a case of peri urban wards in Temeke municipality. A dissertation submitted in partial fulfillment of the Requirements for the degree of master of science in human nutrition of Sokoine Univer.:41–5

Godefay B and Molla B. (2000). Bacteriological quality of raw milk from four dairy farms and milk collection center in and around Addis Ababa. Berl. Münch. Tierärztl. Wschr. 113, 1-3.

Green M. and Bradley A. (2004): *Staphylococcus aureus* mastitis in cattle. Clinical Forum, *UK. VET. J*., 9(4):1-9.

Gwida MM, EL-Gohary FA(2013). Zoonotic bacterial pathogens isolated from raw milk with special reference toEscherichia coliand *Staphylococcus aureus* in Dakahlia. Governorate, Egypt. 2013;2(4).

Gyles, C., Prescott, J., Songer, J. and Thoen, C. (2004): Pathogenesis of Bacterial infections in animals 3rd edition, Pp. 43-50.

Hagstad, H. and Hubbert, W. (1986): Food Quality Control, Foods of Animal Origin. 1st Edition, *Iowa State University Press*, **52**, 87-88.

Hahn G. (1996). Pathogenic bacteria raw milk situation and significance. Proceedings Bacteriological Quality of Raw Milk, IDF, Brussels (Belgium), pp 67–83.

Hein, I., Jorgensen, H., Loncarevic, S. and Wagner M. (2005): Quantification of *Staphylococcus aureus* in unpasteurised bovine and caprine milk by real-time PCR. Research in Microbiology, 156:554–563.

Hennekinne, J., Buyser, M. and Dragacci, S. (2012): *Staphylococcus aureus* and its food poisoning toxins: characterization and outbreak investigation. Federation of European Microbiological Societies (FEMS) Microbiol., 36: 815–836.

Hobbs, B. and Gilbert, R. (1981): Food Poisoning and Food Hygiene. 4th Edition, Edward Arnold, London, Great Britain, Pp 27.

Hulya, T., Senay, E. and Dilek O. (2006): Antibiotic resistance of *staphylococcus aureus* and coagulase-negative staphylococci isolated from bovine mastitis. Bull. Vet. Inst .Pulawy, 50:41-45

ICMSF (1996): Microbiological specifications of food pathogens. Blackie Academic and Professional, London, Pp: 299–333.

Iman, I., Amal, R., Mohamed, E. (2012): Isolation of *Staphylococcus aureus* from Milk and Human with Reference to its Survival on Surfaces. *World Journal of Dairy and Food Sciences,* 7 **(2**): 142-145.30–35.

Intrakamhaeng M. And Komutarin T. (2012): Antibiotics resistance and RAPD-PCR typing of multidrug resistant MRSA isolated from bovine mastitis cases in Thailand. Scie. Asia, 38:

ISO (6888-3: 2003): Microbiology of food and animal feeding stuffs -- Horizontal method for the detection and identification of staphylococci. Part 3: Geneva, Switzerland.

Jay, J. (2000): Modern Food Microbiology. 6th Edition, Aspen Food Science Text Series, Aspen Publishers (Inc), Gaithersburg, Maryland, Pp: 441-456.

Kadariya, J., Smith, T.C. and Thapaliya, D. (2014): Review Article Staphylococcus aureus and Staphylococcal Food-Borne Disease: An Ongoing Challenge in Public Health. BioMed R. Intern., 10: 1-9.

Kerouanton, A. (2007): Characterization of S. aureus strains associated with food poisoning outbreaks in France. Int. J. Food Microbiol., 115: 369-375.

Kloos, W. and Bannerman, T. (1994): Update onclinical significance of coagulase-negative staphylococci. Clinical Microbiology Revolution,7: 117-140.

Lamprell, H., Villard, L., Chamba, J., Beuvier, E., Borges, E., Maurin, F., Mazerolles, G., Noel, Y. and Kodjo, A. (2004): Identification and biotyping of coagulase-positive staphylococci in ripened French raw milk cheeses and their in vitro ability to produce enterotoxins. *Review on Veterinary. Medicine,* **155**: 92-96.

Levinson, W. (2008): Medical Microbiology and Immunology. 10th ed., McGraw-Hill Companies, Inc. San Francisco, California.

Loir, Y., F. Baron and M. Gautier. (2003): *Staphylococcus aureus* and food poisoning .*Genetic Molecular Research*, **2**:63-76.

Loo , I., Huijsdens, X., Tiermersma, E., Neeling, A., van de sande- Bruinsma, N., Beaujean, D., Voss, A. and Klutmans, J.(2007): Emergence of methicilin –resistant staphylococcus aureus of animal origin in humans. Emerging Infectious Disease, 13:1834-1839.

Ludmilla, S., Soares, B., Valdir, C. and Marina, G. (2007): Study of *Staphylococcus aureus* in raw and pasteurized milk consumed in the Reconcavo area of the State of Bahia, *BrazilLílian Porto de Oliveira*[1], **3(2)**: 6.

Magnusson, M., Christiansson, A. and Svensson, B. (2007): Bacillus cereus spores during housing of dairy cows: factors affecting contamination of raw milk. Journal. of Dairy Sci., 90: 2745-2754.

Mansel W. Griffiths, (2010).Improving the safety and quality of milk, Volume1:Milk production and processing Woodhead Publishing Limited, Abington Hall, Granta Park, Great Abington, Cambridge, CB216AH,UK)), Pp28..

Martin R. Adams and Maurice O. Moss,. (2008) .Food Microbiology, 3rd Edition, RSC publishing /www.rsc.org, University of Surrey, Guildford, UK Pp 254-255.

Martın, M., Fueyo, J., Gonzalez, M. and Mendoza, M. (2004): Genetic procedures for identification of enterotoxigenic strains of *Staphylococcus aureus* from three food poisoning outbreaks. *International Journal of Food Microbiology*, **94**: 279– 286.

Megersa, L.(2015). Identification and antimicrobial susceptibility profiles of Staphylococcus species isolated from raw milk, swabs of Udders, milking utensils and milkers hands in small holder and Dairy farms in ambo and guder town, Addis Ababa University ,Thesis Pp 27.

Mekuria, A., Asrat, D., Woldeamanuel, Y. and Tefera, G. (2013): Identification and antimicrobial susceptibility of S. aureus isolated from milk samples of dairy cows and

nasal swabs of farm workers in selected dairy farms around Addis Ababa, Ethiopia. *Afr. J. Microbiol. Res*., 7: 3501-3510.

Mennane Z, Ouhssine M, Khedid K and Elyachioui M. (2007). Hygienic Quality of Raw Cow's Milk Feeding from Domestic. Int. J. Agri. Biol. 9(1):1560–8530.

Michelle, A. and Jeffrey, B. (2011): *Staphylococcus aureus* Mastitis, UK *Veterinary Diagnostic Laboratory and Animal and Food Sciences, cooperative extension service*, University of Kentucky College of Agriculture, Lexington, KY, **40:** 190.

Mokennen, M., Pal, M. and Kyule, M.N. (2011): Isolation and Identification of *Staphylococcus* species from Raw Bovine Milk in Debre Zeit, Ethiopia. Vet. Res.,4: 45-49.

Montville, T. and Matthews, K. (2008): Food microbiology: An introduction. 2nd ed, Press, Washington D.C.,Pp: 43-50.

Morrison, R. (2008): The coagulase test in the identification of pathogenic *staphylococci.* Journal of Applied Bacteriology, 25: 432-435.

Murray, R. (2005): Recognition and management of *S. aureus* toxin-mediated disease. *Intern. Med. J*., 2:S106-S119.

Omoe, K., Liang, D., Omoe, T., Nakane, A. and Shinagawa, K. (2005): Comprehensive analysis of classical and newly described staphylococcal super antigenic toxin genes in *Staphylococcus aureus* isolates. *Microbiology Letters*, **246**: 191–198.

Otter, J. A. And French, G. L. (2008): the emergence of community associated methicillin resistant *Staphylococcus aureus* at a London teaching hospital, 2000-2006 Clinical microbiology and infection , 14: 670-646.

PAHO (2001): Zoonoses and communicable diseases common to man and animals 3rd ed. Washington, D.C. © 2001. 3 vol.—(Scientific and Technical Publication No. 580), Pp: 251-260.

Pal, M. (2007): Zoonoses.2nd Edition, Satyam Puplishers, Jaipur, India, Pp 138-139.

Quinn, P. J., Carter, M.E., Markey , B. and Carter, G.R. (2002): Veterinary Microbiology Microbial Diseases- Bacterial causes of Bovine Mastitis, 8th Edition. Mosby international Limited, London, pp 465-475.

Quinn,.J., Markey, K., Donnelly, W. and Leonard, F. (1999). Veterinary microbiology and microbial disease, Pp: 43-45.

Ray,B.(2004) Fundamental food microbiology, 3rd edition CRC press LLc Pp 347. www.crcpress.com

Rho, M. and Schaffner, D. (2007): Microbial risk assessment of staphylococcal food poisoning. Int. J. Food Microbiol., 116: 332-338.

Roberts, D. and Greenwood, M. (2003): Practical Food Microbiology. 3rd Edition, Blackwel Publishing, USA, pp 173.

Rodostitis OM, Gay DC, Blood, Hinschiff KW (2000) Veterinary medicine: A Textbook of the diseases of cattle, sheep, pigs, goats and horses. W.B, Saunders. Company Ltd., Londen. United Kingdom

Rowland, S., Walsh, S., Teel, L. and Carnaham, A. (1994): Pathogenic and Clinical Microbiology. A Laboratory Manual.Little Brown, USA, Pp 11-20.

Salandra, G., Goffredo, E., Pedarra, C., Nardella, M., Parisi, A., Dambrosio, A., Quaglia, N., Celano, G. and Normanno, G. (2008): Occurrence, characterization and antimicrobial resistance pattern of *Staphylococcus* species isolated from dairy products in southern Italy. *International Journal of Food Microbiology*, **9**: 327-360.

Salyers, A. and Whitt D. (2002): Bacterial Pathogenesis. 2nd Edition, American Society for Microbiology (ASM) press, Washington DC, USA, Pp. 216-229.

Sandel, M. and McKillip, J. (2004): Virulence and recovery of Staphylococcus relevant to the food industry using improvements on traditional approaches. Food Control, 15: 5-10.

Schlundt J, Toyofuku H,Jansen J And Herbsts A(2004), `Emerging food-borne Zoonoses',revscitech, 23,513±533.

Schmid, D., Gschiel, E., Mann, M. et al.(2007): Outbreak of acute gastroenteritis in an Austrian boarding school, September 2006. Euro Surveill. 12(3): 51-3.Sci., 90: 2745-2754.

Shah, M. (2003): Molecular pathogenesis of *Staphylococcus aureus* and other staphylococci. *Journal of Applied Bacteriology*, **59**: 207-221.

Silva W., Destro M., Landgraf M. and Franco B. (2000): Biochemical characteristics of typical and atypical *Staphylococcus aureus* in mastitic milk and environmental samples of Brazilian dairy farms. Brazilian J. Microbiolo., 31: 103-106.

Smith, K. (2007): Food borne pathogenic microorganisms and natural toxins. Food and Drug Administration, Center for Food Safety and Applied Nutrition, 10: 119-150.

Soares de souza, M. M., Shana de Mattos, D. C., Ingrid, A., Lidiane D. S., Bruno R. P. and I rene, D. C. (2012): Antibiotic Resistance in Staphylococcus Species of Animal Origin, Antibiotic Resistance Bacteria-A continuous challenge in the New Mollennium, Dr Marina Pana (ED), In Tech, Pp. 273-302.

Soejima, T., Nagao, E., Yano, Y., Yamagata, H., Kagi, H., and Shinagawa K. (2007): Risk evaluation for staphylococcal food poisoning in processed milk produced with skim milk powder. *International Journal of Food Microbiology*, **115**: 29-34.

Stephen P. Oliver,1 Kathryn J. Boor,2 Steven C. Murphy,2 and Shelton E. Murinda. (2009). Food Safety Hazards Associated with Consumption of Raw Milk, Foodborne Pathogens and Disease Volume 6, Number 7, ª Mary Ann Liebert, Inc.

Stewart, C. (2003): *Staphylococcus aureus* and staphylococcal enterotoxins. Ch 12 In: Hocking AD (ed) Food borne microorganisms of public health significance. 6th ed, Australian Institute of Food Science and Technology (NSW Branch), Sydney, Pp: 359–380.

SU (Standard Unit), (2014): Identification of Staphylococcus species, Micrococcus species and Rothia species. UK St. for Microbiol. Investig., 3:1-32.

Tadesse B., (2014). Isolation and identification of methicilin resistant *S. aureus* From bovine mastitic milk in and around wolaita sodo, Southern Ethiopia. Addis Ababa University, Thesis, Pp-5.

Tassew, A. Negash,M. Demeke,A. Feleke,A. Tesfaye,B and Sisay,T(2015). Isolation, identification and drug resistance patterns of methicillin resistant *Staphylococcus aureus* from mastitic cow's milk from selected dairy farms in and around Kombolcha, Ethiopia, *JVMAH* Vol. 8(1), pp. 1-10,

Tesfay, T., Kebede, A. and Seifu, E. (2013): Quality and Safety of Cow Milk Produced and Marketed in Dire Dawa Town, Eastern Ethiopia. *Int. J. Int sci. Inn. Tech*., 2: 01-05.

Tesfaye, M.(2009): Cattle Milk And Meat Production Systems In Arsi-Negelle Town, Oromia, Ethiopia. a thesis submitted to the department of animal and range sciences hawassa college of agriculture, school of graduate studies, hawassa university, Ethiopia.

Thaker ,H. C., M. N. Brahmbhatt, J. B. Nayak,(2013). Isolation and identification of Staphylococcus aureus from milk and milk products and their drug resistance patterns in Anand, Gujarat Department of Veterinary Public Health, Veterinary College, Anand Agricultural University, Anand 388001, Gujarat, India doi:10.5455/vetworld.2013.10-13

Threlfall, E.J., Ward, L.R., Frost, J.A., Willshaw G.A. (2000). Review: The emergence and spread of antibiotic resistance in food-borne bacteria. *International Journal of Food Microbiology*, 62: 1-5.

Thrustfield, M. (2005): Veterinary Epidemiology. 3rd Edition, Black Well Science Ltd. Cambridge, USA. Pp 225-228.

Tondo EC, Guimaraes MC, Henriques JA, Ayub MA.(2000). Assessing and analyzing contamination of a dairy products processing plant by *Staphylococcus aureus* using antibiotic resistance and PFGE. Can J Microbiol. 2000;46:1108–14.

Waage S, Bjorland J, Caugant DA (2002). Spread of *Staphylococcus aureus* resistant to penicillin and tetracycline within and between dairy herds. Epidemiol. Infect., 129:193–202.

Walderhaug, M. (2007): Food borne pathogenic microorganisms. Food and Drug Administration, *Center for Food Safety and Applied Nutrition*, **28**: 48-65.

Wallenberg GJ, Vanderpoel HM, Vanior JT (2002). Viral infection and bovine Mastitis. J. Vet. Micro. 88: 27-45.

Waters, A. E., Contente-Cuomo, T., Buchhagen, J., Liu, C. M., Watson, L., Pearce, K., Foster, J. B., Driebe, E. M., Paul, S. K. and Price, L. B. (2011): Multidrug-resistant Staphylococcus aureus in US meat and poultry. Clinical. Infection. Dis., 52: 1-4.

White, D. G., Zhao, S., Sudler, R. (2001): In: The road to resistance: Antibiotics as growth promoters for animals: The isolation of antibiotic resistant Salmonella from retail ground meats. *New England Journal of Medicine*. 345: 1147-54.

Worku, T., Negera, E., Nurfeta, A. and Welearegay, H. (2012): Microbiological quality and safety of raw milk collected from Borana pastoral community, Oromia Regional State. *Afr. J. Food Sci. Technol*., 3: 213-222.

Wubet, A. (2004): Bacteriological quality of bovine milk in small holder farms in Debre ziet, Ethiopia. M.Sc. Thesis Faculty of veterinary Medicine, Addis Ababa University.

Wytze ,Brandsma. Dawit, Mengistu. Binyam, Kassa. Mahlet ,Yohannes. Jan van der ,Lee.(2013): The Major Ethiopian Milksheds An assessment of development potential Wageningen UR Livestock Research Lelystad, December 2013

Yilma, Z., Loiseau, G. and Faye, B. (2007): Manufacturing efficiencies and microbial properties of butter and ayib, a traditional Ethiopian cottage cheese. Livestock research for Rural Development, 19: 67.

9. Annexes

Appendix I. Questionnaire format

Farm owner/worker questioner

Name _____Date_____

Address/kebele _____ Age_____

1. Do you consume the milk at home?

A. Yes B. No

2. If Yes, by what form?

A. Raw (fresh whole milk) B. Ergo (naturally fermented milk)

C. Ayib(Ethiopian cottage cheese) D. boiled

3. Did one of your family members become ill after consuming raw milk?

A. Yes B. No

4. If yes, which of the following signs did he or she showed?

A. Diarrhea only B. Vomiting only

C. both diarrhea and vomiting D. abdominal pain and cramp

5. Do you know any milk borne diseases?

A. Yes B. No

6. If, yes to question number 5 can you name them----------

7. How often do you and your family consume the milk and its products?

A. rarely B. As common diet

C. Frequently D. As required

8. Is there any time gap between taking & using the milk?

A. yes B. No

9. For how long do you keep milk at home?

A. 1-4hrs B .4-10hrs C.10-16hrs D. more than 16hrs

10. At what temperature do you keep milk?

a. $<4\,^{o}c$ b. Room temperature

11. What is your level of education(who consume raw milk)?

a. Elementary /Read and write b. college graduate c. high school d. no education

12. Do you know about staphylococcal food born intoxication?

a. yes b. no

13. What kind of floor did you use for housing dairy cows?

A. concrete ground B. natural floor

C. straw bedded floor D. stone and sand E. Made from wood

14. What about for milking areas?

A. concrete ground B. natural floor

C. straw bedded floor D. stone and sand E. Made from wood

15. How often did you clean your cows' house?

A. Three times a day B. two times a day C. one times a day D. weekly E. monthly

16. How often did you clean your milking cows' udder?

A. No cleaning B. Only before milking

C. Only after milking, D. Before and after milking.

17. What kind of milking equipment did you use?

A. Aluminum cans B. Plastic beaker

C. clay pot D. other traditional

18. What is the source water do you use to clean your equipment, teat and udder?

A. River B. well water C. tap water D. stagnant water

19. Do you wash the milk container with soap, detergent?

A. yes B. No

20. Do you wash your hand before milking?

A. yes B. No

Hotels/café owners questioner

Name_____Date_____

Address _____

1. Where do you purchase raw milk?
   a. Farm c. Milk collection centers
   b. Others source
2. What kind of container do you use to collect milk?
   a. Plastic
   b. Metallic c. other
3. What kind of quality assessment do you use before purchasing the milk?
   a. Alcohol test
   b. Lactometer reading
   c. Organoleptic test (smell, test, visualization)
   d. No quality assessment
4. What kind of milk product do you serve for customer?
   a. Boiled milk
   b. Yoghurt
   c. Cheese
   d. Raw milk
5. Do you have awareness about food born disease?
   a. Yes
   b. No
6. Do you have awareness about staphylococcal food born disease?
   a. Yes
   b. No
7. Is there any time gap between purchasing and serving the milk?

   A. yes B. No

9. If yes, how much is the average time gap?

   A. 1-4hrs B .4-10hrs C.10-16hrs D. more than 16hr

10. At what temperature do you keep milk?

    a. $<4\,^{o}c$(refrigerator)

    b. Room temperature

Consumer questioner

Name_____ Date_____

Address_____ Age_____

1. In what form do you consume milk?
   a. Boiled milk
   b. Yoghurt
   c. Cheese
   d. Raw milk
2. Do you have awareness about milk born disease?
   a. Yes
   b. No
3. Do you know about Staphylococcus food born disease?
   a. Yes
   b. No
4. Do you experience illness after consuming milk and milk product?
   a. Yes
   b. No
5. If yes, what kind of clinical sign did you experience?
   a. Diarrhea
   b. Vomiting
   c. Stomach cramp
   d. Other _____
6. Where do you purchase milk?
   a. Farm
   b. MCC
   c. Hotel /café
   d. Other _____
7. What kind of container do you use to collect milk?
   a. Plastic
   b. Metallic
   c. Other _____
8. How long the milk stay at home prior consumption?
   a. <1hr
   b. 1-2hr
   c. >2hr
9. At what temperature do you keep milk?
   a. < 4 ^{o}c /refrigerator
   b. Room temperature

Milk collection center

Name_____Date_____

Address_____

1. What kind of quality assessment does you before collecting milk?
   a. Lactometer reading
   b. Alcohol test
   c. Organoleptic test(smell, test and visualize)
   d. Other _____
2. How long the milk stay at Milk collection centre?
   a. <1hr
   b. 1-2hr
   c. >2hr
3. At what temperature milk kept at the center?
   a. $<4^{o}$c
   b. Room temperature
4. Do you have awareness about milk born disease?
   a. Yes
   b. No
5. Do you know about Staphylococcus food born disease?
   a. Yes
   b. No
6. Do you experience illness after consuming milk and milk product?
   a. Yes
   b. No
7. If yes, what kind of clinical sign did you experience?
   a. Diarrhea
   b. Vomiting
   c. Stomach cramp
   d. Other _____
8. From where do you collect milk?
   a. Farm
   b. Other source _____

9. What kind of container do you use to collect milk?
   a. Plastic
   b. Metallic
   c. Other _____

Appendix II. Check list format used for recording data

Appendix table 1. Sample collection sheet for bacteriological analysis

Serial number	**Date of collection**	**Sample code**	**Type of sample**	**Source**	**Number of samples**

Apendex table 2: Record sheet for laboratory isolation and identification of *Staphylococcus aureus*

Serial number	Type of sample	Sample code	Colony characteristics on BAP	Haemolysis	Gram stain	Catalase test	Coagulase reaction	MSA Mannitol fermentation (MSA)	Growth on Maltose fermentation (PAB)	Staphylococcus

Appendix table 3: Differential tests used for identification of *Staphylococcus aureus* from other *Staphylococcus* species

SN	Staphylococcus species	Haemolysis	Pigment production	Coagulase test	Fermentation of sugar	
					MSA	PAB
1	***S. aureus***	+	+	+	+	+
2	***S. intermidius***	+	-	+	±	±
3	***S. hicus***	-	-	+	-	-
4	**CNS**	-	-	-	-	-

+ = 90% or more strains are positive, + = 90% or more strains are weakly positive, - =90% or more strains are negative.

Source: Quinn, *et al*., 1999

Appendix Table 4: The resistance of each antimicrobial was determined depending on the following measure of zone inhibition diameter.

Antimicrobial agents	Unit	Diameter of zone of inhibition to nearest mm		
		Resistant ≤	Intermediat	Susceptible ≥
Amoxacillin- clavulanic acid	20	19	-	20
Ampicillin	10	28	-	29
Ciprofloxacin	5	15	16-20	21
Erythromycin	15	13	14-22	23
Nalixidic acid	30	13	14-18	19
Penicillin G	10 IU	28	-	29
Streptomycin	10	11	12-14	15
Tetracycline	30	14	15-18	19
Trimethoprim-sulphamethoxazole	25	10	11-15	16
Vancomycin	30	9	10-11	12
Gentamycine	10	12	13-14	15

Source (CLSI, 2012)

Appendix III: **Flow chart of the ISO 6888-3 protocol**

Isolation and identification of Staphylococcus

⇩

Twenty-five grams (25ml) samples will be added
in a stomacher bag containing 225ml of buffered peptone water

⇩

The mixture will be homogenized using a laboratory
blender and incubated aerobically at 37^0C for 24 hours

⇩

A loopful of the cultures of the various dilutions will be streaked aseptically onto sterile BAP and incubated at 37^0C for 24-48 hours under aerobic culture conditions

⇩

Colonies are 0.5 to 1.5 µm in diameter, grey or grey-white to golden-yellow
Gram-positive spherical bacteria that occur in microscopic clusters resembling grapes

⇩

Biochemical tests for confirmation

⇩

Catalase test-3% H2O2 (positive)

⇩

Inoculate colonies on MSA and incubate at 37 ^{0}C for 24-48 hours, growth and change in the PH of the medium is confirmative for Staphylococcus classified as highly fermentative (*S. aureus*), weakly fermentative (*S. intermidius*) and non fermentative (*S. hicus* and CNS)

⇩

Coagulase test to identify the most pathogenic CPS (*S. aureus, S. intermidiusand S. hicus*) from CNS

⇩

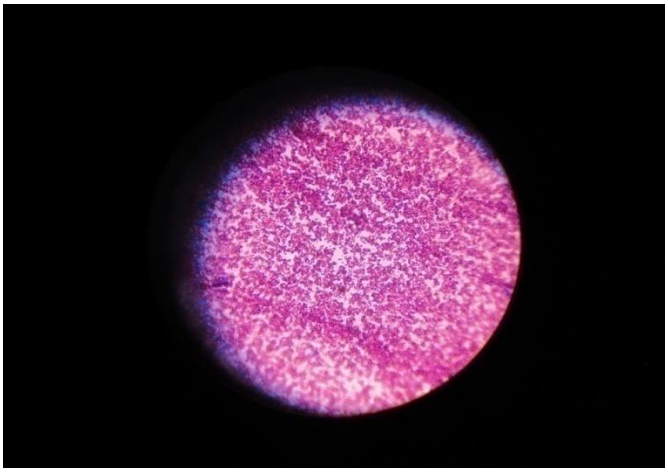
Inoculate CPS isolates on PAB media plate with 1% of maltose and incubate at 37^0C for 24-48 hours to differentiate the pathogenic staphylococci, particularly the coagulase-positive isolate. The identification was based on the fact that *S. aureus* rapidly ferment maltose to change the medium and colonies to yellow. *S. intermedius* gives a weak or delayed reaction and *S. hicus* did not ferment maltose.

Appendix IV: Biochemical tests and procedures.

Apendex Figure 1; growth of *S.aueus* on nutrient agar

Gram's staining procedures

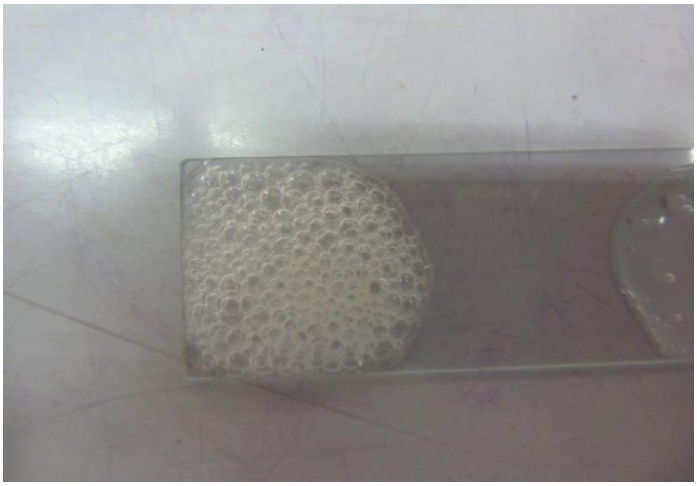
1. Make a thin bacterial colony smear and allow it to dry on the air
2. Fix the dried smear by passing through the Bunsen flame two to three times taking care not to overheat the smear
3. Flood the fixed smear with Gram's crystal violet (primary stain). Let stand for 60 seconds.
4. Pour off the stain and gently wash with tape water.
5. Flood with Gram's iodine (mordant) solution. Allow it to remain for 60 seconds.
6. Pour off the iodine solution and gently wash with tape water.
7. Decolorize with Gram's decolourizer solution 95% acetone alcohol) for 15-20 seconds until the blue dye no longer flows from the smear and gently wash the smear with tape water.
8. Counter stain with Gram's safranin solution or carbolfuschin (counter stain) for 60 seconds.
9. Wash off the red safranin solution with water. Blot with bibulous paper to remove the excess water. Alternatively, the slide may shake to remove most of the water and air-dried.
10. Examine the finished slide under a microscope (oil immersion objective).
11. Interpretation: Bluish purple colour indicate Gram positive and pinkish colour indicate Gram negative bacteria



Appendix Figure 2; gram stain picture of *S.aureus.*

Catalase test procedure

1. Pick a colony from an 18-24 hours culture and place it on a clean glass slide.
2. Put one drop of 3% H2O2 over the organism on the slide.
3. Observe for immediate bubbling (gas liberation) and record the result.
4. Interpretation: A positive result is the rapid evolution of O2 as evidenced by bubbling and a negative result is no bubbles or only a few scattered bubbles.

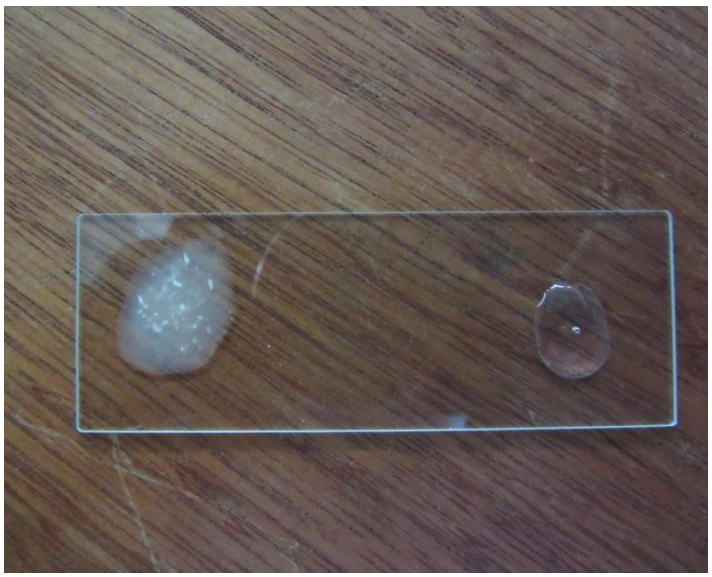


Appendix Figure 3. catalase positive on the left side and test control on the right side.

Coagulase test procedure

1. Three test tubes are taken and labeled “test”, “negative control” and “positive control”.
2. Each tube is filled with 0.5 ml of 1 in 10 diluted rabbit plasma. To the tube labeled test, 0.1 ml of overnight broth culture of test bacteria is added.
3. To the tube labeled positive control, 0.1 ml of overnight broth culture of known *S. aureus* is added and to the tube labeled negative control, only 0.1 ml of sterile broth is added.
4. All the tubes are mixed gently, incubated at 37^{o}C and observed up to four hours. If the test remains negative until four hours at 37^{o}C, the tube is kept at room temperature for overnight incubation.
5. Avoid shaking or agitating the tube during reading. Doubtful or false negative results may occur due to break down of the clot.

Result: Positive result is indicated from a loose clot suspended to a solid clot that is immovable, which remains in place even after inverting the tube. No degree of clotting is observed in negative result.

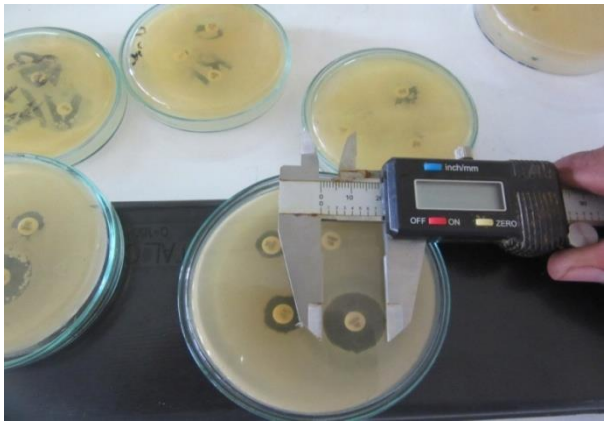


Appendix figure 4, positive coagulase reaction on the left and test control on the right.

Procedure for the disk diffusion methods

1. At least 4-5 well isolated colonies of the same morphological type are selected from non-selective agar plate and just the top of the colonies are touched.

2. Then a suspension was made in a saline or broth without pre incubation.
3. The turbidity of both suspensions is adjusted by comparison with a 0.5 McFarland turbidity standard.
4. The standard and the test suspension are placed in similar 4-6 ml, thin glass tube or vial.
5. The turbidity of the test suspension is adjusted with broth or saline and compared with the turbidity standard, against a white back ground with contrasting black lines, until the turbidity of the test suspension equates to that of the turbidity standard.
6. Inoculation of bacterial suspension
7. A sterile, nontoxic swab on an applicator stick is dipped in to the standardized suspension of bacteria and excess fluid is expressed and rotating the swab firmly against the inside of the tube above the fluid level.
8. The swab is streaked in three directions and continuously brushed over the MullerHinton or by rotating the plate for complete cover of the agar surface.
9. The inoculated plates are allowed to stand for 3-5 minutes but no longer than 15 minutes and the discs are placed on the agar surface using sterile forceps or an antibiotic dispenser.
10. Each disc is gently pressed with the point of a sterile forceps to ensure complete contact with the agar surface. The disc should be placed no closer together than 24 mm (centre to centre).
11. This is equivalent to 6 discs per standard 90 mm Petri plate.
12. After incubation, the diameter of the zones of inhibition are measured to the nearest mm using a ruler or calliper.
13. The diameters are read from the back of the plate, when the test is on the comparatively clear Muller-Hinton medium.
14. The diameter of the zones should be read across the centre of the discs.
15. An interpretation of the size of the zones of inhibition is made with reference.



Appendix Figure 5. Antibiotic sensitivity test on muller hinton agar

Appendix figure 6. *S.aureus* growth on manitol salt agar.

Appendix V: Composition and preparation of media used for the study

Buffered peptone water (Oxoid, England)

Typical formula (g/l):

Peptone ……...…….10.0

Sodium chloride…….5.0

Final PH 7.3 +0.2 at 25^0C

Instructions for use:

Dissolve 15.0g in 1 liter of distilled water. Stirr and dissolve completely. Sterilize by autoclaving at 121^0C for 15 minutes. Cool to room temperature before use.

Blood agar (Himedia, India)

Typical formula (g/l):

'Lab-Lemco'powder…….10.0

Peptone…….10.0

Sodium chloride…….5.0

Agar ……. ……...15.0

Final PH 7.3 +0.2 at 25^0C

Instructions for use:

Suspend 40g in 1 litre of demineralized (distilled) water. Bring to the boil to dissolve completely. Sterilize by autoclaving at 121^0C for 15 minutes. Cool to 45-50^0C and add 7% sterile defibrinated blood.

Nutrient agar (Sisco, India)

Typical formula (g/l):

'Lab-Lemco'powder……..1.0

Yeast extract ……..2.0

Peptone……...5.0

Sodium chloride ……....5.0

Agar ……15.0

Final PH 7.4 +0.2 at 25^0C

Instructions for use:

Suspend 28g in 1 liter of distilled water. Bring to the boil to dissolve completely. Sterilize by autoclaving at 121^0C for 15 minutes.

Mannitol salt agar (Oxoid, England)

Typical formula (g/l):

'Lab-Lemco' powder……...…..1.0

Peptone…….10.0

Mannitol……...10.0

Sodium chloride …….....75.0

Phenol Red ……0.025

Agar……..15.0

Final PH 7.5 +0.2 at 25^0C

Instructions for use:

Suspend111g in 1 litre distilled water and bring to the boil to dissolve completely. Sterilize by Autoclaving at 121^0C for 15 minutes. Mix well before pouring in to sterile petridishes.

Purple agar base (Himedia, india)

Typical formula (g/l):

Proteose peptone ……..10.0

Beef extract……... ……1.0

Sodium chloride……..5.0

Bromcresol Purple……0.02

Agar……..15.0

Final PH 6.8 +0.2 at 25^0C

Instructions for use:

Suspend 31g of the powder in 1 litre of purified water. Mix thoroughly. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder. Autoclave at 121^0C for 15 minutes. When preparing 0.5-1% carbohydrate fermentation, agar dissolves 5-10g of the desired carbohydrate in the basal medium prior to sterilization by autoclaving.

Tryptone soya broth (Sisco, India)

Typical formula (g/l):

Pancreatic digest of casein……..…17.0

Papaic digest of soybean meal……..…3.0

Sodium chloride……..5.0

Di-basic potassium phosphate…….3.5

Glucose……2.5

Final PH 7.3 +0.2 at 25^0C

Instructions for use:

Dissolve 30g in 1 litre of distilled water and distribute into final containers. Sterilize by autoclaving at 121^0C for 15 minutes

Muller Hinton Agar (Oxoid, England)

Composition (g/l):

Beef extracts 2;

Acid hydrolysate of casein 17.5;

starch1.5;

Agar 17.

Direction: suspend 38 g of the powder in 1 liter of distilled water. Mix thoroughly, heat with frequent agitation and boil for 1 minute to completely dissolve the powder. Autoclave at 121^oc for 15 minutes.