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ASSESSMENT OF CONTAMINATION OF MILK WITH *STAPHYLOCOCCUS AUREUS*, MILK CONSUMPTION HABIT AND HANDLING PRACTICES: IMPLICATION FOR PUBIC HEALTH IN SEBETA, CENTRAL ETHIOPIA

MSc Thesis

By

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DEPARTMENT OF MICROBIOLOGY, IMMUNOLOGY AND VETERINARY PUBLIC HEALTH

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June, 2015

Bishoft, Ethiopia

ASSESSMENT OF CONTAMINATION OF MILK WITH *STAPHYLOCOCCUS AUREUS*, MILK CONSUMPTION HABIT AND HANDLING PRACTICES: IMPLICATION FOR PUBIC HEALTH IN SEBETA, CENTRAL ETHIOPIA

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

By

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June, 2015

Bishoftu, Ethiopia

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DEDICATION

I am dedicating this paper to my mother, Bogalech Tola, and to my lovely sister, Engineer Etenesh Ayele, whose words of encouragement and push for tenacity ring in my ears and have never left my side and they are very special. My sister, I will always appreciate all she have done, for helping me in all directions. Thank you for believing in me; for allowing me to further my studies. I am honored to have you. Thank you for giving me a chance to prove and improve myself through all my walks of life. Please don't ever change. I respect and love you.

STATEMENT OF THE AUTHOR

First, I declare that this thesis is my bona fide work and that all sources of material used for this thesis have been duly acknowledged. This thesis has been submitted in partial fulfillment of the requirements for MSc degree at Addis Ababa University, College of Veterinary Medicine and Agriculture and 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.

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LIST OF ABBREVIATIONS

a_w	Water activity
CFU	Colony forming units
CNS	Coagulase-negative *Staphylococcus*
FBD	Foodborn diseases
HACCP	Hazard critical control point
MCCs	Milk collection centres
MSA	Mannitol salt Agar
PAB	Purple agar base
SCC	Somatic cell count
SEA	Staphylococcal enterotoxins A
SEE	Staphylococcal enterotoxins E
SEG	Staphylococcal enterotoxins G
SEQ	Staphylococcal enterotoxins Q
SEs	*Staphylococcus* entotoxins
SFP	Staphylococcal food poisoning
SPSS	Statistical Package for Social Sciences
TSS	Toxic syndrome shock
TSST	Toxic shock syndrome toxin
PVL	Panton-Valentine leukocidin

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ABSTRACT

A cross-sectional study was conducted from November 2014 to April 2015 to assess the contamination of milk with Staphylococcus aureus, milk consumption habit and handling practices using microbiological technique and questionnaire survey in Sebeta town, central Ethiopia. Simple random sampling and purposive sampling technique were used to generate the desired data. The study involved 209 individual cow milk, 27 swab from milking buckets and 25 swab from hands of milkers, 20 milk samples from tank of milk collection centers, 10 pasteurized milk from processing plant and 23 farm owners, 19 milk collectors, 50 consumers, 17 hotel/cafeteria workers and 1 milk processing staff member were included. Bacteriological culture and antimicrobial susceptibility tests were performed following the recommended standard procedures. The study revealed a prevalence of 19.6% (95%CI: 14.5-25.6) at farm milk and contamination of 80% of Staphylococcus aureus at collection centers, and there was statistically significant variation between them with higher contamination at milk collection centers (χ^2=35.599, df=1, p=0.000). There was also significant variation in the proportion of Staphylococcus aureus among collection centers (χ^2= 60.000, df=3 p=0.000). The contamination of milker's hand and milking bucket with Staphylococcus aureus were 32% and 11.1%, respectively. Staphylococcu s aureus was isolated from none of the pasteurized milk samples. The isolates were found to be resistant to cefoxitine (100%), penicillin G (98.5%), and streptomycin (77.9%). 35% of the farmers were consume raw milk, all didn't wash their hands using antiseptic solutions, never washed cow teats/udder and all had no knowledge of Staphylococcal food poisoning. In conclusion, the study showed poor handling practices of milk, raw milk consumption habit, resistance of Staphylococcus aureus to commonly used antibiotics and lack of awareness about staphylococcal food poisoning implicating for public health issue. Eventually, raising awareness on milk handling practices, milk borne staphylococcal poisoning and further study to estimate the risk of staphylococcal poising following consumption of milk contaminated with Staphylococcus aureus at each milk value chain in the study area were recommended.

Key words: *Antibiotics, Contamination, Milk, Sebeta, Staphylococcus aureus, Value chain.*

1. INTRODUCTION

Foodborne diseases (FBD) are diseases of infectious or toxic nature transmitted by the consumption of contaminated food or water. They are among the most widespread public health problems globally (Hubalek, 2003; Pal, 2001). Food normally becomes a potential source of human infection due to contamination that can be occurred during production, collection, transport and preparation or during processing. Feaces, feacally-contaminated soil or water, unhygienic handling and use of unhygienic equipments and utensils are potential sources of food contamination (Slifko *et al.,* 2000). The presence of foodborne pathogens in ready-to-eat foods, milk and milk products, meat, and meat products puts consumers at high risk and imposes grave economic losses to producers (Sofos, 2008; Syne *et al.,* 2013).

Among the bacteria predominantly incriminated in FBD, *Staphylococcus aureus* is a leading cause of gastroenteritis resulting from the consumption of contaminated food. Staphylococcal food poisoning is a FBD due to absorption of Staphylococcal enterotoxin (SE) preformed in the food (Loir *et al.,* 2003). *S. aureus* starts to produce SE when the population density in milk reaches about $10^{6.5}$cfu/ml and thereafter the amount of SE increase linearly with time (Fujikawa and Morozumi, 2006). A small amount of SE ranging from 100–200 ng can cause illness (Makita *et al*., 2012). Staphylococcal organisms alone have found to cause a hospitalization rate as high as 14%. The fatality rate range from 0.03% in the general population to as high as 4.4% for highly sensitive persons such as immunocompromised persons, elderly persons and children (Aycicek *et al.,* 2005; Kerouanton *et al*., 2007).

Milk is very nutritional and nearly balanced food enriched with carbohydrate, proteins, fats, vitamins and minerals (Bradely, 2002; Pal, 2012). As a matter of the fact, it is highly vulnerable to bacterial contamination and support the growth and multiplication of pathogenic organisms leading to food spoilage, food infection and poisoning (Soomro *et al*., 2003; Pal, 2012; Girma *et al.,* 2014). *Staphylococcus aureus* find their way to raw milk and milk products from sub clinical mastitic cow, environment, milkers' hand and unhygienic equipments (Ismael *et al.,* 2009). Milk of the infected animal is the main source of enterotoxigenic *S. aureus* of animal origin

and these toxins are known to cause nausea, vomiting and abdominal cramps when ingested by human and are responsible for staphylococcal food poisoning outbreak (Kerouanton *et al.*, 2007).

S. aureus can become pathogenic when conditions such as pH, temperature and nutrient availability are altered and become favourable for overgrowth (Mims *et al.*, 2009). Milk Storage at temperatures of 37 to 42 ºC favours growth and multiplication of *S. aureus* (Presscott *et al.*, 2002). The optimum temperature for toxin production is 35 to 40ºC (range 10 to 45ºC), pH is 5.3-7.0 (4.8- 9.0), a_w is 0.90 (range 0.86 to 0.99) and greatest toxin production is in the presence of oxygen (Morandi *et al.*, 2007; Ash, 2008).

Heating of milk at normal boiling and pasteurization temperature can kill the bacteria but the enterotoxins remains active (Presscott *et al.*, 2002). Staphylococcal enterotoxn s are highly heat resistant and are thought to be more heat resistant in foodstuffs than in a laboratory culture medium (Bergdoll, 1989). Enterotoxins producing *S. aureus* are most dangerous and harmful for the human health. About 50 % strain of this organism are able to produce enterotoxins that associated with food poisoning (Payne and Wood, 1974).

Although pasteurization or boiling of milk is likely to destroy all pathogens including *S. aureus*, there is a great public health concern when either milk is consumed in raw form or pasteurization is not efficient (Bharathy *et al*., 2015). Majority of Ethiopian population consume raw milk and raw milk products including cheese, cream, butter and yoghurt (Ashenafi and Beyene, 1994). Moreover, production and consumption of raw milk and various dairy products often takes place under unsatisfactory hygiene conditions (Wubete, 2004). As a result, the possibility of incidence of SFP due to the consumption of dairy products is likely (Ashenafi and Beyene, 1994; Yilma *et al.*, 2007; Makita *et al.*, 2012). Other studies conducted elsewhere substantiated the occurrence of *S. aureus* in milk at various points of milk value chain that might attributed to milk contamination from mastitic cow, cross contamination, poor handling practices and use of unhygienic equipments leading to Staphylococcal poisoning in humans following consumption prior to subjecting to adequate heating (Hundera *et al.*, 2005; Desissa, 2010). There is only a report of *S. aureus* as one of the

major bacterial pathogens causing mastitis with no citable information regarding its occurrence in milk ready for consumption in and around Sebeta (Sori *et al*., 2005).

Moreover, there is a paucity or lack of information regarding the prevalence of *S. aureus*, potential sources of contamination, milk consumption habit and handling practices of milk along the milk value chain in Sebeta town. Furthermore, antimicrobial susceptibility pattern of *S. aureus* isolates from different points along the milk value chain in the study area is not known. Therefore, this study was done to address the above aforementioned gaps with general objective of to asses contamination of milk with *Staphylococcus aureus*, milk consumption habit and handling practices in Sebeta , central Ethiopia.

Specific objectives were:

- To identify the milk value chain in the study area
- To estimate the prevalence of *Staphylococcus aureus* along the milk value chain
- To determine the antimicrobial susceptibility pattern of the isolates
- To assess sources of contamination of milk with *Staphylococcus aureus*
- To assess the milk consumption habit and handling practices

2. LITRATURE REVIEW

2.1. General Characteristics of *Staphylococcus* Organisms

2. 1. 1. Etiology

Staphylococcal food poisoning is a foodborne poisoning attributed to ingestion of contaminated food in which the enterotoxigenic strains of *Staphylococcus* can multiply reaching about 105 CFU/g of food. *Staphylococcus aureus* is a major cause of foodborne intoxication and outbreaks occur throughout the world because of its ubiquity and its ability to persist and grow under various conditions (Pal, 2001; Salandra *et al*., 2008).

Staphylococcus aureus is a facultatively anaerobic, Gram-positive coccus, which appears as grapelike clusters when viewed through a microscope and has large, round, golden-yellow colonies, often with hemolysis, when grown on blood agar plates. The golden appearance is the etymological root of the bacteria's name; aureus means "golden" in Latin (Silva *et al.,* 2000). The bacterium multiply by simple division into two, and under suitable conditions of environment and temperature, this occurs every 15-30 minutes. Thus, one cell could become over 2 million in 7 hours and 7000 million cells after 12 hours continuous growth (Jay, 2000).

Staphylococcus aureus is catalase positive and able to convert hydrogen peroxide (H_2O_2) to water and oxygen, which makes the catalase test useful to distinguish from enterococci and streptococci. A small percentage of *S. aureus* can be differentiated from most other staphylococci by the coagulase test: *S. aureus* is primarily coagulase-positive (meaning that it can produce "coagulase", a protein product, which is an enzyme) that causes clot formation while most other *Staphylococcus* species are coagulase-negative (Silva *et al*., 2000). The bacterium do not produce endospores but are highly resistant to high osmotic conditions and desiccation, especially when associated with organic matter such as blood, pus, and other tissue fluids. These properties facilitate its survival in the environment, growth in food, and communicability. However, usually it is readily killed at cooking, pasteurization

temperatures; but survives frozen storage. Heat resistance of *S. aureus* is increased in dry, high-fat and high-salt foods. On the other hand, SEs are extremely resistant to heat (Silva *et al*., 2000; Ash, 2008).

Although *S. aureus* is commonly found on the skin of a wide variety of mammals and birds and on the environment, humans are thought to be the primary source of strains associated with food matrix staphylococcal intoxication (Sandel and McKillip, 2004).

2. 1. 2. Taxonomy and classification

Staphylococci are Gram-positive cocci, 0.5 to 1.5 µm in diameter, which occur either singly or in pairs or tetrads, but the prevalent form is grapelike clusters. The name *Staphylococcus* (staphyle= bunch of grapes) was introduced in 1883 by Ogston. One year later, Rosenbach used the term in a taxonomic sense and provided the first description of the genus *Staphylococcus* (Salyers and Whitt, 2002). The scientific classification of *Staphylococcus* according to and Todar, 2008 is as follows:

Kingdom:	Bacteria	Order:	Bacillales
Phylum:	Firmicutes	Family:	*Staphylococcaceae*
Class:	Bacilli	Genus:	*Staphylococcus*

2. 1. 3. Morphology

The organism has a Gram-positive cell composition, with a unique peptidoglycan structure that is highly cross linked with bridges of amino acids (Hein *et al.*, 2005). The bacterium cells are spherical (cocci), which tend to be arranged in pairs, short chains, or typically occurring in bunched, grape-like irregular clusters when viewed through a microscope, owing to cell division in multiple planes (Shah, 2003). The most obvious morphological characteristic is its marked tendency to occur as masses of cells in grape like clusters. This happens because of the geometry (divide in two planes) (Freeman, 1985). In the genus *Staphylococcus*, *S. aureus* has large, round, golden-yellow colonies, often with haemolysis, when grown on blood agar plates. Some strains of *S. aureus* are capable of producing staphyloxanthin a carotenoid pigment that acts as a virulence factor. This pigment has an antioxidant action that

helps the microbe to evade killing with reactive oxygen used by the host immune system. It is thought that staphyloxantin is responsible for *S. aureus* characteristic golden colour (Tsegmed, 2006; Todar, 2008).

2. 1. 4. Growth requirements

Staphylococci are not highly fastidious in their nutritive requirements and grow readily on the usual meat extract peptone mediums. Growth of *Staphylococcus* is most profuse on sheep blood agar medium (Quinn *et al*., 2002). They are facultative anaerobes that grow best by aerobic respiration or fermentation that yields principally lactic acid (Silva *et al*., 2000). The optimum temperature for growth is 35^0C to 37^0C (they can grow at temperature ranging from 6 to 48^0C) (Aycicek *et al.*, 2005; Ash, 2008). Foods with a pH around 7 are ideal for bacterial growth and most animal food products including meat, fish, poultry, eggs, and milk have a pH around 7 (Rho and Schaffner, 2007).

The optimum pH for growth is 7 to 7.5 (minimum pH for growth is 4.2; maximum pH for growth is 9.3). The organisms are resistant to drying and may grow and produce enterotoxins in foods with water activity (a_w) as low as 0.85. They can grow in foods with 25% NaCl but grows well in 7 to 10% NaCl. The optimum water activity for growth is 0.99. Its ability to grow at low a_w means that it has a competitive advantage on low a_w foods (Ash, 2008). The optimum temperature for toxin production is 35 to 40^0C (range 10 to 45^0C), pH is 5.3-7.0 (4.8- 9.0), a_w is 0.90 (range 0.86 to 0.99) and greatest toxin production is in the presence of oxygen (Morandi *et al*., 2007; Ash, 2008).

2. 2. Epidemiology

Staphylococcus aureus is one of the most important bacterial foodborne pathogen globally. About a quarter of people among the world population carry one or other strain at any one time, and, if they develop an infection, their own colonizing strains are likely to be responsible for such an infection (Johnson *et al*., 2006).

The clinical significance of *S.* a*ureus* is largely due to its ubiquity. It forms parts of the bacterial environment of animals and humans throughout the world and can exist as a persistent or a transient member of the normal flora of the skin and mucous membranes without causing any symptoms of diseases (Acco *et al*., 2003). In humans, most frequently it is present on the mucus membranes of the nose and throat and in the pores and hair follicles of normal skin, particularly in damp areas such as axillae and perineum. Breaks in skin and mucous membranes allow entrance of these organisms into the body where they may cause disease (Acco *et al*., 2003; Lourdes *et al*., 2004).

In many outbreaks of SFP, a human food handler is implicated who contaminates the food and then, under favourable conditions, staphylococci will multiply and produce enterotoxins (Chiang *et al*., 2008). It is estimated that 30-80 % of the human population are carriers of *S. aureus* and of these 50 % carry food poisoning strains. Thus, unhygienic treatment of food has to be considered as a major risk of contamination (Atanassova *et al*., 2001). Approximately 30 % of the human populations have small number of *S. aureus* in the intestine. If the normal flora is disturbed, as can happen after antibiotic therapy, *S. aureus* may become a dominant organism for short periods in the intestine and excreted in very large numbers (Baron, 2007).

Washing the skin with soap and water usually eliminates many of the gram-negative bacteria but gram-positive cocci tend to rise to the surface of the skin from pores and can be present in even larger numbers on the surface after washing. Scrubbing disturbs the superficial layers of the skin and may further spread *S. aureus.* The salt tolerance of the *S. aureus* gives them a selective advantage on the skin, as the sweat has a high salt content (Quinn *et al.*, 1999).

Most commonly clinical isolates are from the respiratory tract and the skin (pimples, carbuncles, furuncles, suppurative wounds etc.) of humans and animals (Bania *et al*., 2006). Because *S. aureus* is major cause of hospital acquired (nosocomial) infection of surgical wounds and community acquired infections, it is necessary to determine the relatedness of isolates collected during the investigation of an outbreak (Foster, 1991).The sources of infection are mainly contaminated foods, water and environment

where the animals are crowded together. The two most important sources to foods and water contamination are nasal carries and individuals whose hands and arms are inflicted with boils and carbuncles and are permitted to handle foods (Smith, 2007).

Staphylococcus aureus is most often transmitted by direct or indirect contact with a person who has a discharging wound (septic and non-septic lesions), a clinical infection of the respiratory or urinary tract, or one who is colonized with the organism. It can be carried on the hands of healthcare personnel and food preparers. Contaminated surfaces and medical equipment are also possible sources of staphylococci (Aycicek *et al*., 2005; Bania *et al*., 2006; Pal, 2007). Foods responsible for SFP outbreaks are often those that have been heated to destroy microorganisms, and then require some food handling and storage at room temperature (Chiang *et al*., 2008).

2. 3. Pathogenesis

Pathogenicity of *S. aureus* is due to the membrane active substances i.e. cytolytic toxins, consisting of four haemilysins and a leukocidin. This genus may have alpha, beta, gamma and delta haemolysin and the pathogenic members of species aureus display beta haemolysis (Presscott, 2002).

The importance of Staphylococci to both the clinical and food settings is associated to the wide variety of specific virulence determinants common to these groups of bacteria (Acco *et al.,* 2003; Sandel and McKillip, 2004). They are important pathogens due to a combination of toxin mediated virulence, invasiveness, and antibiotic resistance (Loir *et al.,* 2003; Soejima *et al.,* 2007).

The major potential virulence factors of pathogenic staphylococci are surface proteins that promote colonization of host tissues, invasions that promote bacterial spread in tissues (leukocidin, kinases, hyaluronidase), surface factors that inhibit phagocytic engulfment (capsule), biochemical properties that enhance their survival in phagocytes (catalase production), immunological disguises (protein A, coagulase, clotting factor), membrane-damaging toxins that lyse eukaryotic cell membranes

(hemolysins, leukotoxin, leukocidin), exotoxins that damage host tissues and inherent and acquired resistance to antimicrobial agents (Martın *et al.,* 2004; Todar, 2008).

2.3.1. Tissue invasion

The event that leads to infection is initiated with carriage of the organism. Then the organism disseminated via hand carriage to body sites where infection may occur (either through overt breaks in dermal surfaces, such as vascular catheterization or operative incisions, or through less evident breakdown in barrier function, such as eczema or shaving associated trauma). The hallmark of staphylococcal infection is the abscess, which consists of a fibrin wall surrounded by inflamed tissues enclosing a central core of pus containing organisms and leukocytes. From this focus of infection, the organisms may be disseminated hematogenously, even from the smallest abscess (Loir *et al*., 2003; Smith, 2007).

The ability to elaborate proteolytic enzymes facilitates the process. This may result in pneumonia, bone and joint infection, and infection of the heart valves. In immunocompromised hosts (e.g. patients with cancer who are neutropenic and have a central venous line), 20-30% develop serious complications or fatal sepsis following catheter related *S aureus* bacteremia. Persistent deep-seated infections have now been linked to small colony variants of the organism. This population is more resistant to antibiotics and grows slowly (Soejima *et al*., 2007; Todar, 2008).

2.3.2. Toxin mediated diseases

Staphylococcus aureus also elaborates toxins that can cause specific diseases or syndromes. Enterotoxin producing strains of *S. aureus* cause one of the most common foodborne illnesses by preformed toxin production with an incubation period of 1-6 hours; as well as by infecting both local tissues and the systemic circulation with variable and indefinite incubation period, most commonly 4-10 days. Enterotoxins are low-molecular-weight extracellular, super antigenic chemicals that initiate nonspecific T-cell proliferation resulting in severe acute onset of watery diarrhea, nausea, vomiting, and abdominal pain (Baron, 2007; Chiang *et al.,* 2008). A rare but well described disorder in neonates and young children is staphylococcal scalded skin

syndrome (Ritter disease). The organism produces an exfoliative toxin produced by strains belonging to phage group II. Initial features include fever, erythema, and blisters, which eventually rupture and leave a red base. Gentle shearing forces on intact skin cause the upper epidermis to slip at a plane of cleavage in the skin, which is known as the Nikolsky sign (Loir *et al*., 2003; Smith, 2007).

The most feared manifestation of *S aureus* toxin production is toxic shock syndrome (TSS). Although first described in children, it was most frequently associated with women using tampons during menstruation. Since the early 1990s, at least half of the cases have not been associated with menstruation. The syndrome is associated with strains that produce the exotoxin TSST1, but strains that produce enterotoxin B and enterotoxin C may cause 50% of cases of non-menstrual TSS. These toxins are super antigens, T cell mitogens that bind directly to invariant regions of major histocompatibility complex class II molecules, causing an expansion of clonal T cells, followed by a massive release of cytokines. This cytokine release mediates the TSS (Salyers and Whitt, 2002; Loir *et al*., 2003).

2. 4. The Role of Bacterial Virulence Factors in *Staphylococcus aureus*

According to the narrowest definition, a virulence factor is a substance that when purified to homogeneity and introduced into a test animal, produces a pathogenic effect. By using this definition factors involved in attachment cannot be considered to be virulence factors. In the broadest sense, any factor produced out of the bacterial cytoplasm allowing survival within or on a host organism in a non-symbiotic manner can be regarded as a virulence factor (Projan and Novick, 1997). *S. aureus* can produce numerous putative virulence factors that allow the microbe to adhere to eukaryotic membranes, resist phagocytosis, lyse eukaryotic cells and trigger the production of a cascade of host immunomodulating molecules. Among bovine *S. aureus* isolated from mastitis, production of enterotoxins (Tollersrud *et al*., 2000) or genes encoding these toxins have been the most studied (Fueyo *et al.*, 2005; Srinivasan *et al*., 2006). Many suggest that virulence of bovine *S. aureus* vary among strains (Sommerhauser *et al*., 2003), but the possible role of specific virulence factors in this phenomenon is poorly understood.

In general, *S. aureus* virulence factors can be divided into those involved in attachment and into those enabling evasion of the host immune system and tissue invasion (maintenance of infection) (Haveri, 2008).

2. 5. Clinical Manifestation and Outcome

Staphylococci are among the most significant pathogens causing a wide spectrum of diseases in both humans and animals (Pal, 2007). There are two kinds of pathogenic staphylococci: those that cause pyogenic infections and those responsible for food poisoning. The same organism may show both these apparently unrelated effects (Salandra *et al*., 2008).

Clinical manifestation of bovine mastitis caused by *S. aureus* following IMI can vary from subclinical to a per acute, gangrenous form (Barkema *et al.,* 2006). Subclinical mastitis is the most common and likely the most problematic. The only detectable response is an increased milk SCC but the pathological changes; reduced secretory cell and luminal areas, lead to lowered quality and quantity of milk production. Unfortunately, milk SCC may remain under threshold limits and infection is often chronic when detected. *S. aureus* mastitis generally responds poorly to treatment (Barkema *et al*., 2006), relapses are common, and the infection is easily transmitted to unaffected quarters of the same cow. Virulence of the infecting strain and host factors, including the efficacy of the immune response of the cow, which may be related to age and lactation stage, are assumed to affect the severity of the clinical signs and persistence of infection. Older cows are more often infected with *S. aureus* compared with primiparous cows and develop more severe inflammation (McDougall *et al*., 2007). High SCC in the affected quarter and multiple infected quarters in the same cow impair the prognosis (Zadoks *et al*., 2001).

2.5.1. Disease in food animals

In food producing animal reservoirs, such as ruminants, staphylococci present on the skin and mucosae. In animals, *Staphylococcus* can cause pustular inflammation of the skin and other organs, mastitis being the most serious (Pal and Seid, 2013). They are frequently associated to subclinical mastitis becoming responsible of contamination of

milk and dairy products and great economic importance to the dairy industry (Salandra *et al*., 2008).

Its large capsule protects the organism from attack by the cow's immunological defences (Hein *et al*., 2005). From the species of organisms that were isolated from both clinical and subclinical mastitis staphylococci were the major pathogens out of which *S. aureus* contribute the major share accounting for 34 % and 49.43% from clinical and subclinical mastitis, respectively (Anderson and Pritchard, 2008). Washcloths, teat cup liners and flies mechanically transmit the infection from cow to cow. Cattle are often infected by humans and the infection is carried from one cow to another by the milkers' hands (Freeman, 1985). There are estimates that 80-100% of all herds have at least some staphylococcal mastitis, with 5 to 10% of cows infected (Anderson and Pritchard, 2008). Herds with excellent milking hygiene practices and management have lower levels of staphylococcal intramammary infections as compared to those herds with poor hygiene or management (Kaloreu *et al.,* 2007). The bacteria produce toxins that destroy cell membranes and can directly damage milk-producing tissues (Jones, 1998). Staphylococcal infections also develop into metritis, enteritis, ear infections and conjunctivitis (Anderson and Pritchard, 2008).

2.5.2. Disease in man

Nasal carriage of *S. aureus* is considered to be a key risk factor for development of staphylococcal infection (Von Eiff *et al.,* 2001). Three bacterial carriage patterns have been distinguished in the healthy adult population: about 20% of individuals are persistent *S. aureus* carriers; around 60% are intermittent carriers and 20% are persistent non-carriers (Moreillon *et al.,* 2005). So far and based on currently available information, there are insufficient data to confirm or refute the existence of host-bacteria genotypes that predispose to carriage (Cespedes *et al*., 2005). However, studies comparing carriage and infecting isolates of *S. aureus* have found that individuals are usually infected with their own carriage isolate. In fact, the temporary eradication of these carriage, has been shown to reduce nosocomial infection in immunocompromised patients (Kluytmans, and Wertheim, 2005).

Skin-related infections

S. aureus is a leading cause of many skin-related infections including keratitis, atopic dermatitis, carbuncles, cellulites, impetigo, psoriasis, furuncles, follicles, mastitis, etc (Pal, 2007). In most cases, a minor rupture of the skin or mucosal barrier may allow the internalization of the bacteria forming an abscess enclosing a central core of pus containing not only the bacteria but also neutrophils and macrophages (Moreillon *et al*., 2005).

Deep-seated infections

Once the bacteria have broken down the natural skin barrier, they can disseminate into more profound sites or migrate directly into the blood. Thus any localized infection has the potential to become the seeding ground for a more severe spread (Moreillon *et al*., 2005). *S. aureus* is able to cause deep-seated related threatening systemic infections such as endocarditis, osteomyelitis, pneumonia, bacteremia and septic shock (Moreillon *et al*., 2005; Pal, 2007) in which intracellular foci are probably present.

Osteomyelitis

S. aureus is responsible for 70% of human osteomyelitis, 80% of cases of joint infections in patients with rheumatoid arthritis and is a common agent in other bone associated diseases (Lew and Waldvogel, 1997). It can invade and persist within osteoblasts causing rapid tissue destruction via the production of toxins (Ellington *et al*., 1999). Antibiotic resistant strains and their intracellular localisation make successful treatment of osteomyelitis very difficult (Ellington *et al*., 2003; Ellington *et al*., 2006).

Endocarditis

S. aureus is a virulent pathogen with a unique capacity to cause difficult-to-eradicate endovascular infections. This facility relies in part on his affinity to colonize and invade endothelial cells and endovascular tissue and its ability to resist killing by

platelet microbicidal peptides (Yeaman *et al*., 1998). A number of cellular changes result from these progression including the release of proinflammatory cytokines and the expression of Fc receptors and adhesion molecules. These cellular alterations may play a crucial role in initiating and amplifying the inflammatory response, which results in a magnified endothelial cell injury (Sinha *et al*., 1999). The bacteria are responsible for about 70% of cases of endocarditis in intravenous drug users and more than 30% cases of native valve endocarditis (Moreillon and Que, 2004).

Pneumonia

Once staphylococci are in the lung, they multiply and sometimes invade the epithelium of the bronchioles (Da Silva *et al*., 2004). The production and release of cytokines and chemokines, including IL-8, due to the presence of *S. aureus* elicit infiltration of polymorphonuclear cells and macrophages, leading to tissue damage and subsequent pneumonia. It has been found responsible for about 10% of community acquired pneumonia and about 30% of cases of hospital acquired-pneumonia. Incidence is high especially in patient's over-75 years at nursing homes. A necrotizing pneumonia caused by PVL leukocidin-positive *S. aureus* in otherwise healthy children and young adults are now recognized as a new nightmare, since the lethality rate is very high. *S. aureus* is also a major pathogen in cystic fibrosis patients (Saiman and Siegel, 2003).

Bacteremia and septic shock

The frequency of the presence of bacteria in blood-stream has increased over the past decades. *S. aureus* bacteremia still carries a 20-25% mortality rate. *Staphylococcal* sepsis and septic shock result from the overproduction of inflammatory mediators as a consequence of the interaction of the host immune system with the bacteria and its wall constituents. The increasing frequency of *S. aureus* sepsis is vastly linked to the increased use of invasive equipment on persons with a weakened immune system and the ability of the bacteria to easily colonize intravascular catheters or surgically implanted materials (Van Amersfoort *et al.,* 2003). The cellular events leading to septic shock are similar in staphylococcal infection and infections with gram-negative

bacteria. *S. aureus* and *S. epidermidis* are responsible for half of cases (Van Amersfoort *et al*., 2003).

2. 6. Diagnosis

The diagnosis of *S. aureus* infection depends upon the symptoms of patient and the healthcare advisor's evaluation. Obtaining an appropriate specimen is the first step of definitive diagnosis of *S. aureus* infection. Based on the type of infection presented, the specimen can be collected accordingly and sent to a laboratory for identification by biochemical, enzyme-based or molecular-based tests (Li, 2010).

Under laboratory condition, a Gram stain can be performed as the first identification step. If Gram positive cocci are observed under microscope, further tests should be performed. Secondly, culturing the suspect specimen on selective medium, such as mannitol salt agar (MSA), blood agar or other chromogenic agars to differentiate staphylococci or *S. aureus* from other bacteria, can be used to obtain the typical single colony. Typical *S. aureus* should show a big creamy yellow colony surrounded by yellow area on MSA or big glossy black colony surrounded by clear hole on blood agar. Under certain cases, selective medium that contain specific antimicrobials may used to identify the antimicrobial resistant ability of the isolates. Minimum Inhibitory Concentration test is also needed to determine the antimicrobial susceptibility (Li, 2010). To differentiate on the species level, several biochemical tests can be performed, such as catalase test and coagulase test. A typical *S. aureus* isolate show a strange catalase positive and coagulase positive results (Li, 2010).

Diagnostic microbiology laboratories and reference laboratories are of important for identifying outbreaks and new strains of *S. aureus*. Recent genetic advances have enabled reliable and rapid techniques for the identification and characterization of clinical isolates of *S. aureus* in real-time (Pal and Seid, 2013). These tools support infection control strategies to limit bacterial spread and ensure the appropriate use of antibiotics. These techniques include Real-time PCR and Quantitative PCR and are increasingly being employed in clinical laboratories (Omoe *et al*., 2005; Ash, 2008).
A number of serological methods based on monoclonal antibodies (e.g. ELISA, ELFA, Reverse Passive Latex Agglutination) for determining the enterotoxigenicity

of *Staphylococcus aureus* isolated from foods as well as methods for the separation and detection of toxins in foods have been developed and used successfully to aid in the diagnosis of illness. These rapid methods can detect approximately 1.0 nanogram of toxin/g of food (Bania *et al*., 2006; Walderhaug, 2007; Ash, 2008).

2. 7. Staphylococcal Food Poisoning

Staphylococcus aureus is an important foodborne pathogen of global significance (Pal and Seid, 2013). It is a versatile pathogen of humans and animals and causes a wide variety of diseases ranging in severity from slight skin infection to more severe diseases such as pneumonia and septicemia (Pal, 2007). Of particular relevance to the food processing industry is the ability of some *S. aureus* strains to produce heat stable enterotoxins that cause staphylococcal food poisoning (SFP), which ranks as one of the most prevalent causes of gastroenteritis worldwide (Dinges *et al.,* 2000). The intoxication is characterized by enteric responses like diarrhea, abdominal cramps, and vomiting within 1-6 h of consumption of contaminated food (Pal, 2001; Leenalitha and Peter, 2007). The toxins are heat stable proteins (Pal, 2001; Leenalitha and Peter, 2007). The bacterium is heat labile and does not compete well with other microorganisms and therefore, contamination usually occurs after the food has been processed when there is little competition from other microorganisms (Kilango, 2011).

The organism usually gains access to foods from food handlers or other surfaces like the processing equipment. Although Staphylococci are commonly found on animal skins, water, soil etc, bacteria from food handlers and other human sources are considered as the most important contributing factors to intoxications associated with food (Leenalitha and Peter, 2007). Food poisoning is of great concern to food industries and regulatory agencies as it represents massive health and economic losses. The foods that are commonly contaminated by staphylococcus entrotoxins (SEs) are baked dessert items such as cream filled pastries, cream pies, chocolate éclairs, meat and meat products, potatoes, tuna, chicken, turkey, ready-to-eat salads, eggs, poultry, dairy and milk products (Leenalitha and Peter, 2007).

Staphylococcus aureus does not form spores. Thus, *S. aureus* contamination can be readily avoided by heat treatment of food. Nevertheless, it remains a major cause of foodborne diseases because it can contaminate food products during preparation and processing. *S. aureus* is indeed found in the nostrils, and on the skin and hair of warm-blooded animals. Up to 30-50% of the human populations are carriers (Le Loir *et al.*, 2003).

Staphylococcus aureus is able to grow in a wide range of temperatures (7° to 48.5°C with an optimum of 30 to 37°C; Schmitt *et al*., 1990), pH (4.2 to 9.3, with an optimum of 7 to 7.5; Bergdoll, 1989) and Sodium chloride concentrations (up to 15% NaCl). These characteristics enable *S. aureus* to grow in a wide variety of foods. This, plus their ecological niche, can easily explain their incidence in foodstuffs that require manipulation during processing, including fermented food products, such as cheeses (Kilango, 2011).

2. 8. *Staphylococcus aureus* in Milk

2. 8. 1. Mastitis in cows as a source of S.aureus

Mastitis is an inflammation of the milk-producing glands causes great pain to the dairy cows (Althaus, 2003). In dairy cows, mastitis is frequently caused by bacterial infections, and less frequently by agents such as yeasts, fungi and algae (Karimuribo *et al.*, 2008). Bacterial pathogens that cause mastitis are generally classified as either contagious or environmental based upon their primary reservoir and mode of transmission. The primary reservoir of contagious mastitis pathogens is the udder of the cow, and they are commonly transmitted among cows by contact with infected milk. The most common mastitis pathogens previously reported in Tanzania are Gram-positive bacteria, with *S. aureus* being the most prevalent (Mdegela *et al.*, 2004).

Mastitis can occur in either clinical or subclinical forms; clinical mastitis is characterized by changes in the udder and milk that are directly observable, whereas the subclinical disease is characterized by an increase in somatic cells in the milk, and the absence of clinical signs (Karimuribo *et al.*, 2008). Although

mastitis occurs sporadically, it assumes a major economic importance in dairy cattle. Losses attributed to mastitis include reduced milk yield, milk discard, premature culling, treatment costs, and increased labour (Fetrow, 2000). The use of dry cow therapy, post milking teat disinfectants, and effective pre-milking hygiene are effective control procedures for most contagious mastitis pathogens (Kilango, 2011).

Exposure to environmental mastitis pathogens may occur continuously because the primary route of exposure is contact with moisture, mud, and manure. Unlike mastitis caused by contagious pathogens, mastitis caused by environmental pathogens cannot be eradicated from a dairy herd (Smith and Hogan, 1993). The most important environmental mastitis pathogens include gram-negative bacteria (such as *E. coli* and *Klebsiella* spp.) and *Streptococcus* spp. (such as *Strep. uberis* and *Strep. dysagalactia*). Mastitis caused by environmental pathogens can be controlled by reducing exposure and by increasing immune resistance of the cow (Kilango, 2011).

2. 9. Food Safety Along Dairy Value Chains

There are different passages or outlets of dairy value chains through which milk products flow from the producer to the consumer. On the way to the consumer, the product change ownership from time to time among the milk-marketing participants (Kohls and Uhl, 1990).

This has implications on quality of milk and transaction costs as well as potential risk of contamination with pathogens. However, an understanding of functional market chains is an important first step towards understanding /dealing with food safety risks (Kilango, 2011).

2. 10. Dairy Production System in Ethiopia

In Ethiopia, milk production systems can be categorized into urban, peri-urban and rural, based on location (Redda, 2001). Located around Addis Ababa and regional towns, urban and peri-urban systems are market oriented and make use of the high

demand in urban areas. The rural system is part of the subsistence farming system and includes pastoralists, agro-pastoralists, and mixed crop-livestock producers mainly in the highlands. As this system is not market oriented, most of the milk produced is retained for home consumption. The surplus is mainly processed using traditional technologies into more shelf stable products such as Ergo (Ethiopian naturally fermented milk), butter, ghee and Ayib (Ethiopian cottage cheese) that are marketed through the informal channel (Redda, 2001).

In Sub Saharan countries the traditional sector, which is characterized by small herd size dominated by indigenous zebu breeds of low milk production with very little or no specialized inputs, is the dominant type of production system accounting to 70 - 80% of Africa's cattle population (Ibrahim and Olaloku, 2000). In Ethiopia, around 97% of the annual milk production is accounted by the traditional milk production system (Felleke, 2003), which is likewise dominated by indigenous breeds. Most of the milk produced in the country is accordingly processed on-farm using traditional technologies that are generally not well understood. Most of the very few enterprises currently operating in and around the capital entirely depend on the traditional sector for their milk intake, while others depend on it for the majority of their intake. These underscore the importance of understanding the traditional sector in order to make improvement interventions (Yilma, 2012).

Cows contribute to about 95% of the total annual milk produced by cows and camels at national level (CSA, 2010). In 2010, the cattle population was estimated at about 50.9 million (99.19% indigenous, 0.72% hybrid and 0.09% pure exotic breeds). The female cattle population accounted for about 55% of the total. The large livestock population; the favourable climate for improved, high-yielding animal breeds; and the relatively animal disease-free environment make Ethiopia to hold a substantial potential for dairy development (Yilma, 2012). In 2010, a total of 2940 million litters of milk was produced from about 9.6 million cows at national level. During the same year, dairying has created an estimated 588,000 full-time on-farm jobs. However, Ethiopia is a net importer of dairy products with import values significantly exceeding export values. In five reference years, for instance, export values increased from about 73000 USD in 2005 to 123000 USD in 2009, while import values increased from

about 5.6 million USD in 2005 to about 10.3 million USD in 2009 (4.7 million USD increment) (Yilma, 2012).

2. 11. Public Health and Economic Importance

Staphylococcal infections are frequent but are usually contained by immune mechanisms to the site of entry. The highest incidence of disease usually occurs in people with poor personal hygiene, overcrowding and in children (Rho and Schaffner, 2007). In developing countries, the surveillance system of FBD hardly exists and it is therefore, difficult to estimate the real magnitude of the problem (Hocking and Doyle, 1997). 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 (Walderhaug, 2007).

Milk, either raw or processed, is a well known vehicle of a number of human pathogens. Milk and milk products have, therefore, pose a health risk to consumers if it is contaminated by any pathogens and subjected to temperature abuse where these organisms can multiply to high counts and may produce toxins (Radostits *et al*., 1994). In countries where foodborne illness are investigated and documented, the relative importance of pathogens like *S. aureus*, *E. coli*, *Salmonella* species and *Listeria* species is well known (Godefay and Molla, 2000).

Staphylococcus aureus is the leading cause of foodborne illness throughout the world. Milk and milk products can became contaminated unless good hygiene (including mastitis) control occurs on farms, the milk is adequately pasteurized, and precautions are taken to prevent contamination and subsequent growth of staphylococci during the manufacturing process and the finished product. The pathogenicity of *S. aureus* has been recognized for many years and it may cause mastitis or skin disease in milk producing animals or lead to foodborne intoxication in milk and milk products (Asperger, 1994). Human carriers can also contaminate milk. Five serologically distinct enterotoxin (A, B, C, D, and E) are

recognized, with enterotoxin A most frequently involved in food poisoning outbreaks. The minimal intoxication dose is 100 nanogram and sometimes less (Asperger, 1994).

The change in food supply, the identification of new FBD, and the availability of new surveillance data have changed the morbidity and mortality figures (Loir *et al*., 2003). A study from the US Centres for Disease Control and Prevention (CDC) reports that FBD cause approximately 76 million illnesses, 325,000 hospitalizations, and 5000 deaths and costs annually 5-6 billion USD in the United States each year (Jay, 2000). Identified pathogens account for an estimated 14 million illnesses, 60,000 hospitalizations, and 1800 deaths. *Salmonella, Listeria*, and SFP organisms are responsible for 1500 deaths. Unidentified pathogens account for the remaining 62 million illnesses, 265,000 hospitalizations, and 3200 deaths. Overall, FBD appear to cause more illnesses but fewer deaths than previously estimated (Baron, 2007).

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 immunocompromised persons, elderly persons and children (Aycicek *et al.*, 2005; Kerouanton *et al.*, 2007).

The public health standard for milk ordinance provides chemical, bacterial and temperature standards as well as sanitation requirements for production and processing of grade "A" raw and pasteurized milk and milk products. Some processors may often monitory incentives for producers to meet more stringent standards to improve milk quality (Hagstad and Hubbert, 1986).

The microorganisms present can originate from interior of the udder, its exterior and/ or milking equipment. High initial microbial count in milk of $>10^5$ cfu/ml in evidence of serious faults in milk production hygiene, whereas production of milk having counts consistently below 10^5 cfu/ml reflects good hygiene practices (Ombui *et al*., 1995). A standard plate counts of $1x10^5$ cfu/ml has been widely adopted for good quality raw milk intended for treatment before liquid consumption. However, some other countries have adopted different standards suited to local conditions. For

example, the standard plate count for America is no more than $3x10^5$ cfu/ml, while the standard for Kenya is no more than $2x10^6$ cfu/ml (Ombui *et al.,* 1995). The standard plate count for pasteurized milk should be less than 30,000 cfu/ml (Saskatchewan, 1997).

Coliforms can rapidly build up in moist, milky residues in milking equipment, which then becomes the major source of contamination of milk produced. Coliform counts regularly in excess of 150 cfu/ml are considered generally as evidence of unsatisfactory production hygiene. However, relatively low coliform counts in milk don't necessarily indicate effectively cleaned and disinfected equipment (Ombui *et al*., 1995).

2. 12. Antimicrobial Resistance

Antibiotics have been broadly used in farm animals for the purpose of antimicrobial therapy, prophylaxis and growth promotion (Mathew *et al*., 2007; Waters *et al.,* 2011). This increasing handling of antibiotics has led to a worldwide problem in the development of antibiotic resistance among bacterial populations during recent decades (Shryock and Richwine, 2010). The improper antibacterial treatment and overuse of antibiotics for agricultural purposes have contributed to the increased incidence of multiple antibiotic resistances in farm animals (Ding and He, 2010; Shryock and Richwine, 2010). Introduction of antimicrobials such as tetracycline, aminoglycosides and macrolides into mastitis therapy has been accompanied by the emergence of corresponding resistance in bovine *S. aureus* strains (Myllys *et al.,* 1998). Acquisition of modified penicillin-binding proteins (PBP) has made some *S. aureus* strains resistant to all β-lactams. This property, called methicillin resistance, has been uncommon among bovine *S. aureus* isolates to date (Monecke *et al*., 2007). The recent emergence of vancomycin resistant *S. aureus* strains in human medicine has made control of *S. aureus* infections increasingly difficult (Jones, 2008).

Staphylococcus aureus exhibits resistance to a wide range of antimicrobial agents including disinfectants (Bjorland *et al.,* 2001). Staphylococcal infections are frequently treated with antibiotics and consequently resistance to it and or

acquired resistance develop (Normand *et al.,* 2000). Currently, medical attention focuses to both coagulase-positive and coagulase-negative staphylococci because they represent a serious therapeutic problem. Moreover, they may develop multi antimicrobial resistance (Normand *et al.,* 2000; Blahova *et al.,* 2004; Authier *et al.,* 2006). In recent years, much has been written about the emergence of methicillin-resistant *S. aureus* and methicillin-resistant coagulase-negative staphylococci (Normanno *et al.,* 2007; Leonard and Markey, 2008; Springer *et al*., 2009; Weese, 2010; Huber *et al*., 2011).

2. 13. Management Strategies

2. 13. 1. Treatment

Many approaches have been taken to the treatment of *S. aureus* mastitis including intramammary antimicrobial therapy, systemic antimicrobial therapy, and vaccination in conjunction with antimicrobial therapy. Cure rates have ranged from 3-74% depending treatment product, length of treatment and whether treatment was administered during lactation or during the dry period, or in the case of heifers, shortly before calving (Barkema *et al.,* 2006). Barkema *et al.* (2006), in their review concluded that "the probability of cure depends on cow, pathogen, and treatment factors". Cure rates are impacted by increasing cow age, increasing SCC (Somtic cell count), increasing chronicity of infection, increasing bacteria counts, and increasing numbers of mammary quarters infected. They concluded that "the most important treatment factor affecting cure was treatment duration". Further, they suggest "treatment of young animals with penicillin-sensitive *S. aureus* infections is often justified based on bacteriological cure and economic outcome, whereas treatment of older animals, chronic infections, or penicillin-resistant isolates should be discouraged." Similarly, Roy and Keefe (2012) in a systematic review of the literature on the treatment of *S. aureus* mastitis during lactation concluded that extended intramammary therapy for 5-8 days was the best therapeutic option. However, this recommendation should be tempered with the knowledge that extended therapy regimens can be associated with clinical mastitis with secondary organisms such as yeast and coliforms in some cases (Middleton and Luby, 2008; Roy and Keefe, 2012). Furthermore, overall long-term cure rates in some herds even with 8

days of intramammary antibiotics can be quite poor. In one study, Middleton and co-workers, showed that while cure rates were quite high 4 days after treatment (80%), by 28 days post-treatment most infections had recrudesced with the overall cure rate dropping to 29% (Middleton *et al.*, 2007).

Pre-partum treatment of heifers with intramammary antibiotics has been extensively studied. Generally heifers are treated with an intramammary antibiotic 2-4 weeks prior to parturition in an attempt to clear IMI caused primarily by coagulase negative staphylococci, but some efficacy has been noted against *S. aureus*. While cure rates can be quite promising, the impact of such therapy on 1st lactation performance including milk production and SCC over no treating varies from herd to herd with some herds showing no benefit (Middleton *et al*., 2005).

2. 13. 2. Prevention and control

Principles of control of staphylococcal mastitis should be focused on three areas: preventing new infections, elimination of existing infections and monitoring progress after implementation (Kaloreu *et al*., 2007). The single most important step in preventing new infections is to dip each quarter of every cow after milking with an effective teat dip. Dry cow treatment is an essential step in eliminating existing infections, and is also a step, which can reduce new infections by 50-75% (Jones, 1998).

Control is both important and difficult as *staphylococci* can persist for months in dust, curtains and human carriage is often permanent. Reservoirs and routes of spread differ, so different measures are appropriate in different circumstances. Prevention is much concerned with the destruction of the bacteria and with the inhibition of growth (Loir *et al*., 2003; Baron, 2007; Chiang *et al.*, 2008).

Effective methods for preventing SFP are aimed at eliminating food contamination through high standards of personal hygiene to prevent food contamination by food handlers. This is through public education in relation to hand washing, wearing gloves during food preparation and storing foods at proper temperature to inhibit growth or destroy the pathogen and minimize toxin production as heating food after toxin is

formed will not be an effective control measure. Moreover, persons with lesions containing purulent exudates should not be permitted to handle food until proper medical advice is sought. In general, measures such as serving hot meal immediately after cooking, reheating cooked foods thoroughly, rapid refrigeration of cooked foods, proper washing of hands before and after food preparation, avoiding food service worker with skin infections in food establishments and using clean utensils and equipments will certainly reduce the incidence of food poisoning outbreaks due to *Staphylococcus* (Jay, 2000; Acco *et al*., 2003; Baron, 2007).

3. MATERIALS AND METHODS

3. 1. The Study Area

The study was conducted in Sebeta town from November 2014 to March 2015. Sebeta is a town and also separate district, located in the Oromia Special Zone surrounding Finfinne (Addis Ababa) of the Oromia Region. Sebeta town is located in Oromia regional state 24km south west of Addis Ababa at a geographical coordinate of $8^0$55'N $38^0$37'E latitude and 8.917^{0}N 38.617^{0}E longitude and an altitude of 2780m.a.s.l. Sebeta has total area of 102,758km^2 and the annual mean rainfall registered in area ranges from 860 to 1200 mm (CSA, 2013). The minimum and maximum mean temperature ranges from11.3 ^{0}C to 28 ^{0}C with relative humidity of 49.3%. The population and housing census of CSA of 2010 estimated the total population of Sebeta town to be 61,461. Majority of the dairy farms in the area are kept under small holder intensive farms and animal products, especially dairy products, play a headstone role in household food security both by direct consumption and purchasing of other food items in the area (CSA, 2011).

3. 2. Study Population

The study population consisted of all dairy cows, farm owners, milk collectors, milkers' and staff member of processing plant found in Sebeta town.

3. 3. Study Design

A cross-sectional study was conducted from November 2014 to April 2015 to asses to asses contamination of milk with *Staphylococcus aureus,* milk consumption habit and handling practices in Sebeta town.

3. 4. Sample Size Determination and Sampling Technique

Milk samples were taken from individual cow's, storage tanks and processing plant. Swab samples were taken from milking buckets and milkers for isolation and identification of *S. aureus*. To calculate the total sample size required at farm level, the following parameters were pre-determined: 95% level of confidence (CL), 5% desired level of precision, expected prevalence of 16.2% (Mekuria *et al.*, 2013) reported in and around Addis Ababa, Ethiopia. The sample size was determined using the formula for recommended by Thrusfield (2005).

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

n = required sample size, Pexp = expected prevalence, d=desired absolute precision Accordingly, the study involved 209 cow milk samples. A total of 52 swab samples were taken purposively from milking buckets and hands of milkers' each accounted for, 27 and 25 samples, respectively. Twenty tank milk samples were sampled based on the number of tanks per collection centers from four MCCs purposively from a total of 19 MCCs (Table.1). The main reason for doing it was these centers supply milk both for the consumers and processing plants and the remaining centers directly supply to the processing plant. Similarly, 10 pasteurized milk samples from the processing plant were sampled purposively to assess pasteurization survival rate of *S. aureus*.

Simple random sampling technique was used to select 23 small holder dairy farms and lactating cows and purposive sampling technique was used to sample from processing plant, milking buckets, milkers from the farms and milk collection center. A total of 110 individuals from the town were included in the study to assess the milk consumption habit and handling practices: dairy farm owners (23), processing plant worker (1), milk collectors (19), hotel/Cafeteria workers (17) and consumers (50). Interview using pretested questionnaire was used to generate the data.

Table 1. Number of milk samples collected from milk collection tanks at collection centers.

Collection centers	No of tanks	No of raw bulk milk
Center 2	4	4
Center 3	3	3
Center 5	6	6
Center 7	7	7
Total	20	20

3. 5. Sample Collection and Transportation

Procedure for collection of milk was according to Quinn *et al.* (2002); strict aseptic procedures were adopted when collecting milk samples in order to prevent contamination with microorganisms present on the body of animal and from the barn environment. Cows udder were first cleaned with soap then dried with clean towels and teats were cleaned, disinfected with 70% alcohol before swabbing. Swabs from hands of the milking personnel and milking bucket were collected using sterile, cotton-tipped swabs. Samples were taken from the hands of the milkers' by rotating the swab 360^{o} between the bases of the fingers of both hands. Swab samples were transported by sterilized universal bottles containing 9 ml of peptone water. The first stream of milk from each quarter was discarded. Then, about 2 ml milk from each cow was taken into sterile universal bottles. Briefly, milk samples in the bulk containers were agitated before collection, and samples taken from the top of the bulk tank using a sanitized dipper from MCCs. Pasteurized milk samples were purchased from the milk processing plant and immediately transported to laboratory. After collection of the milk sample, all samples were clearly labeled with the appropriate identification of samples were made by date of collection and sources of the milk, using permanent marker on the test tube. All samples were transported with in an icebox containing ice packs and taken to the Laboratory of Microbiology at the College of Veterinary Medicine and Agriculture of Addis Ababa University, Bishoftu, without delay for microbiological analysis. Upon arrival, the samples were stored overnight in a refrigerator at +4°C until examined the next

day. The bacteriological media used in different stages were prepared according to the manufacturer's recommendations.

3. 6. Isolation and Identification

Isolation and identification of *S. aureus* was conducted in the Microbiology Laboratory of the College of Veterinary Medicine and Agriculture of Addis Ababa University. The bacteriological culture was performed following the standard microbiological technique recommended by Quinn *et al.* (1999). A loopful of milk was streaked on sterile 5% sheep blood agar and swab samples were streaked on medias by using cotton applicator and the plates were incubated aerobically at 37^{o}C and examined after 24-48 hrs of incubation for growth. The colonies were provisionally identified based on morphology characteristics, hemolytic pattern and Gram's staining reaction. The representative colonies were sub cultured on nutrient agar plates and incubated at 37^{o}C for 24 hrs. Pure colonies were preserved and maintained on nutrient slants for further characterizing the isolates.

3. 6. 1. Catalase test

Pure culture of the isolates were picked using a sterile loop from the agar slant and mixed with a drop of 3% H_2O_2 on a clean glass slide. If the organism was positive, bubbles of O_2 were liberated within a few seconds and the catalase negative isolates did not produce bubbles. The catalase positive cocci were considered as staphylococci

3. 6. 2. Coagulase test

The tube coagulase test was performed in sterile tubes by adding 0.5 ml of selected isolates of *Staphylococcus* grown on tryptone soya broth (TSB) at 37^{o}C for 24 hours to 0.5 ml of citrated rabbit plasma. After mixing by gentle rotation, the tubes were incubated at 37^{o}C along with a negative control tube containing a mixture of 0.5 ml of sterile TSB and 0.5 ml of rabbit plasma. Clotting was evaluated at 30 minutes intervals for the first 4 hours of the test and then after 24 hours incubation. 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.

3. 6. 3. Mannitol salt agar

The colonies that were identified by Gram-staining reaction and catalase test as *Staphylococcus* were streaked on MSA plates and incubated at 37^{o}C and examined after 24- 48 hours for growth and change in the colour of the medium. The presence of growth and change of pH in the media (red to yellow colour) were regarded as confirmative identification of staphylococci. Phenol red pH indicator detected the acidic metabolic product of mannitol. Fermentation of mannitol by *S. aureus* causes yellow discoloration of the medium. Colonies that develop weak or delayed yellow colour after 24 hours of incubation were taken as *S. intermedius* and colonies that failed to produce any change on the medium were considered as *S. hyicus* and CNS.

3. 6. 4. Purple agar base

Purple agar base (PAB) with the addition of 1 % 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^{o}C for 24-48 hours. The identification was based on the fact that *S. aureus* rapidly ferment maltose and the acid metabolic products cause the pH indicator (bromocresol purple) to change the medium and colonies to yellow. *S. intermedius* gives a weak or delayed reaction and *S. hicus* did not ferment maltose but attacks the peptone in the medium producing an alkaline reaction (deeper purple) around the colonies.

3. 7. Antimicrobial Susceptibility Test

Antimicrobial susceptibility testing of isolates was performed using disk diffusion method on Muller-Hinton agar plates as per the National Committee for Clinical Laboratory standards (NCCLS, 2002). Single colony was selected and emulsified in 3ml sterile normal saline solution in a sterile test tube. The turbidity of the suspension was then adjusted to the density of a barium chloride standard (0.5 McFarland) in order to standardize the size of inoculums. A sterile cotton swab was dipped into the

standardized suspension of the bacterial culture, squeezed against the sides of the test tube to remove the excess fluid and inoculated onto Mueller-Hinton agar and allowed to dry the flood. Thereafter, antimicrobial discs were placed on the agar with forceps and gently pressed down to ensure contact. The plates were then allowed to stand for 30 minutes for diffusion of active substance of the agents. Plates were inverted and incubated at 35-37^{o}C for 24 hrs. An inhibition zone diameter of each antimicrobial was then measured and interpreted as 'Resistant', 'Intermediate' and 'Sensitive' by comparing with recorded diameters of a control organism, ATCC25923 (CLSI, 2007). The antimicrobial agents tested were the following vancomycin (30µg), penicillin G (10IU), tetracycline (30µg), sulphamethoxazole (25µg), ciprofloxacin (5µg), nalidixic acid (30µg), amoxicillin (10µg), cefoxitin (30µg), erythromycin (15µg), and streptomycin (10µg).

3. 8. Data Management and Analysis

Data was entered into excel spread sheet and analyzed using SPSS statistical software version 20. Frequency tables and graphs were used to present the data. Chi-square test and descriptive statistics were used to analyze the data. The significance level was set at α=0.05.

4. RESULTS

4. 1. Milk Value Chain

The milk value chain in Sebeta town involves various routes in which milk from farms reaches to the consumers. Actors in the milk value chain include farm owners, milk collectors and processing plant. Milk consumers can get access to raw milk from farm and milk collection centers. The following figure depicts the milk value chain, possible sources of contamination and handling practices of the milk at each steps leading to exposure to the consumers in the area (Figure 1).

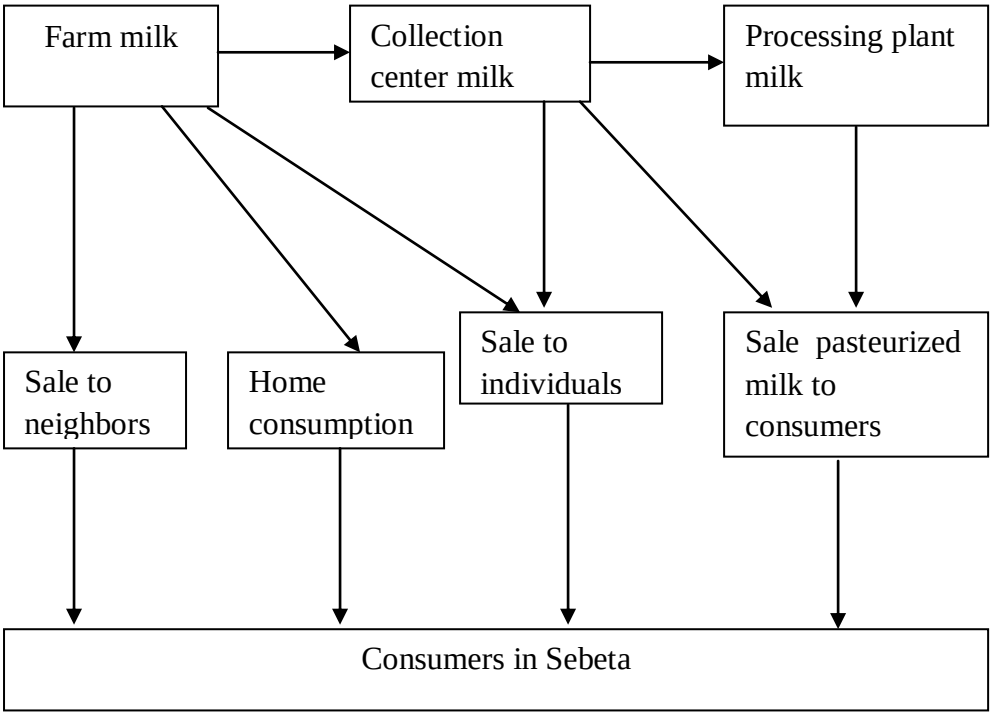

Figure 1. Milk value chain of sources of contamination in Sebeta Town.

4. 2. Prevalence of *Staphylococcus aureus*

Attempt was made to isolate and identify *S. aureus* from 209 cow milk, 20 tank milk, 10 pasteurized milk, 27 milk bucket and 25 milk handlers' hands. A total of 68 *S. aureus* isolates were identified from all the samples considered in this study.

The frequency of isolation of *S. aureus* varied and ranged from 19.6% (41/209) and 80% (16/20) from farm milk and tanks milk of MCCs, respectively. The prevalence *of S. aureus* from milk samples taken from individual cows was 19.6% (95% CI: 14.5-25.6).

Among the 20 raw milk samples taken from MCCs, 80% (16/20) (95%CI:56.3-94.3) of them were found to be contaminated with *S. aureus*. Among the four collection centers, high contamination rate (100%) was observed at milk collection center 3 and 7. There was statistically a significant difference in the isolation among the milk collection centers (χ^2= 60.00, df=3, p=0.000) (Table 2).

Table 2. Proportion of *S. aureus* isolated among different milk collection centers

Collection centres	**Sample**	**Positive**	**Percentage (%)**	**χ^2**	**P-value**
Centre 2	4	2	50	60.000	0.000
Centre 3	3	3	100		
Centre 5	6	4	66.7		
Centre 7	7	7	100		
Total	20	16	80		

The study showed a relatively higher contamination rate of *S. aureus* in milk samples taken from MCCs than that of the farms. There was statistically a significant variation in the isolation rate of *S. aureus* between farms and milk collection centers (χ^2= 35.599, df=1, p=0.000) (Table 3).

Table 3. Proportion of *S. aureus* isolated at farm and milk collection centers

Source of samples	Samples	Positive	Percentage (%)	χ^2	P-value
Farm milk	209	41	19.6	35.599	0.000
MCCs milk	20	16	80		

S. aureus was found in 32% (n=8) of the total 25 swab samples taken from the hands of milkers. Among the 27 milking bucket swab samples, 11.1% (n=3) yielded *S. aureus* (Table 4). *S. aureus* was isolated from none of the pasteurized milk samples taken from the Processing plant.

Table 4. Proportion of *Staphylococcus aureus* isolated from the potential sources of contamination.

Type of samples	Samples	Positive	Percentage (%)
Milker's hand	25	8	32.0
Milking bucket	27	3	11.1

4. 3. Antimicrobial susceptibility

All the 68 isolates of *S. aureus* were tested for their susceptibility to 10 selected antibiotics. The highest sensitivity in ciproflaxin (82.4%), followed by SXT (67.6%) and naldixic acid (42.6%) was observed. The highest resistance was seen in cefoxitine (100%), penicillin G (98.5%), and streptomycin (77.9%), erythromycin(69.1%) and tetracycline (64.7%) (Table 5).

Table 5. The susceptibility pattern of *S. aureus* isolates

Antimicrobials	**Susceptible No (%)**	**Intermediate No (%)**	**Resistant No (%)**
Ciproflaxin	56 (82.4)	5 (7.4)	7 (10.3)
Naldixic acid	29(42.6)	27(39.7)	12(17.6)
Vancomycin	24(35.3)	0(0.0)	44(64.7)
Penicillin G	1(1.3)	0(0.0)	67(98.5)
Amoxicillin	10(14.7)	11(16.2)	47(69.1)
Cefoxitin	0(0.0)	0(0.0)	68(100)
Streptomycin	11(16.2)	4(5.9)	53(77.9)
Erythromycin	14(20.6)	7(10.3)	47(69.1)
SXT	46(67.6)	0(0.0)	22(32.4)
Tetracycline	24(35.3)	0(0.0)	44(64.7)

SXT- Sulphamethoxazole-Trimethoprim

Table 6. Antimicrobial susceptibility pattern of *S. aureus* isolates according to the types of samples

Antimicrobial	**Unit**	**Type of samples susceptible to different antimicrobial agents**											
		Farm milk No (%) n=41			Hand swabs of milker's No (%) n=8			Milking bucket swab No (%) n=3			MCCs No (%) n=16		
		R	I	S	R	I	S	R	I	S	R	I	S
Ciproflaxin	5 µg	3(7.3)	5(12.2)	33(80.5)	4(50)	0(0.0)	4(50)	0(0.0)	0(0.0)	3(100)	0(0.0)	0(0.0)	16(100)
Naldixic acid	30 µg	8(19.5)	23(56.1)	10(24.4)	2(25)	0(0.0)	6(75)	0(0.0)	2(66.7)	1(33.3)	2(12.5)	2(12.5)	12(75)
Vancomycin	30 µg	26(63.4)	0(0.0)	15(36.6)	4(50)	0(0.0)	4(50)	2(66.7)	0(0.0)	1(33.3)	12(75)	0(0.0)	4(25)
Penicillin	10 units	41(100)	0(0.0)	0(0.0)	8(100)	0(0.0)	0(0.0)	2(66.7)	0(0.0)	1(33.3)	16(100)	0(0.0)	0(0.0)
Amoxicillin	30 µg	25(60.9)	8(19.5)	8(19.5)	8(100)	0(0.0)	0(0.0)	2(66.7)	1(33.3)	0(0.0)	12(75)	2(12.5)	2(12.5)
Cefoxitin	30 µg	41(100)	0(0.0)	0(0.0)	8(100)	0(0.0)	0(0.0)	3(100)	0(0.0)	0(0.0)	16(100)	0(0.0)	0(0.0)
Streptomycin	10 µg	36(87.8)	0(0.0)	5(12.2)	4(50)	4(50)	0(0.0)	3(100)	0(0.0)	0(0.0)	10(62.5)	0(0.0)	6(37.5)
Erythromycin	15 µg	28(68.3)	5(12.2)	8(19.5)	4(50)	0(0.0)	4(50)	3(100)	0(0.0)	0(0.0)	12(75)	2(12.5)	2(12.5)
SXT	25 µg	15(36.6)	0(0.0)	26(63.4)	4(50)	0(0.0)	4(50)	1(33.3)	0(0.0)	2(66.7)	2(12.5)	0(0.0)	14(12.5)
Tetracycline	30 µg	28(68.3)	0(0.0)	13(31.7)	8(100)	0(0.0)	0(0.0)	2(66.7)	0(0.0)	1(33.3)	6(37.5)	0(0.0)	10(62.5)

Sulphamethoxazole-Trimethoprim (SXT) R= Resistant I=Intermediate S=Susceptible

4. 4. Questionnaire Interview Survey

Among the total of 23 dairy farmers, 57% (n=13) were illiterate and among this, 38.5% of them consume raw milk. About 35% of the farmers were found to consume raw milk (Table 7). (60.9 %), of the farmers were clean barn twice per day and 82.6% (n=19) didn't wash udder and teat and all of them didn't wash their hands using antiseptics but all were washed equipments with hot water and Ajax before milking (Figure 2 and Figure 3).

Table 7. Raw milk consumption habit of the farmers according to their education status.

Educational status	**Consumption of raw milk**		
	Yes	Total	Percentage %
Illiterate	5	13	38.5
read and write	1	5	20
Secondary education	2	2	100
collage and above	0	3	0
Total	8	23	34.8

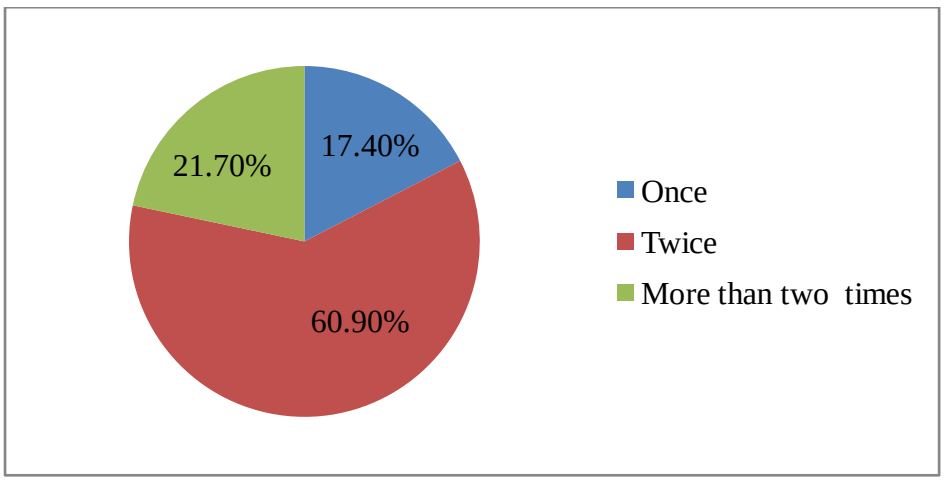

Figure 2.The frequency of barn cleaning practices by the farmers

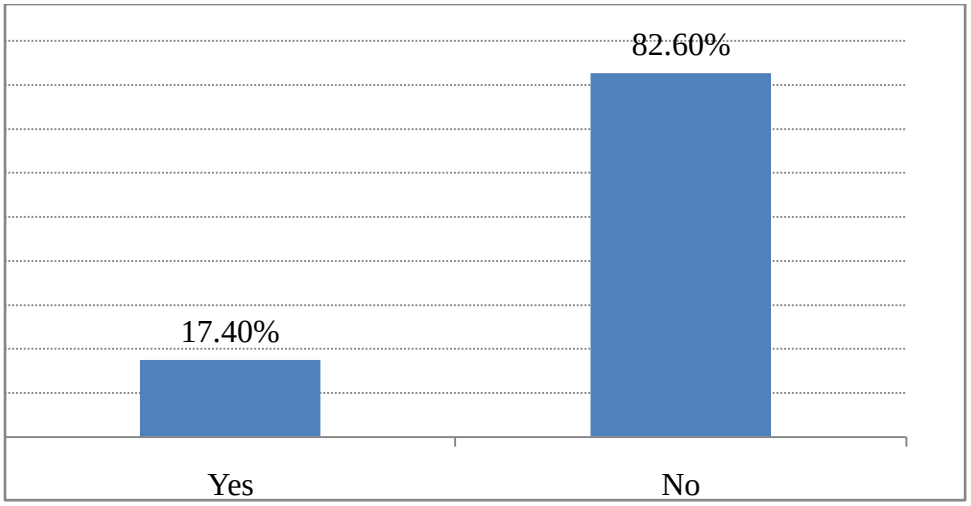

Figure 3.Udder and teats washing of practices of the farmers before milking

All of the farms were used plastic containers for both milking and storage. Only 13% (n=3) of the 23 dairy producers surveyed were aware of the occurrence of foodborne poisoning due to raw milk consumption and all the 23 producers had no knowledge of staphylococcal food poisoning associated with consumption of raw milk and milk products. Among 87 % (n=20) of farmers who had no awareness about food poisoning, 40 % (8/20) of them had habit of raw milk consumption (Table 8).

Table 8. Raw milk consumption habit of the farmers according to their awareness of food poisoning

Awareness of food poisoning	**Consumption of raw milk**			
	Yes	No	Total	Percentage (%)
Yes	0	3	3	0
No	8	12	20	40
Total	8	15	23	34.8

There were 19 milk collection centers in Sebeta town that collect milk from different dairy farms twice a day: morning time from 6:30-8:30 Am and evening time from 2:00-6:30 pm. Among these, 79 % (n=15) collection centers were owned by Sebeta Agro Industry. Most of the centers were located near the main roads made of concrete asphalt whereas the others were located some distances away from the main roads. All the milk collectors check for the quality of the milk prior to receiving the milk using lactometer reading and Alcohol tests. Stainless metallic cans for long distance and plastic containers for short distance were used by the 15 collection centers and the cans were cleaned with soda ash and hot water at the processing plant and dispatched to the collection centers by vehicles used for transportation of milk to the plant. All the milk daily collected at each center directly utilized for further processing by the processing plant. The other four centers not owned by Sebeta Agro Industry used Ajax and hot water to wash milking tanks and they sold raw milk to any consumers and processing plants.

The average daily quantity of milk bought by the consumers was1.5 liters. Among the consumers, 58% (n=29), 24% (n=12) and 18% (9) were buy milk from farms, cafeteria and from collection centers, respectively. Of the consumers, 66% (n=33) used plastic containers and 34% (n=17) metallic containers to transport milk to their homes. Ten percent were keep milk in a refrigerator while 90% (n=45) of them keep milk at room temperature. Of the consumers, 88 % (n= 44) consume boiled milk and the other 6% (n=12) consume milk in the form of raw milk and raw milk products like yogurt. Out of 50 consumers 54% (n=27) were don't have awareness about food poisoning associated with drinking of raw milk.

All hotels/ cafeterias/ restaurants (n=17) were buy raw milk from farms about 15 to 25L per day twice a day (morning and afternoon). Among them, 29% (n=5) use metallic container while 71% (n=12) use plastic containers for transportation. The respondents indicated that they use different methods of quality assessments like boiling (47%) and visualizing and smelling 5 (29.4%) before purchasing milk. The rest 23.6% (n=4) of them directly buy the milk without undertaking quality assessment. Milk was found to be kept in a refrigerator by all hotels until

consumption. Seventy seven percent of the respondents had awareness about the occurrence of food poisoning associated with drinking of raw milk.

Sebeta Agro Industry processing plant is a modern milk processing plant located in Sebeta town. On average, 6300 L milk was collected and processed per day. Milk from collection centers were further subjected to lactometer reading and alcohol test prior to transportation to the plant. The arrival time of milk to the plant was 8:30 AM in the morning and 6:30 PM in the evening. Laboratory tests (Petri film paper test) were conducted immediately up on arrival. Stainless metallic and plastic containers were used for long distance and short distance during transportation. All the milk tested and accepted were processed to produce pasteurized milk, cheese and butter per day. Milk that undergone fermentation due to long distance were used only for the production of cheese. Plastic and aluminum covered cartons were used for packaging of pasteurized milk.

5. DISCUSSION

In this study, from 209 lactating caw milk samples subjected to bacteriological examination, 19.6% (41/209) were found to be positive for *S. aureus.* This finding is in agreement with the findings (21.13%) observed in Addis Ababa by Abunna *et al.* (2013). However, the present finding was similar with reported by Mekuria *et al.,* (2013) in which he reported 16.2% in and around Addis Ababa, Ethiopia.

In the present study, 80% (16/20) milk tank samples from MCCs was found to be contaminated with *S. aureus*. The results showed a highly contaminated in tanks milk of MCCs with *S. aureus* than farm milk samples. There was statistically significance between them. This might be attributed to cross contamination of milk while bulking and poor handling across the milk value chain. Also the number of personnel working at MCCs were higher which might have contributed to milk contamination (Addis *et al*., 2011). The contamination of *S. aureus* at collection centers was nearly in agreement with the previous work (Wubete, 2004) and (Desissa, 2010) where *S. aureus* was isolated at recovery rate of 75% and 72% respectively. This findings were disagree with (Addis, 2009) 46% of *S. aureus* were isolated from milk from tanks.

The present study found absence *S. aureus* from pasteurized milk samples. This might show that *S. aureus* were inactivated during pasteurization process and absence of post pasteurization contamination. *Staphylococcus* species can indeed be easily eliminated from foods by heat treatment (in pasteurized foods) or by competition with other flora (in fermented foods) (Addis, 2009).

The isolation of *S. aureus* from hand's of the milker's and bucket milk were 32% and 11.1%, respectively. These indicates that milk handlers and milk buckets were the potential sources of contamination of milk with *S. aureus*. The isolation rate from mikers hand was relatively in agreement with the prevalence rate reported by Deandrade and Zelante (1989) and Tondo *et al.* (2000) whose results were 35.7% and 35.2%, respectively. In contrary, lower percentages of 11.67 and 4.2 were previously recorded by Samaha *et al*. (2004) and El- Khawas and Amani (2008), respectively. This may be attributed to staphylococci are ubiquitous organisms and at least 50% of

individuals carry the organism in their nasal passages, throat and through coughing or sneezing they contaminate their hands (Gwida and EL-Gohary 2013) and also may be due to variation in milk handling and washing practices of hands, buckets and teats/udder before milking.

Antibiotic therapy is an important tool in the treatment of *S. aureus* related infection and poisoning. However, the misuse or intensive use of antibiotics can lead to the development of resistance among different bacterial strains (Radostits *et al.*,2000). The antimicrobial susceptibility tests carried out in this study indicated the existence of susceptibility and resistance of *S. aureus* to some of the antimicrobials. This study presents the sensitivity of the *S. aureus* isolates towards life saving drugs, ciproflaxin (82.4%), SXT (67.6 %) and naldixic acid (42.6%). However, these isolates were highly resistant to cefoxitin (100%) which was used in both dogs and cats to treat a variety of bacterial infections (Authier *et al*., 2006), Penicillin similarly, the present investigation indicated that the resistance pattern of penicillin was found to be (98.5%) which is similar to the finding (96.7%) made by Mekuria *et al*. (2013) and Tariku *et al*. (2011) (87.2%) in Ethiopia, Landin (2006) (80%) in Sweden, Gooraninejad *et al.* (2007) (57%) in Iran and Myllys *et al.* (1998) (50%) in Finland. This is in contrast to findings observed by Adesiyun (1994) who reported 23% of resistance to pencillin G in West India. Moreover, the present study showed the resistance of *S. aureus* to streptomycin (77.9%), erythromycin (69.1%), tetracycline (64.7%) followed by vancomycin (64.7%), but the present study disagree with the observation made by Abera *et al*. (2013) (72.4%) who said that vancomycine was sensitive for all gram positive and resistance for all gram negative. But agreed with Abera *et al*. (2013), tetracycline, penicillin and streptomycin were showed very poor efficacies on many isolates, where by only 20.6%, 51.7% and 44.8% respectively. Antibiotic resistance development among the bacteria poses a problem of concern. Effectiveness of current treatments and ability to control infectious diseases in both animals and humans may become hazardous.

Result from interviewee questionnaire showed that in farm milk production practices majority of farmers did not employ good milking practices to minimize contamination of milk on the farm. All of them were did not wash their hands with antiseptic solutions which is agreed with (Hofi , 2011) many farmers did not sufficiently clean

their hands before milking and the udder before milking. This practice and the prevalence of *S .aureus* on milkers hand might contribute to milk contamination. Yet pre milking udder preparation plays an important role in the contamination of milk during milking. Lack of potable water, antiseptic solutions and use of detergents was a major constrain to hygienic practices on the farms. Most of the farmers clean barn twice a day.

In the present study all the farms used plastic containers both for milking and storage until sold. Plastic container have the characteristics that make them unsuitable for milk handling. Plastic containers scratch easily and provide hiding places for bacteria during cleaning and sanitization and plastic containers are poor conductor heat and hence will hinder effective sanitization by heat (Soomro *et al*., 2003). Plastic containers are not recommended for handling milk as they are known to be vulnerable to bacterial contamination. Milk handling problems coupled with lack of quality assurance of milk delivered to most of the retailers and household consumers pose potential sources of public health risks to consumers (Gratian, 2011). Omore *et al.* (2005) reported that the use of plastic containers was associated with high coliform counts in raw milk. This is likely due to the fact that plastic containers are difficult to clean and sterilize (Gratian, 2011).

Among the farmers 34.8 % (n=8) have a habit of drinking of raw milk and didn't have awareness about diseases which can be transmitted by drinking of raw milk. A study in the USA also reported that 42.3% of dairy producers consumed raw milk (Jayarao *et al*., 2006). The remaining didn't drink raw milk 65.2% of these 20% do have awareness of food poisoning and 80% don't have awareness. Though the result showed relatively a lower percentage of raw milk consumers, still these individuals are at a greater risk of contracting staphylococcal intoxication than those who do not consume raw milk.

All the milk collectors check for the quality of the milk prior to receiving the milk using lactometer reading and alcohol test to test adulteration with water and freshness. Steeliness metal for long distance and plastic containers for short distance were used for collection of milk. The cans were cleaned with hot pipe water and soda

ash before starting of the collection and dispatched to the collection centers by vehicles used for transportation of milk to the plant.

Consumers form the last group of the food chain i.e. farm to fork and therefore they are at risk of any malpractice occurring in the chain. In the current study among the consumers which were found in Sebeta town, 72 % n=36 consume boiled milk and other 28 % n=14 consume in the form of raw milk and raw milk products like yogurt. 66% (n=33) used plastic containers and 34% (n=17) metallic containers to transport milk to their homes. 27 (54%) of the consumers were don't have awareness and 23 (46%)have awareness about FP/GIT disturbance associated with drinking of raw milk. Findings of this study showed that the level of knowledge and awareness of health risks associated with drinking milk was high when compared with results reported by Karimuribo and co-workers (2005) who found 20.6% out 96 people when doing a similar study. Most of them reported stomach problems/diarrhoea as major health risk one can encounter from drinking milk. This could be the reason why most of them prefer boiled milk served hot, as they believe boiling of milk kills most of pathogenic bacteria.

All hotels and cafeterias were check the milk prior to purchase ; by boiling 47% (n=8) similar with (Gratian, 2011) 40.9% (n=9); other respondents said, they know whether it is pure milk or not just by seeing and smelling 17.6% (n=5) this finding was in agreement with other studies about 57.1% (n=7) of vendors used viscosity and color as main technique for assessing quality of milk before buying (Gratian, 2011); others purchased from honest customers they never test it 23.6% (n=4). This observation disagreed with the findings reported by Omore and others (2003), who found that 58% of milk traders in Tanzania did not do any quality control before purchasing of milk.76.5% (n=13) have awareness and 23.5% (n=4) don't have awareness about FP/GIT disturbance associated with drinking of raw milk, especially about staphylococcal poisoning in relation to unhygienic handling of milk and milk products in the studied area.

6. CONCLUSION AND RECOMMENDATIONS

The present study revealed the contamination of milk with *S. aureus* at farms and MCCs and on milker's hand and milk buckets. Lactating cows, milker's hand and milk buckets were found to be the potential sources of milk contamination with the agent. High contamination rate of the milk with *S. aureus* at MCCs indicated the possibility of an increase in contamination rate along the milk value chain in its way from farms to the processing plant. In nut shell, the study showed poor handling practices of milk, raw milk consumption habit, resistance of *S. aureus* to commonly used antibiotics in animals and humans, and lack of awareness about staphylococcal food poisoning implicating for public health issue in the Sebeta town.

Based on the above conclusion, the following recommendations were forwarded:

- Training on good animal husbandry, milk hygiene, and milk handling practices should be given to all actors in the milk value chain regularly by concerned body.
- Collaborative awareness should be created about the occurrence of food borne diseases in general and staphylococcal food poisoning in particular that might associated with raw milk consumption.
- Regular antimicrobial sensitivity testing ought to be done to reduce the development of drug resistance towards commonly used antibiotics
- Further study should be conducted to identify the toxigenic strains of *S. aureus*, production and concentration of eneterotoxins at each milk value chain, and estimate the risk of consuming milk contaminated with *S. aureus* in the study area.

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8. ANNEXES

Annex 1. Flow chart of the ISO 6888-3 protocol

- Isolation and identification of *Staphylococcus*
- A loopful of the milk samples were streaked aseptically onto sterile blood agar media and incubated at 37^0C for 24-48 hours under aerobic culture conditions.
- Colonies were 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% H_2O_2 (positive)
- Inoculate colonies on MSA and incubate at 37^0C 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. intermidius* and *S. hicus*) from CNS.

Inoculate CPS isolates on PAB media plate with 1% of maltose and incubate at 37^oC 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 49 change the medium and colonies to yellow. *S. intermedius* gives a weak or delayed reaction and *S. hicus* did not ferment maltose.

Annex 2. Sample collecting sheet for bacteriological analysis

S.N	Date of collection	Type of samples collected	Source	Number of samples examined	Sample code

Annex 3. Laboratory data collecting sheet

Isolation and identification							
S.N	Sample code	Type of sample	Gram stain	Catalase test	Mannitol Salt agar	Coagulase test	Purple agar

Annex 4. Drug sensitivity test

Drug		Zone of inhibition (mm)	Result			Remark
S. No	Name		S	R	I	
1						
2						

S=Sensitive, I= Intermediate, R= Resistance

Annex 5. Differential tests used for identification of *S. aureus* from other *Staphylococcus* species

SN	Staphylococcus species	Haemolysis	Pigment production	Coagulase test	Fermentation of sugar	
					MSA	PAB
	S. aureus	+	+	+	+	+
	S. intermidius	+	-	+	±	±
	S. hicus	-	-	+	-	-
	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).

Annex 6. Questionnaires used to assess the milk handling and consumption practices of actors in milk value chain

Farm Questionnaire

Date_____ Sample number _____
Owner name :_____ Kebele _____

1. Educational status a) Illiterate b) Read and write c) Primary education d) Secondary education e) College and above

2. Occupation a) Gov't employer b) Merchant c) Farmer

d) Student e) Daily laborers f) House wife g) Others

3. Barn cleaning frequency per day _____ Bedding condition _____

4.Type of barn you own? a)Stanchion type b)Free

5. Do you wash udder or teats before milking? a)Yes b) No

5.1. If yes, udder or teat cleaning material
a) Warm water d) Cold tap water d) if any specify_____

6. Do milker's wash hands/milking equipments before milking? a)Yes b)No

6.1. If yes, hand cleaning mechanisms:
a) Tap water b) Detergent c) Antiseptics d) Other (specify) _____

7. Do have a habit of drinking milk? a) Raw b) Boiled c) Both d) none

8. Do you know a milk can be source of disease/infection? a)Yes b)No

Milk Collection Centers Questionnaire

Date: _____ Name: _____

1. Where do you collect the milk? How many liters do you collect a day?

2. How many days do you work/collect/week?

3. Do you test milk when you buy/collect? What kind of test?

4. What kind of containers do you use to collect/store milk?

5. Do you wash equipments? a) Yes b) No

6 .Which cleaning agent do you use?
a) Detergent b) Disinfectant c) Combination of the listed options
d) Other (specify) _____

7. How long the milk stay at centre? At what temperature?

8. Time of milk collection (morning and evening)?

9. How many liters of raw milk do you sell to individuals and others (e.g. hotels a day)?

10. Do you consume raw milk?

Milk consumers questionnaire

Date_____
Name:_____

1. Where do you get the milk? How many liters do you use a day?

2. What kind of containers do you use?

3. How long the milk stay at home prior consumption? At what temperature?

4. In what form do you consume (raw, boiled, others)? What is the rationale?

5. Do you know any food poisoning/GIT disturbance associated with drinking of raw milk?

Questionnaire for managers/coordinators of hotels and cafeterias

Date: _____

Name: _____

1. Where do you buy the milk?

2. How many liters do you buy a day?

3. What kind of containers do you use? When do you buy?

4. How long the milk stay prior sale? At what temperature?

Containers?

5. What kind of tests used to buy milk?

6. What form of milk consumption (raw, boiled, boil with coffee, yogurt etc)? What is the rationale?

7. Do you know any food poisoning/ GIT disturbance associated with drinking of raw milk?

Milk processor questionnaire (milk handling at processing plant)

Date: _____

1. What are method of milk transportation from collection centers?

2. How many liters do you collect from the centers? Quantity processed?

3. Arrival time of milk (morning, evening)

4. How long the milk stay prior processing? Storage tank and temperature?

5. What kind of tests used up on arrival?

6. What kind of packaging used for pasteurized milk?

7. Of the milk collected and supplied to the plant, how many percent are processed to produce pasteurized milk.

Annex 7. 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^{0}C

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

Blood agar (oxoid england)

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

Nutrient agar (oxoid, england)

Compositions:
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 ^{0}C
Instructions:
Suspend 28g in 1L of distilled water. Bring to the boil to dissolve completely. Sterilize by autoclaving at 121 ^{0}C for 15 minutes.

Mannitol salt agar (oxoid, england)

Compositions:
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 ^{0}C
Instructions:
Suspend111g in 1L distilled water and bring to the boil to dissolve completely. Sterilize by autoclaving at 121^{0}C for15 minutes. Mix well before pouring into sterile Petri dishes.

Purple agar base (difco, france)

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

Tryptone soya broth (Oxoid, England)

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^{0}C
Instructions for use:
Dissolve 30g in 1 liter of distilled water and distribute into final containers. Sterilize by autoclaving at 121 ^{0}C for 15 minutes