ISOLATION OF *STAPHYLOCOCCUS AUREUS* FROM CAMEL MILK, ANTIMICROBIAL SUSCEPTIBILITY TESTS AND ITS PUBLIC HEALTH IMPORTANCE IN BABILE DISTRICT EAST HARARGE *ZONE*, ETHIOPIA

MSc.THESIS

BY

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I hereby certify that I have read and evaluated this thesis entitled "**Isolation of *Staphylococcus Aureus* from Camel Milk, Antimicrobial Susceptibility Test and its Public Health Importance in Babile district, East Hararge, Ethiopia"** prepared under my guidance by Adem Yusuf. We recommend that it be submitted as fulfilling the thesis requirements for the Degree of Master of Science in Veterinary Public Health.

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BIOGRAPHICAL SKETCH

Adem Yusuf was born in 1989 from his father Yusuf Ahmed and his mother Qamara Jibrehil in Badano woreda of Eastern Hararghe *Zone,* Oromia Regional State, Ethiopia.He completed his primary education in 2004 G.C in kacha keble , After completing his secondary and Preparatory education in 2008 in Badano town. During the year of 2009 he attended the Jigjiga University, School of Veterinary Medicine. He has received Doctor of Veterinary Medicine (DVM) degree from Jigjiga University on 28th June 2014. During the following three years, he was employed as Veterinary Clinician in Qumbi Woreda. In academic year of 2018/2019, he entered the Post-graduate program at Haramaya University, College of Veterinary Medicine and Department of Veterinary Public Health. Currently, he has been doing his MSc research on the title of "Isolation of S*taphylococcus aureus* from raw camel milk, antimicrobial susceptibility tests and its public health importance in East Hararge *Zone*, Ethiopia".Funded by Haramaya University and Qumbi Woreda livestock and fishery bureau.

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ABBREVIATION

BAP	Blood Agar Plate
BLDA	Babile Livestock Resource, Development and Health Agency
CDC	Center of Disease Control
CFU	Colony Form Unity
CSA	Central Statistics Agency
FAO	Food and Agricultural Organization
FBD	Food Borne Disease
FSA	Food Standards Agency
MRSA	Methicillin Resistant *Staphylococcus aureus*
MSA,	Manitol Salt Agar
PAHO	Pan American Health Organization
SE	Staphylococcal Enterotoxin
SFP	Staphylococcal Food Poisoning
S. aureus	*Staphylococcus Aureus*

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ABSTRACT

A cross sectional study was conducted from January 2021 to June 2022 in Babile District, East Hararge *Zone*, Oromia, Ethiopia, with aim of *S. aureus* isolation from raw camel milk, to assess antimicrobial resistance and public health importance.The study was conducted on a total of 223 milk samples from producers, collectors and retailers. For this study both laboratory and questionnaire based data were collected and analysis by chi-square test(x^2). The overall prevalence of *S. aureus* was found to be 18.4 % (41/223).The frequency of isolation of *S. aureus* were varied between sources of sample and ranged from 11.7-34.6 %. The prevalence of *S. aureus* was 11.7 % (16/137), 20.6 %(7/34) and 34.6% (18/52) from udders at milk producers, milk from container at collectors and retailers, in the increasing order. There was statistically a significant difference (P=0.001) in isolation of *S. aureus* from milk udders at producer, milk from containers at collectors and retailer. Of all (n=41) isolates of *S. aureus* were subjected against fourteen antibiotics susceptibility testes by using the kirby-bauer disk diffusion method, high susceptible to Ciprofloxacine (100%) Gentamycin(92.7), Erythromycin(92.7) and Kanamycine (90.2),while high resistant *S.aureus* isolate to Penicillin G (100%) Ampicillin (85%) and Tetracycline (68.3) were observed. The prevalence of multidrug resistance of *S.aureus* isolates from raw camel milk was 63.4% (26/41). Milk handling practice and consumer behavior at milk producers, sellers and consumers would be assessed. A 60% of respondent had no clean milk container, 62.5 % could no wash hand, 70% could no wash udders before milking and 57.5respondent were used plastic containers under milk producers. Majority of sellers were pooling the milk obtain from different producers and collectors that stored in plastic containers (86.7%) without refrigerator .A 100 % of milk consumers had habit of raw milk consumption and have not awareness about milk borne disease(100%) were observed at all respondents. The study showed that, high prevalence of *S.aureus* from raw camel milk and multidrugs resistant were observed, which could be public health risk in Babile district, East Hararge *Zone*. Creation of public awareness about good milk handling practices, boiling of milk prior to consumption and periodic assessment of the drugs sensitivity test prior to use.

Key words: Raw Camel milk, *S. aureus*, Antimicrobial resistant, Public health

1. INRODUCTION

1.1. Back Ground

Staphylococcal food poisoning is one of the most common food-borne diseases in worldwide resulting from the ingestion of staphylococcal enterotoxins preformed in food by coagulase-positive staphylococci, mainly *S. aureus* (Daka *et al*., 2012; Thaker *et al*., 2013). It is a gram-positive, catalase-positive, usually oxidase-negative, facultative anaerobic which belongs to the family of *Micrococcaceae* and the group of *Staphylococci* (Aqib *et al*., 2018). It can be distinguished from other staphylococcal species on the basis of gold colony pigmentation, production of coagulase, fermentation of mannitol (Tessema, 2016).The organism is commonly hemolytic in blood agar due to hemolysin production and it is a salt-tolerant, which is able to grow in mannitol-salt agar medium containing 7.5% of sodium chloride (Arumugam *et al*.,2017). As staphylococcal enterotoxins are heat stable, they may be present in food when *S. aureus* are absent (Balaban and Rasooly, 2000).

Staphylococci are ubiquitous in the normal flora, nasal cavity and the skin of warm-blooded animals. The mucous membranes, udders and teats of milking animals are the most important reservoir of this contaminant (Befekadu *et al*., 2016). It is an important food-borne pathogen that usually associated with raw unpasteurized milk of dairy animals suffering *Staphylococcal* associated mastitis (Rahimi *et al*., 2013).

There are many paths by which the pathogen can enter into dairy food destined for human consumption, especially raw milk (Ana *et al*., 2020). Bacterial contamination of milk usually occurs during the milking process which depends on the sanitary conditions of the environment, utensils used for milking, the milking personnel hygiene and microorganisms that introduced from udder through the teat canal and also during milk handling, pooling milk from different herds, transportation and displaying at the selling points. Under any of these condition, microorganism get into the milk and multiply in milk may contain pathogenic microorganisms and their source may lie either within or outside the udder (Kalsoom *et al*., 2004; Ahmad *et al*., 2012;Thaker *et al*., 2013).

The prevalence of *S.aureus* isolate from raw milk is varied in different district of Ethiopia,11.45% in Jigjiga (Serda *et al*., 2018), 12.8% in Borena (Regassa *et al*., 2013), A 54.3%

from udder milk and 60 % from market milk sample were *S. aureus* positive in Afar region of Ethiopia by Wasie *et al*(2015).

Staphylococcus aureus has been reported to frequently show multiple antimicrobial resistance patterns (Enright, 2003). Antimicrobial resistance of *S. aureus* isolate from raw camel milk for tetracycline-was 69%, Amoxicillin-clavulanic acid 34.5%, oxacillin 31%, cephalothin 31%, chloramphenicol 27.6%, sulphamethoxazole-trimetthoprim 24.1% and clindamycin 13.8%. The highest resistance rate was observed in penicillin (93.1%) and tetracycline (68%) (Teshome *et al*., 2016). A 55.2 % of *S. aureus* isolated were found to be multidrug-resistant (Melese *et al*., 2016).

1.2. Statement of the Problems

A Babile district was important camel milk producing area in East Hararge *Zone* of Ethiopia. Milk had been reported as common food, which is a help the growth of many bacteria specially *S.aureus*. About 50% of strains of *S.aureus* are able to produce enterotoxins associated with dairy food poisoning (Befekadu *et al*., 2016). As camel milk is consumed in its raw state, the contamination and intoxication of raw milk concerning pathogenic *S.aureus* were public health importance (Mulugojjam and Aleme, 2014). Among various risk factors associated with this pathogen are unhygienic milking procedures, poor milk handling practice, milk storage at ambient temperature after milking (Ayoup *et al.*, 2020) and contamination of milk when the dairy animals suffering from *S.aureus* induced mastitis (remaz *et a.,l* 2017).

Antibiotic resistance is an important health problem (Eiz *et al*., 2019).Antimicrobal resistant of *S.aureus* to methicillin, macrolides and aminoglycosides are serious problem (Tessema,2016). The multiple antibiotic resistance of *S.aureus* was reported by Befekadu *et al (*2016).

In developing countries like Ethiopia were high prevalence of clinical and sub clinical mastitis mainly caused by *S.aureus* and high consumption of raw camel milk with poor hygienic practice (Carruth *et al*., 2017) and also common misuse of antibiotics was leads to public health problem (Yenealem, 2020). Besides there were a limited studies on prevalence of *Staphylococcus aureus* from raw camel milk and antimicrobial susceptibility in this study area. Therefore, this study needs to isolation of *Staphylococcus aureus* from raw camel milk, antimicrobial resistance and its

public health importance in East Hararge, Oromia Regional State, Ethiopia.

1.3. Significance of the Study

Raw Camel milk uses as food for consumer, source of income for producers and traders whose handle the product under different condition. On the other hand, only single village of Babile namely Erer were done on Bacteriological qualities that are not representatives to all this studies area, therefore to prevent public health pathogens to the consumer. Additionally, the status of milk handling practices and utilization of the camel milk from producers (milking practice) till consumer need attention because this leads to public health importance. Thus, the significance of this study would be community awareness on milk handler and users as a baseline data for awareness creation in the forms of training. Furthermore, the study would be helping the researchers as baseline for conducting another study and to recommend for Babile agricultural and livestock bureau to improve their efforts in dairy animals and community milk handling practice.

1.4. Objective of study

General objective

The general objective of this study was to assess prevalence of *S. aureus* in camel milk, antimicrobial susceptibility and also to assess its Public health significance in Babile district, East Hararge zone.

Specific Objective

- To assess the prevalence of *S. aureus* isolate from ra*w* camel milk in Babile district.
- To determine antimicrobial susceptibility of *S. aureus* isolate from raw camel milk..
- To assess milk handling practice and consumer behavior.

2. LITRATURE REVIEW

2.1. General Description of *Staphylococcus Aureus*

2.1.1. General Characteristics of *Staphylococcus Aureus*

Staphylococci are Gram-positive cocci, approximately 1 mm in diameter that tends to occur in irregular clusters resembling bunches of grapes. Most Staphylococci are facultative anaerobes and catalase-positive. They are non-motile, non-spore forming, and cannot produce endospores, but are highly resistant to drying, especially, when associated with organic matter such as blood, pus and other tissue fluids (Quinn *et al*., 2005). *S. aureus* produce golden yellow colonies (Bhunia, 2008) on blood agar; they appear as glistening, smooth, entire, raised, translucent colonies that often have a golden pigment. The colonies are 2-3mm in diameter after 24hr incubation and most strains show β-haemolysis surrounding the colonies (SU, 2014). *S. aureus* is one organism of particular important in food safety. This bacterium is a major cause of food borne intoxications and outbreaks throughout the world because of its ubiquity and its ability to persist and grow under various conditions (Salandra *et al*., 2008).

They are quite resistant to desiccation and high osmotic conditions. These properties facilitate their survival in the environment and growth in food products. Staphylococcal cells are destroyed by heat but if they have already produced enterotoxin in a food, the toxins can survive approved doses of irradiation and some thermal processes, including pasteurization (Melese and Addisu, 2015). The organisms are able to grow in a wide range of temperatures (7°C to 48°C with an optimum of 30°C to 37°C) and resistant to freezing , water activity , the presence of oxygen and composition of the food, pH (4.2 to 9.3, with Optimum of 7.0 to 7.5); and sodium chloride concentrations(up to 15%(NaCl) (Stewart,2003). The organism produces catalase and coagulase. The Staphylococcal cell wall is resistant to lysozyme and sensitive to lysostaphin, which specifically cleaves the pentaglycin bridges of Staphylococcus spp (Leloir *et al*., 2003).

2.1.2. Isolation and identification of Staphylococcus aureus

The isolation and identification of *S. aureus* is conducted on the basis of colony morphology, hemolytic properties,Gram-stain, catalase production, coagulase production and ferment mannitol (Quinn *et al*., 2005). Blood agar is the medium of choice for isolation of the organism from specimens and on 24 hours incubation. Staphylococci give good growth of creamy, often deeply pigmented colonies that is surrounded by the narrow zones of clear haemolysis, a broader zone of incomplete haemolysis or none depending on the species (Bendahou *et al*., 2008). Some species of Staphylococcus synthesize the enzyme haemolysin. Haemolysin is an exoenzyme that lyses red blood cells. If a colony of bacterial cells is producing haemolysin and secreting it into the medium, there will be a round, clear zone surrounding the colony because the red blood cells in that area have been lysed. The presence or absence of haemolytic properties, therefore, cannot be used as a definitive identification of Staphylococcus species as some species and strains of Staphylococcus species may not cause haemolysis (Salandra *et al*., 2008).

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

A common medium used for the isolation of pathogenic staphylococci is the mannitol salt agar. Some organisms cannot tolerate high osmotic pressure. Mannitol salt agar contains a high salt concentration so only salt tolerant staphylococci will grow on high salt concentration of this medium that inhibits the growth of most other organisms. Additionally, MSA contains the sugar mannitol. Staphylococcal organisms can utilize mannitol as a fermentable carbohydrate (food source) and will produce acid end products from this metabolism. Since this process is invisible an

indicator is added to the media to detect changes in pH. Phenol red is the indicator used in MSA. It is red at a neutral pH but turns yellow if conditions in the media become acidic. Pathogenic staphylococci not only grow on the medium, but they also produce acid from it. This acid production turns the pH indicator from red to yellow. Non-pathogenic staphylococci can grow on the medium but produce no acid from it and the medium remains pink (Jay, 2000; Quinn *et al*., 2005).

Pathogenic organisms require mechanisms to help them overcome host defense mechanisms. One mechanism involves coating the bacterial cells in a body substance, such as fibrin, to fool the immune system. The coating of a natural body substance will not trigger an immune response and this is accomplished through the production of coagulase. The coagulase test are used both slide coagulase and tube coagulase tests. The slide coagulation test for clumping factor is very rapid but up to 15% of *S. aureus* strains are negative, so isolates negative in slide tests should be confirmed with a tube coagulation test (Brown *et al*., 2005). Coagulase is an exoenzyme that causes fibrin of blood plasma to be deposited on bacterial cells resulting in clot formation. Pathogenic staphylococci produce coagulase, while non-pathogenic strains are coagulase negative (Shah, 2003; Morrison, 2008).

Selective bacteriological media containing one or more agents that are inhibitory to microorganisms other than the target pathogen (staphylococci) can be applied on MSA. The two selective agents most commonly used for these pathogens are sodium chloride and potassium tellurite. The microorganism of interest is not inhibited by the presence of these components in the medium, which can form visible colonies during incubation (Baird and Lee, 1995; Pal, 2007).

2.1.3. Toxins produced by *Staphylococcus aureus*

Many foods can support growth of staphylococci and toxin production. While S. *aureus* is allowed to grow in foods, it can produce a toxin that causes illness. Although cooking destroys the bacteria, however the toxin produced is heat stable and may not be destroyed. In most of the time the contaminated foodstuff reaches a temperature that allows *S. aureus* to growth because of a failure in the refrigeration process (CPH, 2011). The SEs are short proteins belonging to a large family of pyrogenic toxin super antigens with a disulphide bridge secreted in the medium and soluble in water and saline solutions (Salandra *et al*., 2008). They are highly stable, resist most proteolytic enzymes, such as pepsin or trypsin, and thus keep their activity in the digestive tract after ingestion. They are highly heat resistant as well, which can resist 100^0C for at least 30 minutes and probably longer (Walderhaug, 2007). Although pasteurization and cooking kill staphylococci cells which are heat labile, thermo stable SEs generally retain their biological activity. Thus, cases of illness might occur, although no viable bacteria can be isolated from the suspected foodstuff and since SEs are more heat stable than the staphylococci bacteria, it is possible to test a food product and obtain negative staphylococci culture results and positive SEs tests ((FSA,2017).

The amount of enterotoxins produced is determined by factors such as the composition of the food, competition from other microorganisms (the presence of other bacteria affects the production of enterotoxin apparently by limiting the multiplication of the staphylococci), temperature and time (Salyers and Whitt, 2002). To date, a family of 14 different SE types have been identified, which share structure and sequence similarities, of which the six antigenic types (named SE-A, B, C, D, E and G) are most commonly encountered in SFP. In general, SE-A is recovered from food poisoning outbreaks more often than any of the others, with SE-D being second most frequent and the fewest number of outbreaks are associated with SE-E (Salandra *et al*., 2008). *S. aureus* food poisoning is a self-limiting disease, which usually lasts 24-48 hours. Rarely, *S. aureus* food poisoning can develop into systemic disease (FSA, 2017).

2.1.4. Antimicrobial resistance of *Staphylococcus aureus*

Antimicrobial resistance of *S. aureus* to some of the commonly used antimicrobial is present in raw milk (Befekadu *et al*.,2016). *S.aureus* isolates from soft drink were resistant to Erythromycin (64%),Ampicillin(32.2%). Equal proportions(21.4%) of *S.aureus* were resistant to Chloramphenicol, Streptomycin and Amoxicillin (Adem, 2018).

Staphylococcus aureus isolate from raw camel milk was resistance to Tetracycline (50) ,Polymixin B (75%) and Nalidixic acid (100%) and also multiple drug resistance to three and more Antimicrobial resistances of *S.aureus* was found in 69.6% reported (Befekadu *et al*., 2016). Another study showed that *S. aureus* isolate from raw cow milk was resistance to Penicillin (93.1%),Tetracycline (69 %), Amoxicillin-clavulanic acid (34.5 %), Oxacillin (31%), Cephalothin (31%), Chloramphenicol(27.6 %), Sulphamethoxazole-Trimethoprim (24.1 %) and Clindamycin (13.8 %). However the highest resistance rate was observed in Penicillin (93.1%) and Tetracycline (69 %). On the other hand, about 55.2 % (16/29) of *S. aureus* isolated were found to be multidrug-resistant that reported by Melese *et al* (2016) in Jigjiga,Ethiopia. *S.aureus* isolates from fresh raw milk in and around Asela was high resistance to Penicillin G (87.3%) followed by Tetracycline (82.2%), Ampicillin (55.1%), and Cefoxitin (58.1%) and also 65.18% of the isolates were found to be multiple antibiotic resistance phenotypes (Elemo *et al*., 2017) .

2.2. Camel Milk Contamination in Ethiopia

In Ethiopia low lands (arid and simi-arid area) camel milk plays an important role as a primary source of subsistence for pastoral and agro-pastoralists (Tura *et al*., 2010). Milk is a well-known medium that favors the growth of several microorganisms (Farah and Fischer, 2004). Even if milk produced from mammary gland of healthy mammals is sterile fluid, contamination of microbes' starts from udder of milking animal until it is ready to consumption (Farah *et al*., 2007). Camel milk is exposed to several sources of contamination due to milk handling practices. This leads to the deterioration of the milk before it reaches the market (Mulaw *et al*., 2011). The sources of contamination include: unclean milker hands during milking, poor milk handling, pooling milk from different herd(milk from unhealthy camel/container (Farah and Fischer, 2004; Bonfoh*et al*., 2006).

The constraints of milk contamination are different in different pastoral community (El-Ziney and Al-Turki, 2007). There are several constraints in camel milk contamination, clean water for washing containers is scarce or unavailable, common use of recycled oil plastic *jerry-cans* with small opening are very difficult to clean in pastoral areas and long duration during transportation without refrigerators are among other factors (Akweya *et al.*, 2010). Majority of the consumers specially the Somali community, believe that the raw camel milk has medicinal properties which would otherwise be lost due to heating (Eyassu, 2007). Milk producers/traders are extensive hygiene risk factors contributing to milk contamination at the farm level, milk collectors and retailer and also at milk containers(Eyassu, 2007). In Ethiopia camel milks are produced and handled under unhygienic condition and informal improper transport (Farah, *et al*., 2007).

Camel milk production and consumption in Ethiopia was confined to the pastoral areas.However, in the recent year, it is introduced in the urban centers through informal marketing(Farah, *et al*., 2007). Other communities have taken up the consumption of raw camel milk. There are no adequate milk handling practices during production and processing since there is no safety standards set for camel milk in Ethiopia(Farah, *et al*., 2007). Milk handling practices can be danger starting from producer up to the mouth of consumers. In line with that, it is inadequate hygiene condition of dairy camel, poor milking procedure, poor animal health service and lack of proper attention to health of the mammary gland cause of the contamination of milk in the dairy farms (Mekibib *et al*., 2010). Information on microbial safety of camel milk procurement and marketing chain in peri-urban and urban markets is lacking and research outputs on microbial evaluation of raw camel milk in Ethiopia is limited (Semereab and Molla, 2001). Milk hygiene is important to ensure high quality raw milk through the production of milk from health animals and good handling practice(Girma *et al*., 2014).

2.3. Epidemiology of Staphylococci

2.3.1. Source of contamination and reservoir

Staphylococci exist in air, dust, sewage, water, milk, food, or on food equipment, environmental surfaces, humans, and animals. Staphylococci are present in the nasal passages and throats and on the hair and skin(Bergdoll and Lee wong,2006). Although food handlers are usually the main

source of food contamination in food poisoning outbreaks, equipment and environmental surfaces can also be sources of contamination with *S. aureus* (Bennett and Monday,2003). Ruminants are carriers of staphylococcal strains on their skin, which includes the teat skin. The development of mastitis is related to the entrance in the teat duct of staphylococci colonizing the teat apex (Gyles *et al*., 2004).

The principal reservoir of *S. aureus* is the human carrier. A high proportion of healthy people have staphylococci in the nasopharynx and on the skin(Bergdoll and Lee wong,200). The organism has been isolated from the head, body, legs and nose of animals, from the hands and nose of people, and from the environment such as the milking equipment, bedding materials and water courses (Ludmilla *et al.*, 2007).

2.3.2. Prevalence of *Staphylococcus aureus* in Ethiopia

In Ethiopia, the prevalence of *S.aureus* in raw camel milk was exactly unknown because of limited studies. Some outer have reported the prevalence of *S.aureus* in Ethiopia in a limited area with varied percentages that ranged from 6.45% in Jigjiga (Teshome *et al*., 2016) up to 60% from camel milk at market in Afar Region (wasie *et al.*, 2015).

The different studies conducted in different part of Ethiopia showed variable prevalence of *S. aureus.* The prevalence of *S. aureus* isolate from raw camel milk were reported as 11.4% in Borena (Amenu *et al.*, 2019) ,54.3% udder milk sample and 60% market milk were isolate in Afar by Wasie *et al* (2015) . 11.45% in Jigjigga (Serda *et al*, 2018), 32.14% from raw cow milk in Wolayita sodo (Tessema, 2016), 16.5% in Gondar (Moges *et al.*, 2011), 21.13% in Addis Ababa (Abunna *et al.*,2013), 27.7% in Tigray (Gebrewahid *et al*., 2012) , 44% in Bishoftu (Desissa *et al*., 2013) and 48.75 % in Hawassa (Daka *et al*., 2012).

2.4. Public Health Importance

Milk is an adequate medium for multiplication of food borne infection, especially staphylococci. *S. aureus is* producing various toxins that can cause food poisoning via the production of enterotoxins in milk, where there is a lack of appropriate temperature/time control. *S. aureus* food poisoning is a self-limiting disease, but some time food poisoning can develop into systemic disease and severe illness (FSA, 2017).

Staphylococcal food poisoning is one of the most common food borne diseases both in humans and animals globally, resulting from the ingestion of Staphylococcal enterotoxin preformed in food by strains of coagulase-positive staphylococci, mainly *S. aureus* (Hennekinne *et al*., 2012). In developing countries, the surveillance system of FBD hardly exists and it is therefore, difficult to estimate the real magnitude of the problem (Hennekinne *et al*., 2012).

Staphylococcus aureus is important to cause of Food borne disease, causing an estimated 241,000 illnesses per year in the United States (Kadariya *et al*., 2014) and at least before the 1980s, it was implicated in many outbreaks. However, in recent years, the number of staphylococcal food poisoning outbreaks has declined. CDC reports indicate that during 1972 to 1976, it was associated with 21.4% of the food borne disease outbreaks affecting 29.7% of the total cases; in contrast, between 1983 and 1987, there were 5.2% staphylococcal food borne outbreaks with no deaths. This decline is probably a reflection of the better use of refrigerators to store food and improved sanitary practices that can control contamination and growth of *S. aureus.* Even then, the number of outbreaks and number of cases of staphylococcal gastroenteritis is much higher than several other microbial food borne disease outbreaks. In Japan, the annual average of food poisoning outbreaks from 1976 to 1980 was 827 of a total of 8,742 cases, 28.2% were caused by staphylococcal poisoning (PAHO, 2001). Globally, an estimated 2 million people died from diarrheal diseases in 2005;approximately 70% of diarrheal diseases are food borne diseases. It is estimated that up to 30% of the population suffer from food borne illnesses each year in some industrialized countries (WHO, 2011).

Among FBD, SFP is major concern in global public health programmer. Staphylococcal organisms alone have found to cause hospitalization rates as high as 14%. Although not

considered especially lethal, death can ensue if large amounts of SE are ingested: fatality rates range from 0.03% in the general population to as high as 4.4% for highly sensitive persons such as immune compromised persons, elderly persons and children (Kerouanton, 2007). *S. aureus* in camel milk that resistant to antimicrobial agents is an important public-health risk (Lencho, 2015).

Staphylococci are human and animal pathogen. In human it can cause typically local skin and wound infections but can occasionally cause more severe infections in the body and causes superficial skin lesions (boils, styes) and localized abscesses in other sites and deep-seated infections, such as osteomyelitis and endocarditic and more serious skin infections (furunculosis) (FSA, 2017 ; Matofari *et al.,* 2013).

Staphylococcal food poisoning was caused by a filterable enterotoxin (Martin and Maurice, 2008). There are currently 27 species and 7 subspecies of the genus Staphylococcus; enterotoxin production is principally associated with the species *S. aureus*, although it has also been reported in others including *S. intermedius* and *S. hyicus*. As a relatively mild, short-lived type of illness, staphylococcal food poisoning is perhaps more likely to be under-reported than others. Most reported cases are associated with outbreaks and only a few sporadic cases are detected. In the United States between 1983 and 1987, staphylococci accounted for 7.8% (47) of the 600 bacterial food poisoning outbreaks that were recorded(PAHO, 2001; Martin and Maurice,2008).

Staphylococcal Food poisoning is characterized by a short incubation period, typically 2–4 h. Nausea, vomiting, stomach cramps, retching and prostration are the predominant symptoms, although diarrhea is also often reported, and recovery is normally complete within 1–2 days (Lamprell *et al*., 2004). The short incubation period is characteristic of intoxication where illness is the result of ingestion of a pre-formed toxin in the food. The toxins are small single chain polypeptides which share considerable amino acid homology (Normanno,*et al*., 2007).

According to recent studies, a high proportion of strains isolated from staphylococcal mastitis produce ‗enterotoxin A', which causes many human outbreaks. Several studies were successful in isolating the *S. aureus* from skin lesions and camel milk, which is related to epidemic infections in man (Regassa *et al*., 2013). An important causal factor in poisoning is keeping

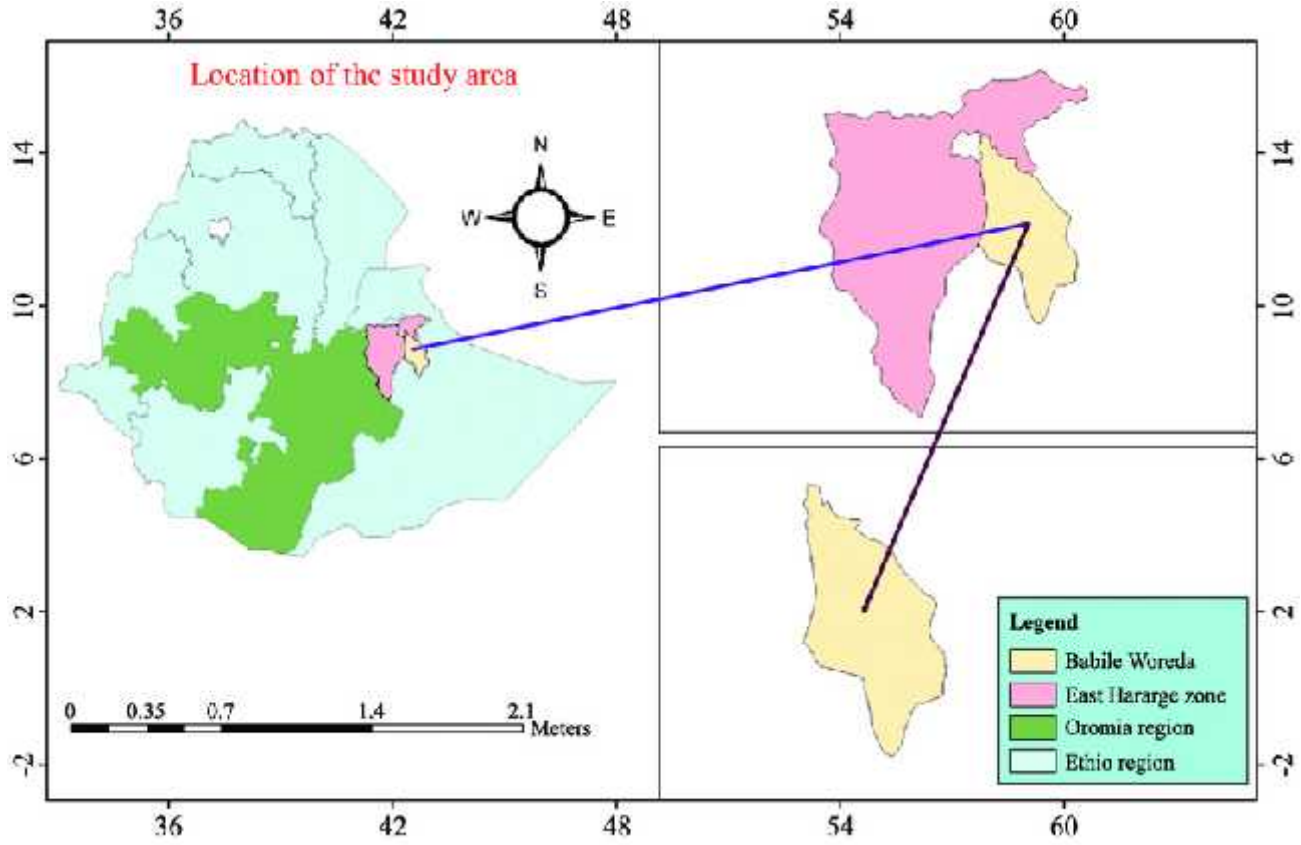
food at room temperature or inadequate refrigeration, practices which allow staphylococci to multiply. Lack of hygiene in food handling is another notable factor. Pasteurization of milk does not guarantee safety if toxins were produced prior to heat treatment, as the toxins is heat-resistant (Walderhaug, 2007).

The presence of large numbers of enterotoxigenic Staphylococci is a good circumstantial evidence that the food contains SEs. The most conclusive test is the linking of an illness with a specific food or in cases where multiple vehicles exist, the detection of the toxin in the food samples (Martın *et al*., 2004; Chiang *et al*., 2008).

3. MATERIALS AND METHOD

3.1. Description of the Study Area

The area of study was in Babile District of East Hararge zone of the Oromia regional state, Ethiopia. Its located 31km away from the town called Harar and about 557 km east from Addis Ababa. It has 20 rural *kebeles* and 2 urban administrative units. It lies between 8°, 9' - 9°, 23'N altitude and 42°, 15'-42°, 53'E longitude and is characterized by semi-arid and arid climate with average annual rain fall of 410-800 mm and the annual temperature ranges from24-28°C. The agro- climatic zones of the district are tropical (85%) and (15%) sub-tropical. The district is bordered by Fadis in the west and Somali in the South and East and Gursum in the north. The vegetation of the study area was sparsely distributed and dominated by Cactus and Acacia species and bushy wood lands. The altitude ranges from 950 to 2000 m above sea level. The total human population of the woreda is estimated to be 118,537 of which 59,298 were males and 59,139 were females (CSA, 2015). Total numbers of camel in Babile district are 18,317(BLDA, 2018). The numbers of camels used for milking at Babile district are 4700 and annual Camel milk in this area is estimated to be 7614 metric ton which is estimated as the study population (BLDA, 2018).



3.2. Study Design

A cross-sectional study was conducted from January 2021 to June 2022, for isolation of *S.aureus* from raw camel milk, antimicrobial susceptibility and its public health importance.

3.3. Study Population

The study population was lactating Camels from household in three *kebeles* (Dekata,Gamechis and Erer),primary milk collectors, retailers, milk handlers and users in Babile District.

3.4. Study Methodology

3.4.1. Sample size determination

The sample sizes were determined using the formula given by Thrusfield (2007) for random sampling. A 95% confidence level, 5% desired level of precision with the expected prevalence of S. *aureus* 16.2% reported in Erer Babile district Eastern Hararge zone,Oromia Regional State, Ethiopia (Mulugojjam *et al*, 2013).

$$\mathbf{n} = \frac{1.96^2 \text{ x P exp (1- Pexp)}}{d^2}$$

Accordingly to this formula, 208 minimum sample sizes were calculated using stated formula to increased sample size by 7.1% in order to increased precision 223 milk samples from three sources were tested for the presence of *S. aureus.*

3. 4. 2. Sampling Technique

Data were collected in to two ways; laboratory and questionnaire interview. A type of sample includes, milk from camel udders at producers (household), milk from containers at collectors and Retailers. A137 milk sample from individual camels were randomly selected from 43 herds (group). A34 milk samples were taken from 5 collectors out of 12 and 52 milk sample collected from 10 retailers out of 22 were selected random sampling by using lottery method from listed sampling frame. So, a total of 223 milk samples from three sources were tested for the presence of *S. aureus.*

3.4.3. Collections, transportation and handling of sample

About 10 ml volume of raw camel milk sample was collected according to the procedure recommended by Quinn *et al (*2005). Strict aseptic procedures were followed when collecting milk samples in order to prevent contamination with microorganisms present on the skin udder and on the hands of samplers. Teat ends were cleaned and disinfected with ethanol (70%) before sampling (Quinn *et al*., 2005). Sterile bottle with tight fitting cups were used. The bottles were labeled with permanent marker before sampling. The first few drop of milk were discarded to avoid

contamination while sampling from camels. After agitating the bulk tank milk, sample was taken from the top of bulk milk using a sanitized dipper from milk containers at collectors/retailers. The bottles were labeled with permanent markers before sampling. All samples were placed in separate sterile plastic bags to prevent spillage and cross-contamination and sample-containing bottles were transported in an icebox to the Haramaya University Veterinary Microbiology Laboratory within a maximum of two hours after collection. Upon arrival, the samples were cultured immediately or stored in a refrigerator at 4ºC for a maximum of 24 hours until they were inoculated onto a standard bacteriological media (Yenealem, 2020).

3.4.4. Isolation and identification of *Staphylococcus aureus*

Prepared the Nutrient medium used for isolation and identification according to the manufacturer's recommendations and milk samples were subjected to bacterial culture according to the procedures of ISO(ISO-6888/1/2003). Briefly, loop full milk sample streaked on blood agar base enriched with 5 % sheep blood. The inoculated plates were incubated aerobically at 37^{O}C for 24 hours. Then the plates were examined for the presence of Staphylococcus colonies. Colonies characterization was based on their morphological aspects. Thus, colonies with morphological features such as B-hemolysis within 24 hours under aerobic culture conditions on the surface of Blood agar plates were suspected. Presumed staphylococcal colonies were then sub-cultured on nutrient agar plates (NAP) and incubated at 37^{0}Cfor 24 hours to get a pure culture (clone of cells derived from a single cell). The pure isolates in the nutrient slant were maintained at 40 ^{0}C for further need (Quinn *et al*., 2005).

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

Final identification of *S. aureus* assignment were done based on biochemical tests such as catalase test, oxidase test, Mannitol sugar fermentation and Coagulase test as described by (Quinn *et al.*,2005).. Accordingly, pure cultures of the isolates were mixed with drop of 3% H_2O_2, and subsequently, the formation of bubbles originating in the microbial colony is verified in catalase test. For oxidase test, the disappearance of dark purple color along the streak on the filter paper in

moistened petridish with 1 percent aqueous solution of tetra methyl –p-phenylene diamiane dihydrochloride was considered as Staphylococcus oxidase test. The presence of growth and change of pH in the medium of mannitol salt agar (red to yellow) was regarded as presumptive identification of *S. aureus* during mannitol fermentation test. Coagulase test consists of inoculating a suspension of the bacterial strain in rabbit plasma in a test tube, which will be incubated in a bacteriological incubator at 37 ºC, for 24 hours. Over this time period, it will be observed whether or not there is the formation of a clot in the plasma, in which the presence of coagulation will indicate a positive result.

3.4.5. Antimicrobial Susceptibility of *Staphylococcus aureus*

Antimicrobial susceptibility testing was performed by the kirby-bauer disk diffusion method using national committee for Clinical Laboratory Standards institute (CLSI, 2012) on all *S. aureus* isolates. Fresh overnight cultures prepared and using Muller Hinton agar for antibiotic sensitivity tests. For susceptibility testing, direct colony suspension of the isolates were adjusted to a turbidity equivalent to a 0.5 McFarland standard. The criteria used to select the antimicrobials were based on the availability of the drugs. Erythromycin (15µg), Ampicillin(10µg), Penicillin G(10µg), Streptomycin (10µg),Tetracycline(30µg),Vancomycine(30µg),Gentamycine(30µg),Ciprofloxacin(5µg),Sulphamethoxazole/Trimethoprim(25µg),Kanamycine(30µg),Cefoxitin(30µg),Amikacin(30µg),Chloramphenicol(30µg) and Oxacillin (1µg**)**. Then the standardized suspension will be streaked in to the Muller-Hinton Agar and allowed to dry. Next, the antibiotic discs were placed on the medium and incubated at 37°C for 24 hours and the zones of inhibition were measured by clipper. The interpretation of the results of the antimicrobial susceptibility was interpreted based on the Laboratory standards institute (CLSI, 2012). Isolates of *S. aureus* was classified as susceptible, intermediate, or resistant depend on inhibition zone for this category of susceptibility test as set in (Annex-4).

3.5. Questionnaires surveys

Structured questionnaire was used to assess the knowledge, attitude and practices of study participants or the target population which are camel milk producers (household) at a farm, sellers and consumers at the handling and consumption of milk in the study area. Risk factors in this study includes clean milking equipment, types of container used, pooling milk, average time gap between milk collected and selling, raw milk consumption, washing hands and udders, awareness on milk born disease and other risk factors were assessed to determined milk handling practice at milk producers (household), seller and consumers. The questions were originally written in English and translated into (*Afaan Oromo*) when interviewed (Annex 1). The answers were translated to English and entered into the original form. For this sample size were calculated by using the formula given by Arsham, (2002) which is N = 0.25/SE2, Where: N= sample size, SE (standard error) = 5%).Therefore, by using the above formula, the sample size were calculated as 100 participants to be interviewed. Purposive sampling was used to select the respondents based on their accessibility and point along the milk handling.

3.6. Data Management and Quality control

For laboratory analysis, Pre-analytical, analytical and post-analytical stages of quality assurance that were incorporated in Standard operating procedures of the microbiology laboratory were strictly followed. In addition, the reading of the growing culture of *S. aureus* was confirmed by the principal investigator and one senior microbiologist after overnight incubation at 37°C. New batches of stain and reagent was checked for correct staining reaction using a smear containing known gram positive and gram negative *S. aureus* as a control. Preparation and performance evaluation of culture media were improved by strictly following standard operating procedures and the manufacturer‘s instruction manual. All culture plates and antibiotic discs were stored at recommended refrigeration temperature (2–8°C). Sterility of culture media was assessed by incubating 3-5% of a batch of prepared culture media at 37^0 for 24 hrs and checked for any growth. Reference strain of *S. aureus* ATCC 25923 was used for quality control of antimicrobial susceptibility test. Quality control of culture media was checked by inoculating quarter plates of the medium with a five hours broth culture control organisms and incubated aerobically at 37 ^{O}C

for 24 hours.

3.7. Data Processing and Analysis

The all data obtained from field survey and laboratory analysis were coded and entered in to Microsoft Excel and exported to SPSS version 20 for descriptive analysis. Difference among and between proportions of the groups with certain determinant factors was determined by chi-square ($\chi 2$) test. A P-value <0.05 was considered indicative of a statistical significant difference. Finally, data was organized, summarized, and presented in tables.

4. RESULTS

4.1. Prevalence of *Staphylococcus aureus*

Among 223 samples examined, 41(18.4 %) were positive for *S. aureus*. From this, 16(11.7%), 7(20.6%) and 18(34.6%) were milk from camel udder at milk producer, milk from container at collectors and retailer respectively (Table 2). The result showed that the highest isolation of *S. aureus* was milk from retailer 34% while less was milk from camel udder 11%. The result showed that significant difference (P=0.001) in prevalence from the three source of milk sample.

Table1: Prevalence of *S. aureus* in raw camel milk from udders at household, containers at primary collection centers and retailer in Babile district.

Source of milk	**Number of tested**	**Prevalence (%)**	X^2	P-value
Household	137	16(11.7)		
Primary milk Collection center	34	7(20.6)		
			13.3449	0.001
Retailers	52	18(34.6)		
Total	223	41(18.4)		

4.2. Antimicrobial susceptibility of *Staphylococcus aureus* isolated from raw milk

All 41 isolates of *S. aureu*s from raw milk were tested for antimicrobial susceptibility testing on fourteen different antimicrobials. Results of antimicrobial susceptibility test against S. aureus showed high susceptibility to Ciprofloxacine (100%),Gentamycin (92.7%),Erythromycin (92.7%), Kanamycin (90.2%) followed by Cefoxitin(68.3%) and Amikacin(65.9%) whereas resistance was recorded against PenicillinG (100%),Ampicillin (85.4%),Tetracycline (65.9%),Streptomycin

(51.2%) and Oxacillin (51.2%) While Sulphamethoxazole/Trimethoprim (80.5%) and Vancomycine (34.1%) were intermediate susceptible (Table 2).

The overall prevalence of multidrug resistance patterns (resistance to at least one antimicrobial drug in three or more antimicrobial categories) of *S. aureus* isolates from raw camel milk were 63.4% (26/41). Among 41 *S. aureus* isolate from raw camel milk, six (14.6%) isolate were resistance to only one drug, Seven (17.07%) isolate were resistance to six drugs and two (4.9%) isolate were resistance to ten drugs. The most frequent multidrug resistance isolates were those exhibiting resistance to Penicillin and Ampicillin, Tetracycline, Chloramphenicol and Streptomycin (Table 3).

Table 2. Antimicrobial susceptibility test against *S. aureus* isolates from raw camel milk.

Antimicrobial	**Unit**	**Antimicrobial susceptibility of *S. aureus* isolate from raw camel milk (n=41)N%**		
		R	I	S
Ampicillin	10 µg	35(85.4)	6(14.6)	0(0.0)
Ciprofloxacin	10 µg	0(0.0)	0(0.0)	41(100)
Erythromycin	15 µg	2(4.9)	1(2.4)	38(92.7)
Gentamycin	10 µg	0(0.0)	3(7.3)	38(92.7)
Penicillin G	10 µg	41(100)	0(0.0)	0(0.0)
Streptomycin (S)	10 µg	21(51.2)	13(31.7)	7(17.1)
Sulphamethoxazole / Trimethoprim	25 µg	2(4.9)	33(80.5)	6(14.6)
Tetracycline	30 µg	28(68.3)	12(29.3)	1(2.4)
Vancomycine	30 µg	19(46.3)	14(34.1)	8(19.5)
Chloramphenicol	30 µg	23(56.1)	10(24.4)	8(19.5)
Amikacine	30 µg	6(14.6)	8(19.5)	27(65.9)
Kanamycine	30 µg	0(0.0)	4(9.8)	37(90.2)
Oxacillin	1 µg	21(51.2)	9(22.0)	11(26.8)
Cefoxitine	30 µg	13(31.7)	0(0)	28(68.3)

R=Resistant, I=Intermediate, S=Susceptible

Table 3: Multidrug resistance combination of *S. aureus* isolates from raw camel milk in Babile district (n=41)

Resistant to drug combination	**Antimicrobial phenotypes**	**Resistant *S.aureus* isolates**	
		Number	**%**
single drug	P	6	14.6
Two drugs	TE,P	1	2.4
	P,S	2	4.9
	P, AMP	4	9.8
Three drugs	AMP P,VA	2	4.9
	P, TE, STX	1	2.4
Four drugs	P, TE, S, VA	2	4.9
	P, AMP ,CHL, VA	1	2.4
	P, TE, FOX ,VA	2	4.9
Five drugs	PEN,TE, S, AMP, STX	2	4.9
	CHL ,P, TE, CHL, S	2	4.9
Six drugs	P , OX ,TE, AMP, S,CHL	3	7.3
	P ,TE, AMP,CHL,S,FOX	4	9.8
Seven drugs	P,TE,CHL,AMP,S,OX,CIP	3	7.3
Eight drugs	P,TE,CHL,AMP,VA,S,OX,ER	2	4.9
Nine drugs	P,TE,AMP,VA,CHL,FOX,S,OX,AMK	1	2.4
Ten drugs	P,TE,AMP,VA,CHL,FOX,S,OX,AMK,CIP	2	4.9
Total		41	100

Abbreviation: ER=Erythromycin, P=PenicillinG, AMP=Ampicillin, TE =Tetracycline, FOX = Cefoxitin, OX=Oxacillin, CHL= Chloramphenicol, STX=Sulfamethoxazole/Trimethoprim, S=Streptomycin, VA=Vancomycin, CIP=Ciprofloxacin

4.3. Results of Questioner Survey

Among the total of 40 interviewed of the household on milk handling practice, 57.5% (23) were used plastic containers, 7(17.5%) and 10(25%) were used traditional containers and stainless steel, respectively and also 40 %(n =16) of them practiced cleaning of milk container by boiling water and 60% of the respondents were no clean milk containers, 28(70%) did not wash udder, A 37.5% of them washing hands before milking but not use disinfectant, majority of respondent showed that average time of milk production and selling were 1-4 hours and have not awareness on milk borne diseases(Table 4).

Milk handing practice at seller site, 86.7% (n= 27) of the respondents use plastic containers, 100% (n=30) of them were cleaning milk containers with detergent, 86.7 %(n=27) of them pooling milk from different producer and collector, 80%(n=24) of respondent were 1-4 hours during milk collected and selling and also 100% of them (n= 30) had no aware of milk borne diseases (Table 5).

Among the 30 consumers, 100% (n= 30) of the interviewed consume raw milk. 100% of them (n= 30) had no aware of milk borne disease, 10% (n=3) of the illness after consumption of raw camel milk. Of the consumers, 56.7% (n=17) and 43.3% (n= 13) of them purchased milk from producer and sellers, respectively. 86.7% of them (n= 26) used plastic containers while the rest 13.3% (n= 4) of them used stainless steel that containers to hold milk to their homes and 60% (n=18) of respondent consumed milk between 5-10 hours (Table 6).

Table 4: Hygienic practices during milking and handling by household in Babile District, East Hararge *Zone*

Question items	Categories	Frequency	Percentage
milk consumption behavior	Raw milk	40	100
	Boiling	0	0.0
Milk storage containers	Plastic containers	23	57.5
	Stainless steel	10	25
	tradition containers	7	17.5
Washing hand before milking	Yes	15	37.5
	No washing hand	25	62.5
Clean milk containers	Yes	16	40
	No	24	60
Clean udders before and after milking	Yes	12	30
	No clean	28	70
Hygienic condition of the environmental	good	0	0.0
	medium	17	42.5
	poor	23	57.5
Mixing milk from different herds	Yes	32	80
	No	8	20
Average time gap between milking and selling	1-4 hours	18	45
	5-10 hours	14	35
	>10 hours	8	20
Any illness after consumption of raw milk	Yes	6	15
	No	34	85
Have awareness on milk borne diseases	Yes	0	0.0
	No	40	100

Table 5: Information on Milk handling practice by sellers in Babile district, East Hararge *Zone*

Question items	Categories	Frequency	Percentage
Type of container usually used to collect milk	Plastic containers	26	86.7
	Stainless steel	4	13.3
clean milk containers	Yes	30	100
	No	0	0.0
mix the milk obtain from different producers/collectors	Yes	26	86.7
	No	4	13.3
Use of refrigeration	Yes	0	0.0
	No	30	100
Average time gap between taking and serving milk	1-4 hours	4	13.3
	5-10 hours	24	80
	>10 hours	2	6.7
Are you Consume raw milk	Yes	30	100
	No	0	0.0
Any illness after consumption of raw milk	Yes	3	10
	No	27	90
Have awareness on milk borne diseases	Yes	0	0.0
	No	30	100

Table 6: Information on milk consuming/use and milk handling activity by consumer in Babile district, East Hararge Zone

Question items	Categories	Frequency	Percentage
Type of container usually used	Plastic containers	18	60
	Stainless steel	12	40
clean milk containers	Yes	30	100
	No clean	0	0.0
Where do you purchase raw milk	Milk producer	17	56.7
	Sellers	13	43.3
Consume raw milk without boiling	Yes	30	100
	No	0	0.0
Use of refrigeration	Yes	2	6.7
	No	28	93.3
Duration of milk stay at home prior to consumption	1-4hours	4	13.3
	5-10hours	18	60
	>10hours	8	26.7
Any illness after consumption of raw milk	Yes	4	13.3
	No	26	86.7
Awareness on milk borne diseases	Yes	0	0
	No	30	100

5. DISCUSSION

The overall prevalence of *S. aureus* isolate from camel milk in the study area was found to be 18.4% (41/223) which varied between source of sample (milk from udder at producers, milk from container at collectors and retailers) and ranged from 11.7-34.6%. This finding was in line with study conduct in Erer 16.2% (Mulugojjam *et al*, 2013) and 22.6%(Tawfiq *et al*.,2019) in kafr Elsheikh governorate of Egypt and also nearly in agreement with a study conduct in Borena 12.8% by Regassa *et al (*2013),11% (Rahimi and Alian, 2013) in Iran and 10.2% (Aydin, *et al*., 2011) in Turkey. However, the results of the present study showed lower than another study conducted in Pakistan by Aqib, *et al (*2017) who reported 48.15% prevalence of *S.aureus* from raw milk. The variation might be due to the hygienic practice, long distance transportation from production to marketing and bulking of milk from different herds.

In this study, 11.7% of the milk sample from udders at the producer, 20.6 %(7/34) of the milk sample from containers at the collectors and 34.6% (18/52) of the milk samples from containers at the retailers that indicate increasing *S. aureus* from producer to retailer. This result was agreement with study conduct in Jigjiga 7.03% at household, 11.7 at milk collection centers and 15% at retailer by Serda, et *al (*2018).

The susceptibility of *S. aureus* to antimicrobial agent has varied in worldwide (Daka *et al*., 2012; Thaker *et al*., 2013; Mekuria *et al*., 2013).

Staphylococcus aureus isolates in the present study showed that high susceptible to Ciprofloxacin (100%),Gentamycin (92.7%), Erythromycine (92.7%) Kanamycin (90.2%), Cefoxitine (68.3%) and Amikacin (65.9%)(Table 3). This result is comparable with study conducted in Nigeria (Okpo *et al*., 2013) who reported susceptible to Ciprofloxacine (100%) and Gentamycin (88.8%) (Alamin *et al*., 2013) and also slightly similar with (Teshome *et al*., 2016) who reported Ciprofloxacin (75%), Cefoxitin(100%), Erythromycine (50%) and Kanamycine (50%). In contrast to the present study, the Sensitivity of *S. aureus to* some antibiotics is much different. For instance, (Mekuria *et al.*, 2013) who reported that the susceptibility rate of *S. aureus* isolates to Erythromycin was 21.6% and (Tofaily *et al*., 2011) who cited a sensitivity percentage of 16.6%

to Erythromycin. However *S. aureus* isolated in the present study found to be highly susceptible to these antibiotics. This might be due to limited usage of these antimicrobials for the treatment of diseases of these dairy camels, including mastitis in the study area.

Staphylococcus aureus isolate exhibited resistance to Penicillin G, ampicillin, streptomycin, tetracycline, oxacillin, Chloramphenicol. Intermediate resistances to vancomycine and Sulphamethoxazole/ trimethoprim were presented (Table 3). In this study some of Antimicrobials resistant were comparable with study (Tariku *et al*., 2011) who reported Penicillin G (87. %), (Balemi *et al*., 2021) who reported Penicillin G (100%), (Rathore and Kataria, 2012) who reported Ampicillin (100%). In contrast to the present study, the percentage of resistant is not comparable with (Alian *et al*., 2012) who reported 17.4% of resistance to pencillin G in Iran and not agreement with (Teshome *et al*., 2016) who reported 50% susceptible to tetracycline. Increase resistance in present study might be due to repeated therapeutic and indiscriminate use of these drugs in this study area. *S. aureus* isolates in present study were resistant to Vancomycin and chloramphenicol, this result were not agreement with (Teshome *et al*., 2016) who reported susceptibility to chloramphenicol(75%),Sulphamethoxazole/trimethoprim(75%) and vancomycin(100%).

The overall prevalence of multidrug resistance (resistance to at least one antimicrobial drug in three or more antimicrobial categories) of *S.aureus* isolates from raw camel milk was 63.4% (26/41). The most frequent multidrug resistance isolates were those exhibiting resistance to Penicillin G Ampicillin, Tetracycline, Chloramphenicol and Streptomycin.The present study finding was in line with the study conducted in Jigjiga city of Somalia Regional State (69.6%) by Befekadu *et al*. (2016). Furthermore the result was in line with a study done by Daka *et al*.(2012) who reported 62.8% multidrug resistance of S.aureus isolates from cow milk in Hawassa town. The presence of multidrug resistant of *S. aureus* illustrates an alarming situation, which needs special attention. The increasing number of MDR might be due to extensive misuse of antibiotic treatment.

Poor milk handling practice and raw milk consumption might be serous implication on public health importance (Yilma, 2007). Maintaining the hygienic conditions of camel udder, milk containers and milker‘s hand is important for good milk handling practice (Magnusson *et al*., 2007). Cleaning the udder of camel before milking is important since it could have direct contact with the ground during lay down, urine, dung and feed refusals while resting. Not washing udder

before milking can import possible contaminants into the milk.

In the present study, majority of them use plastic containers during milking at milk producer, milk sellers/Milk collection center and milk consumer were observed. This result was which agree with (Rahimi and Alian, 2013) who consent handling of milk by plastic containers and the use of unclean water for washing of milk containers may cause contamination of milk. The Plastic containers scratch easily and Provide hiding places for bacteria during cleaning and poor conductor heat leading to bacterial contamination of the milk.

The current study revealed that, 70% of the milk producer did n‘t wash udder and 62.5 % of them did n‘t wash their hands before milking. This result was in agreement with study done around Jigjiga city of Somalia Region that reported 92% of respondents did not use udder washing before milking and 80% of farmers did not washing hand before milking (Serda *et al*., 2018) . A 100% of them were consumed raw milk and also 80% of respondent were mixing milk obtain from different herds at the milk producers. This is agreement with (Wayua *et al.,* 2012) who reported the practice of mixing milk from different herds has been present in the pastoral milk producers. Poor personal hygiene and containers at the milk producers contributed milk to contaminate. This finding in line with (Ahmed *et al*., 2010, Kaindi *et al*, 2011) who reported that poor personal hygiene and milk container at milk producers than milk sellers and consumers. However high prevalence of *S. aureus* occurs at seller site, this might be contaminated during mixing of milk from different producers or collectors and prolonged exposure to high environmental temperatures.

In this study, 100% of the consumers were consuming raw camel milk. This observation is in line with (Serda *et al*., 2018) who reported 100% of them consumed raw camel milk without being subjected to any sort of processing treatment. In this area, majority of consumers were long duration of milk stay at home prior to consumption without refrigerator and 100% of them didn‘t have awareness about food borne disease. Disease history following consumption increase at final consumers purchased from sellers but consumers believe that drinking of raw camel milk could be clean digestive system and had no health risk. However Consumption of raw camel milk should be major concern in public health point of view.

6. CONCLUSION AND RECOMMENDATIONS

In conclusion, the overalls prevalence of *S. aureus* isolated from raw camel's milk in Babile district was found to be 18.4%. The high prevalence of *S. aureus* were assessed at milk retailers whereas lower at household milk from udders. The isolated *S. aureus* from raw camel milk were showed high multidrugs resistant.The isolated *S. aureus* was more resistance to Penicillin G, Ampicillin and Tetracycline whereas, the most effective antimicrobial agents against *S.aureus* was Ciprofloxacin, Gentamycin, Erythromycine, Kanamycin, and Cefoxitin. Majority of respondents were not cleaned milk containers,not washing hand and udders before and after milking/handling practice and also used plastic containers were observed. Besides, most of respondents were consumed raw milk without any heat treatment and milk cooling was not done both after milking and before delivery to the market due to lack of chilling facilities. This indicates the presence of *S. aureus* in raw camel milk could be a risk for consumers, causing a public health problem. In general, the study has showed that increasing prevalence of *S. aureus* from producer to retailers, multidrugs resistance and possibility of the public health risk from careless milk handling practice in Eastern Hararge *Zone* that need attentions with the following recommendations were made

- Good milk handling practice should be maintained from producers up to consumer to minimized prevalence of *S.aureus*
- Monitoring, rational use of drugs and periodic assessment of the antimicrobial sensitivity of drugs prior to use.
- Raw milk intended for human consumption must be subjected to boiling
- Awareness should be created among the public for the implementation of better control and subsequent reduction of Staphylococcus food poising.

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8. ANNEXES

Annex 1. Questionnaire format

I. Questioner for milk producers

Respondents Name_____ Age____ Sex_____ Educational level_____

Adress/Keble_____ Date_____

1. What kind of milking equipment did you use?

   A. Stainless steel B. Plastic containers

   C. Other containers

2. Are wash the milk container by boiling water with detergent?

   A. Yes

   B. No

3. Are you wash your hand prior to milking?

   A.Yes

   B.No

4. How to clean udders before milking?

   A. Clean udder using towel wetted by disinfectants

   B. Clean udders by water

   C. Not clean

5. Hygienic condition of milking environment

   A. good B. medium C. poor

6. Milk Consumption behaviors at home?

   A. Raw B. Boiling

7. Are you/ your family members become ill after consuming raw milk?

   A. Yes B. No

8. Do you know any milk borne diseases?

   A. Yes B. No

9.How long do you keep milk at home?

A. 1-4hrs B .5-10hrs C.>10hrs

10. Are you milking camel under antibiotic treatment?

A. Yes B.No

11.Do you mix the milk obtain from different herds

A. Yes B. No

II. Milk Sellers

Respondents Name_____ Age____ Sex_____ Educational level_____ Address/Kebele___ Date_____

1. What kind of milking equipment did you use?

A. Stainless steel B. Plastic containers

C. Other containers

2. Are you clean the milk container?

A. Yes B. No

3. Where do you purchase milk?

A. producer
B. collectors

4. Do you mix the milk obtain from different producers/collectors

A. Yes B. No

5. Do you wash your hand during selling milk?

A. Yes B. No

6. Do you consume the raw camel milk ?

A. Yes B. No

7. Are you/ your family members become ill after consuming raw milk?

A. Yes B. No

8. Have you Awareness on milk borne illness?

A. Yes B. No

9. How long do you keep milk at selling site?

A. 1-4hrs B .5-10hrs C.>10hrs

10. Do you use refrigerator

A. Yes B. No

III. Milk consumer

Respondents Name_____ Age____ Sex_____ Educational level_____Address/Kebele Date_____

1. What kind of milking equipment did you use?

A. Stainless steel

B. Plastic containers

C. Other containers

2. Are you clean the milk container by boiling water with detergent?

A. Yes

B. No

3. Where do you purchase raw milk?

A. producers

B. collectors

C. sellers

4. Do you consume the raw camel milk?

A. Yes

B. No

5. Have you/your family members become illness after consumption of raw camel milk?

A. Yes

B. No

6. Have you Awareness on milk borne illness?

A. Yes

B. No

7. How long do you keep milk at home?

A. 1-4hrs

B. 5-10hrs

C. >10hrs

8. Do you use refrigerator

A. Yes

B. No

Annex 2. Check list format used for recording data

Serial Number	Date of Collection	Sample code	Source	Number of samples

Annex 3: Record sheet for laboratory isolation and identification of *Staphylococcus aureus*

Serial	Sample code	Colony characteristic	Haemolysis	Gram stain	Catalase test	Oxides test	Mannitol fermentation	Coagulase	*S. aureus*

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

Antimicrobial agents	**Unit**	**Diameter of zone of inhibition to nearest**		
		Resistant ≤	**Intermediat**	**Susceptible ≥**
Ampicillin	10 µg	13	14-16	17
Penicillin G	10 µg	28	-	29
Ciprofloxacin	10 µg	15	16-20	21
Erythromycin	15 µg	13	14-22	23
Amikacin	30 µg	14	15-16	17
Kanamycin	30 µg	13	14-17	18
Streptomycin	10 µg	11	12-14	15
Tetracycline	30 µg	14	15-18	19
Trimethoprim-sulphamethoxazole	25 µg	10	11-15	16
Vancomycin	30 µg	9	10-11	12
Gentamycine	10 µg	12	13-14	15
Chloramphenicol	30 µg	12	12-17	18
Cefoxitin	30 µg	21	-	22
Oxacillin	1 µg	10	11-12	13

Source (CLSI, 2012)