STAPHYLOCOCCUS: EPIDEMIOLOGY AND ITS DRUG RESISTANCE IN CATTLE, FOOD CHAINS AND HUMANS IN CENTRAL ETHIOPIA

MSc Thesis

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Bishoftu, Ethiopia

STAPHYLOCOCCUS: EPIDEMIOLOGY AND ITS DRUG RESISTANCE IN CATTLE, FOOD CHAINS AND HUMANS IN 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 Tropical Veterinary Epidemiology

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As members of the Examining Board of the final MSc open defense, we certify that we have read and evaluated the Thesis prepared by: **Fikru Gizaw** entitled*: **Staphylococcus:*** **Epidemiology and its drug resistance in cattle, food chains and humans in central Ethiopia.** And recommend that it be accepted as fulfilling the Thesis requirement for the degree of: Masters of Science in Tropical Veterinary Epidemiology.

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LIST OF ABBREVIATIONS

ARDRA	Amplified ribosomal DNA restriction analysis
BTM	Bulk tank milk
CHP	Centre for health protection
CMT	California mastitis test
CNS	Coagulase-negative *Staphylococci*
CPS	Coagulase positive *Staphylococci*
DHI	Dairy herd improvement program
DNA	Deoxyribonucleic acid
FBD	Food borne disease
G+C	Guanine plus cytosine
IDF	International Dairy Federation 'fermented milks'
IMI	Intramammary infection
LA-MRSA	Livestock associated methicillin resistant *Staphylococcus aureus*
m.a.s.l	Meters above sea level
MARAN	Monitoring of antimicrobial resistance and antibiotic usage in animals in the Netherlands
PCR	Polymerase chain reaction
PMN	Polymorphonuclear leukocyte
SCC	Somatic cell count
SFP	*Staphylococcal* food poisoning
SPSS	Statistical package for social sciences
TNase	Thermonuclease
TSS	Toxic shock syndrome

ABSTRACT

*The aim of this study was to quantify the prevalence, determine associated risk factors and isolate Staphylococci species and determine its resistance to various antimicrobial agents in cattle, food, equipments and humans in central Ethiopia. In order to isolate and identify Staphylococci species from abattoir and dairy cows a cross- sectional study was conducted from October 2013 to May 2014. The results showed that the prevalence of Staphylococci species were 47.1% (140/297) udder milk, 58% (29/50) tank milk of the selected dairy farms, 34.4% (17/50) cow bucket swab, 38% (19/50) farm tank swab, 38% (19/50) hand and 70% (12/17) nasal swab of milkers, 38.5%(139/361) carcass swab, 37.8% (14/37) knife swab, 48.6% (18/37) slaughter line swab, 37.8% (14/37) hand and 46.7% (7/15) nasal swab of butchers with over all prevalence of 42.8%(428/1001). In addition, the prevalence of Staphylococci were 48.6% (88/181), 47.7% (92/193), 40.7% (103/253), 40.2% (78/194) and 37.2% (67/180) in Assela, Addis Ababa, Bishoftu, Holeta and Adama respectively. The S. aureus was the most frequently isolated species among different samples accounting for 172 (17.2%) followed by S. hyicus 82 (8.2%); S. intermedius **74** (7.4%) and CNS 96 (9.6%). Risk factors analysis revealed that prevalence of Staphylococcus significantly differed with source of sample ($P < 0.05$) and type of sample ($P < 0.05$) in central Ethiopia. Prevalence of Staphylococcus were significantly higher ($p<0.02$) in farm (45.9%) than in abattoir (39.4%). Similarly risk factors analysis for Staphylococcus from udder milk revealed that prevalence significantly differed ($P<0.05$) with the family size, breed, .income level, herd size and lactation stage. In the antimicrobial resistance trials, out of 428 Staphylococcus species isolates 244 (50.9%) were subjected to antibiotic susceptibility tests. 97.5% (238/244) strains revealed antimicrobial resistance properties to at least one of the antibiotics tested. A large proportion of the isolates were resistant to Penicillin G (10units) (90.2%), Cloxacillin (5µg) (70.9%), and Erythromycin (15µg) (70.9%), Nalidixic Acid (30µg) (59.8%), Cefoxitin (30µg) (53.7%), Vancomycin (30µg) (52.9%) and Nitrifurantoin (50µg) (47.5%). Only a small proportion of the isolates from total sample were resistant to Ciprofloxacin (5µg) (1.6%) and Gentamycin (10µg) (2%). This study revealed that, although the prevalence of Staphylococcus species have no statistically significant difference among geographic region of the area sampled, antimicrobial resistance to the isolates have statistically significant*

difference between them except for Penicillin G, Tetracycline, Streptomycin, Gentamycin and Sulphamethoxazole-trimethoprim. In addition, there was no statistically significant difference ($p>0.05$) between source and type of samples in determining resistance pattern to each antimicrobial except for Amoxacillin, Vancomycin, Streptomycin and tetracycline. Multidrug resistance was also observed in 89.3% (218/244) of the total tested isolates. The indiscriminate use of those antimicrobial agents might account, at least in part, for such a high resistance. Milk, meat, equipment and hand and nasal of human contain resistant Staphylococcus species posing a potential risk to consumers. Hence, antimicrobial susceptibility should be conducted before treating cattle. As a result, reduction in transfer of resistant Staphylococci strains between humans and animals could possibly be made. The indifferent distribution of the prevalence of Staphylococci over different geographical area is indicators for lack of proper personal, environmental hygiene and sanitation; and absence of difference in animal husbandry practice in all study area. Hence, implement strict hygienic control measures both in abattoir and dairy farm is important in order to guarantee the quality of cattle derivative food products.

Key words: Staphylococcus, epidemiology, dairy farm and abattoir, antimicrobial resistance, Ethiopia

1. INTRODUCTION

Staphylococcus is a genus of worldwide distributed bacteria correlated to several infectious of different sites in human and animals. Its importance is not only because of its distribution and pathogenicity but especially due to its ability to overcome antimicrobial effects (Souza *et al*., 2012). The species of the *Staphylococci* genus are ubiquitously disseminated in the environment, with a number of species inhabiting specific ecological niches. They are found living naturally on the skin and mucous membranes of warm-blooded animals and humans, which generally imply a commensal or symbiotic relationship with their host. *Staphylococci* are also isolated from a wide range of foodstuff such as meat, cheese and milk (Heikens *et al*., 2005). They are Gram-positive cocci which can be isolated, paired and most often aggregated, forming unmoving grapelike structures (Kloos and Bannerman, 1995). They can be divided into two groups, coagulase positive *Staphylococcus* (CPS) and coagulase negative staphylococci (CPS), according to production of coagulase enzyme, which is capable of coagulating blood plasma(Cunha and Calsolari, 2008).

Infections due to *Staphylococci* are of major importance to veterinary and human medicine (Zadoks, 2002). Long-term surveys suggest that the significance of *Staphylococcus* in the dairy industry has remained unchanged (Swinkels *et al*., 2005). According to the literature data prevalence varies from 2% in Denmark to the 63% in Finland, two studies carried out in Germany reported 16% and 42%, a study in Finland reported 38%, in Estonia 58% while reported prevalence in Switzerland was 34.5% (Pavlak *et al*., 2008).

Food-borne diseases are of a major concern, worldwide. Milk and milk products are the prime habitat to complex microbial ecosystems; these are responsible for the broad variations in taste, aroma and texture of milk and milk products. Contamination of milk and milk products with pathogenic bacteria is mainly due to processing, handling and unhygienic environment (Soomro, 2003). Meat processing at retail level is likely to contribute for the higher levels of contamination in minced beef as compared to carcasses (Tegegne and Ashenafi, 1998). The presence of even small numbers of pathogens in carcass meat and

edible offal may lead to heavy contamination of minced meat when it is cut into pieces; as more microorganisms are added to the surfaces of exposed tissue (Ejeta *et al*., 2004). Staphylococcal food poisoning includes symptoms such as sudden onset of nausea, vomiting, abdominal cramps and diarrhea. On heating at normal cooking temperature, the bacteria may be killed but the toxins remains active (Thaker *et al*., 2013).

The cattle derivative foods that are most often involved in Staphylococcal food poisoning have been documented by a number of workers in developed country. In the United Kingdom, for example, 53% of the Staphylococcal food poisonings were due to meat products, meat-based dishes, 8% were due to milk products (Wieneke *et al*., 1993), in France, among the Staphylococcal food poisonings milk products and especially cheeses were responsible for 32% of the cases and meats for 22% (Haeghebaert *et al*., 2002). In the United States, 36% were due to red meat, and only 1.4% to milk products and sea foods (Larsen *et al.,* 2000).

Antimicrobial resistance is a major public health problem in many countries due to the persistent circulation of resistant strains of bacteria in the environment and the possible contamination of water and food (Normanno *et al*., 2007b). This antimicrobial resistance has been documented by a number of workers in different areas. For example, 20–30% for Denmark and Norway, 85% in Ireland (De Oliveira *et al*., 2000) and Brazil (Costa *et al.,* 2000), in UK 60% (Muhammad *et al.,* 1993), in United state 30-70% (De Oliveira *et al*., 2000), 100% Gujarat (Thaker *et al*., 2013), 30.5% Portugal (Soares *et al.*,2011) , 67-94 Lebanese (Zouhairi *et al*., 2010) has been recorded. Several authors have suggested that administration of antibiotics to food-producing animals for therapeutic purposes or as growth promoters may be a primary factor in selecting for antimicrobial-resistant bacterial pathogens (Barber *et al.,* 2003).

In developing countries like Ethiopia, the production of milk and various dairy products often takes place under unsanitary conditions and the consumption of raw milk and cheese, which

are typically manufactured in small dairy farms under unsatisfactory hygienic conditions (Wubete, 2004). In addition, the widespread habit of raw beef consumption is a potential cause for food borne illnesses besides, the common factors such as overcrowding, poverty, inadequate sanitary conditions, and poor general hygiene (Siddiqui *et al*., 2006).

In Ethiopia quantification and determination of the prevalence of *Staphylococcus* from cattle and cattle derivative food chain has been conducted in Borena 29.2% (Bedane *et al*., 2012), in Assela 58.6% (Birhanu *et al.*, 2013), in Adama 35.8% (Mekonnen and Tesfaye, 2010), in Bushoftu 44% (Alehegn, 2008), in 28.7% Addis Abeba (Zeryehun *et al.,* 2013), in Holota 77.1% (Mekibib *et al*.,2010), in 24% Bishoftu (Mekonnen *et al*., 2011), in 12% Jimma (Haimanot *et al*.,2010). But, little published information about the importance of risk factors of *Staphylococus* pathogens and its risks of antimicrobial resistance development in cattle, food chains and humans. In addition a systematically organized coordinated study to generate information should be conducted to explore the magnitude of the problem and to develop an effective strategy to reduce the outbreak of food borne illnesses and resistance burden in the community and for effective management, control and prevention of *Staphylococcus* in dairy cattle farms. Therefore, the study was carried out with general aim to quantify prevalence, investigate risk factors for *Staphylococcus* from cattle derivative food (milk and meat), cattle and human and its frequency of resistance to various antimicrobial agents, and the relationship between antimicrobial resistances of the isolates and risk factors. Therefore, this study was designed:

- To quantify the prevalence and distribution of *Staphylococcus* in cattle, food and humans in central Ethiopia;
- To identify risk factors of *Staphylococcus* in abattoir, dairy farm and humans in central Ethiopia;
- To evaluate the drug susceptibility pattern of *Staphylococcus* in abattoir, dairy farm and humans in central Ethiopia

2. LITERATURE REVIEW

2.1. Microbiology of *Staphylococci*

2.1.1. Historical aspects

Among the toxic substances produced by the *Staphylococci* are the enterotoxins, the causative agents of staphylococcal food poisoning. The illness can be serious, but usually lasts only a few hours with no sequela (Bergdoll and Lee Won, 2006). Although Ogston is credited with applying the name '*Staphylococcus*' to these organisms in 1881 because of the grapelike clusters of cocci he observed in cultures, it was Rosenbach who in 1884 obtained pure cultures of the microorganisms on solid media and accepted the name *Staphylococcus*. Dack (1956), in his book *Food Poisoning*, relates several descriptions of food borne illnesses similar to staphylococcal food poisoning. A number of food items were involved – sausage, rabbit pie, 'pork brawn', milk, ice cream, and of course cheese, where Vaughan and Sternberg first associated *Micrococcus* with the illnesses in 1884. In 1914, Barber was the first investigator actually to relate staphylococcal food poisoning to a toxic substance produced by the staphylococci (Barber, 1914). He discovered that milk from a mastitic cow caused illness when left unrefrigerated, and showed that the illness was due to growth in the milk of the *Staphylococci* isolated from the mastitis.

2.1.2. Classification of Staphylococci

The name *Staphylococcus* is derived from the Greek *staphylo* (bunch of grapes) and *coccus* (a grain or berry), hence *Staphylococcus* = the grape-like coccus. *Staphylococci* are spherical cells, 0.5-1.5 pm in diameter which can occur as single cells, in pairs, or as clusters. Cluster formation occurs mainly during growth on solid medium and results from cell division occurring in a multiplicity of planes, coupled with the tendency for daughter cells to remain

in close proximity. *Staphylococci* are strongly gram-positive, non motile, and asporogenous; capsules may be present in young cultures but are generally absent in stationary phase cells (Hui *et al*., 2001).

Taxonomically the *Staphylococci* have been placed in the Family *Micrococcaceae*. Baird-Parker (1963) proposed a system of classification of the *Micrococci* and *Staphylococci* based on certain physiological and biochemical tests. He divided the Family *Micrococcaceae* into Group I (*Staphylococcus* Rosenbach emend. Evans) and Group II (*Micrococcus* Cohn emend. Evans). These groups were then divided into subgroups on the basis of pigment production, coagulase and phosphatase reactions, acetoin production, and formation of acid from glucose (both aerobically and anaerobically) and other sugars. Six subgroups were recognized within the genus *Staphylococcus* and seven within the genus *Micrococcus*. Further divided the pathogenic *Staphylococci* found in Baird-Parker's subgroup I into six biotypes with characteristic biochemical and biological properties. *Staphylococcus* can be differentiated from the other three members in the family, *Micrococcus, Stomatococcus*, and *Planococcus*, on the basis of the guanine plus cytosine (G+C) content of the DNA, cell wall composition, and the ability to grow and ferment glucose anaerobically. Only three species of *Staphylococci (S. aureus, S. epidermidis* and *S. saprophyticus*) were included in the genus in 1974 (Buchanan and Gibbons, 1974).

They were differentiated primarily on the basis of the ability to produce coagulase, ferment mannitol (both aerobically and anaerobically) and produce heat-stable endonuclease, and by the cell wall composition (Baird-Parker, 1974). Kloos and Schleifer (1986) outlined a simplified scheme for the routine identification of human *Staphylococci species*. They divided these into 11 species on the basis of coagulase activity, hemolysis, nitrate reduction, and aerobic acid production from several sugars. Since then the number of species and sub-species had increased to 32 as of 1994 (Holt *et al*., 1994). This increase included the elevation of two of the *S. aureus* biotypes to species status, biotype E (from dogs) to *S. intermedius* and biotype F (from swine) to *S. hyicus*. An additional coagulase-positive

species, *S. delphini* from dolphins, has been added. Currently, the genus Staphylococcus consists of more than 40 species (Devriese *et al.,* 2002) (Table 1).

Table 1: The different species of *Staphylococcus* so far recognized in taxonomy

S. aureus	*S. hyicus*	*S. epidermidis*	*S. chromogenes*
S. aureus subsp. *anaerobius*	*S. sciuri*	*S. warneri*	*S. lentus*
S. aureus subsp. *aureus*	*S. gallinarum*	*S. hominis*	*S. lugdenensis*
S. capitis	*S. pasteuti*	*S. auricularis*	*S. caprae*
S. capitis subsp. *Capitis*	*S. equorum*	*S. caseolyticus*	*S. arlettae*
S. capitis subsp. *urealyticus*	*S.felis*	*S. kloosii*	*S. piscifermentans*
S. haemolyticus	*S. schleiferi*	*S. simulans*	*S. delphini*
S. sacchrolyticus	*S. muscae*	*S. intermedius*	*S. vitulus*
S. cohnii	*S. Nepalensis*	*S. hominis*	*S. fleurettii*
S. cohnii subsp. *Cohnii*	*S. carnosus*	*S. hominis* subsp.	*S. pasteuri*
S. cohnii subsp. *urealyticus*	*S. carnosus* subsp. *Carnosus*	*Hominis*	*S. piscifermentans*
	S. carnosus subsp. *utilis*	*S.hominis*subsp.	*S. pulverei* (=*S. vitulinus)*
	S. condimenti	*novobiosepticus*	*S. xylosus*
	S. soprophyticus	S. auricularis	*S. succinus*
		S. kloosii	

Adopted from: Götz *et al*. (2006)

Most of the *Staphylococcal species* recognized to date do not produce coagulase, with the exception of *S. aureus, S. delphini, S. hyicus, S. intermedius* and *S. schleiferi* subspecies *coagulans* (Roberson *et al*., 1998).

2.1.3. Hosts and reservoirs

The *Staphylococci* are ubiquitous in nature, with humans and animals as the primary reservoirs. They are present in the nasal passages and throat, in the hair, and on the skin of probably 50% or more of healthy individuals. *Staphylococci* can be isolated from animals, with the bovine being the most important because of the involvement of staphylococci in mastitis. Although animals and humans are the major source, staphylococci also can be found in the air, dust, water, and human and animal wastes (Bergdoll and Lee Won, 2006).

2.1.4. Ability to survive and grow in the environment

The *Staphylococci* can survive indefinitely in the nasal passages and throats of humans and animals. From these sources they can be transferred to meat and other foods. Essentially all meats can be contaminated with *Staphylococci*; however, the organisms may persist on raw meats but grow very poorly. In foods that provide a satisfactory medium they can grow to sufficient numbers to produce enterotoxin if the foods are not refrigerated. These organisms can be transferred to equipment; if the equipment is then not adequately cleansed before use, the organisms can be transferred to foods. A common source of contamination of dairy products is cows' udders, particularly in animals with *Staphylococcal* mastitis. The organisms are destroyed when the milk is pasteurized, but any enterotoxin in the milk will not be inactivated (Evenson *et al*., 1988).

2.1.5. Staphylococcal enterotoxins characteristics

Staphylococcal enterotoxins are a group of single chain, low-molecular weight (27,000-34,000) proteins produced by some species of *Staphylococci*, primarily *S. aureus*, but also by *S. intermedius, S. hyicus, S. xylosus and S. epidermidis*. To date, 14 distinct enterotoxins have been identified based on their antigenicity and they have sequentially been assigned a letter of the alphabet in order of their discovery (SEA to SEO). There is no enterotoxin F as this letter was assigned to a protein that proved not to be an enterotoxin(Bhatia and Zahoor, 2007).

The temperature range for enterotoxin production varies with the medium, but in general the minimum temperature for production is 10°C and the maximum is 45°C. There are variations with the different enterotoxins and with different media. Most strains of *Staphylococci* will grow at pH values between 4.5 and 9.3, with the optimum being 7.0–7.5; however, the conditions for enterotoxin production are more restricted than for growth. Most experiments

have been done by adjusting the medium to a specific pH value with no attempt to control the pH during incubation (Bergdoll and Lee Won, 2006).

2.2. Epidemiology and prevalence of *Staphylococci*

2.2.1. Prevalence and significance

The most prevalent mastitis causing pathogen was *Staphylococcus*, of which the predominant hemolytic, coagulase positive *S. aureus* (47.1%), followed by Coagulase Negative Staphylococcus (CNS) 30.1% (Mekibib *et al*., 2010). CNS are commonly isolated from cases of subclinical and clinical mastitis, and also from teat canals, teat skin, and teat ducts (Sampimon *et al*., 2007). *S. intermedius* strains and *S. hyicus are* found in milk samples from cows with subclinical mastitis, in certain animal products and in slaughter house effluents. As far as is known to date *S. intermedius* and *S. hyicus* do not occur in man, nor have they ever been shown to be involved in food poisoning in man (Devriese and Hajek, 1980).

According to study conducted by Goli *et al*. (2012) in Iranian, the highest (44.2%) and the lowest (13.4%) prevalence of sub-clinical mastitis caused by contagious pathogens belong to traditional and industrial farms, respectively. In the study of Ethiopian cottage cheese (Ayib) by Mekonnen *et al.* (2011) using routine bacteriological investigation isolates 48 (24%) positive of *staphylococcus species*.

In The Netherlands, 16% of the heifers obtain clinical mastitis in their first lactation of which 38% will occur in the first week after calving. Signs of clinical mastitis caused by CNS are generally milder than in cases with major pathogens, with slight swelling and a limited increase in rectal temperature (Barkema *et al*., 2006). Out of fourteen species of CNS identified, the most frequently isolated species was *S. chromogenes* (30.3%) followed by *S. epidermidis* (12.9%) and *S. capitis* (11.0%) (Sampimon *et al.,* 2008).

The surfaces of equipment used for food handling or processing are recognized as possible support for microbial growth, and biofilms residing on such surfaces are recognized nowadays as potential sources of contamination (Barkema *et al*., 2006). Study conducted on small manufacturing dairy plant in Portugal by Soares *et al*. (2011) using different molecular methods, viz. Multiplex-PCR, amplified ribosomal DNA restriction analysis (ARDRA), and *sodA* gene sequencing, revealed that the main species encountered corresponded to *S. equorum* (41 isolates, 39.0%), *S. saprophyticus* (28 isolates, 26.7%) and *S. epidermidis* (15 isolates, 14.3%). The presence of MRSA isolates in BTM may present a potential public health risk, and MRSA may spread between animals if the numbers of isolates in dairy environments were to increase. Further, resistance to multiple antibiotics is a more realistic risk to animal and public health if found to cause invasive diseases other than MRSA. Herd prevalence of methicillin-susceptible *S. aureus* (MSSA) was 84%, while MRSA herd prevalence was 4% (Haran *et al.*, 2011).

S. aureus is one of the most frequently isolated pathogens in bovine IMI worldwide and the most common contagious mastitis pathogen isolated from raw milk (Olde Riekerink, 2006). Studies from about 25 years ago estimated that 100% of the dairy herds in California (Gonzalez *et al*., 1988), 48% in Vermont (Goldberg *et al*., 1991) and 93% in Great Britain (Wilson and Richards, 1980) included cows infected with *S. aureus*. The proportion of cows with *S. aureus* IMI has been approximately 21% in Great Britain (Wilson and Richards,1980), 11% in Norway (Bakken, 1981), 10% in Denmark, (Aarestrup *et al*., 1995), 10% in Finland (Myllys *et al*., 1998), 9% in California (Gonzalez *et al*., 1988) and 5% in Vermont, USA (Goldberg, 1991). Long-term surveys suggest that the significance of *S. aureus* in the dairy industry has remained unchanged (Swinkels *et al*., 2005). Zakary *et al*. (2011) work indicate the incidences of *S. aureus* in Egyptian Kareish cheese, ice-cream and raw milk by using the traditional methods were 68, 50 and 40%, respectively and the lowest percentage was detected from yoghurt (14%).

2.2.2. Epidemiologic features in dairy and dairy product and meat

Food-borne diseases are of a major concern, worldwide. *Staphylococcus* species are prevalent food borne bacterial pathogens that cause food poising in human when it ingested in contaminated foods including dairy product such as cheese and yoghurt. The organism can gain access to raw milk and milk products either by direct excretion from udders having clinical and sub clinical *Staphylococcus* mastitis or by contamination from food handlers. Potential transmission of staphylococcal food poisoning to consumers via milk of cows affected by subclinical mastitis, mainly when raw milk is ingested is common in Brazil. A two-hundred and eight samples of milk from individual cows showing subclinical mastitis, and 37 samples of bulk tank milk from dairy farms located in São Paulo, Brazil were analyzed and *S. aureus* strains were detected in 18 (7.3%) milk samples: 14 (6.7%) from samples of individual cows, and 4 (10.8%) from bulk tank milk (Fagundes *et al.,* 2010).

Proper sanitary measures are needed to improve the hygienic conditions during milking, storage, transport and manufacturing of dairy product in order to guarantee the quality of these dairy products (Salandra *et al*., 2008). Martin *et al*. (2004) found out that 15% of 157 *Staphylococcus* isolate from dairy products were enterotoxigenic where as Morandi *et al.* (2007) reported that 28.6% of *Staphylococci* isolated from milk and dairy products were enterotoxin producers. From the 200 *Staphylococcus* species isolates, 116 originated from American and colonial cheeses and 84 from colonial sausage. 102 (51%) strains were characterized as coagulase positive *Staphylococci* (CPS), being 67 from colonial sausage and 35 from American and colonial cheeses. The remaining 98 isolates (49%) were coagulase-negative *Staphylococci* (Pelisser *et al*., 2009).

The consumption of meat has grown significantly during the last few years, with one estimate of the global average in 2000 to be 38 kg/capita. The United States of America has the highest consumption rate of meat in the world, estimated to be around 82 kg/capita in 2000 alone. While the majority of meat is cooked before eating. The risk of disease from ingesting pathogens found in raw meat is significantly higher than cooked meat, although

both can be contaminated. Meat can be incorrectly or insufficiently cooked, allowing disease-carrying pathogens to be ingested (Smil *et al*., 2002).

Also, meat can be contaminated during the production process at any time, from the slicing of prepared meats to cross-contamination of food in a refrigerator. All of these situations lead to a greater risk of disease. There are a variety of methods used to detect and kill pathogens. The most effective, as expected, is to cook the meat to a high enough temperature to kill all growth, but meat can be re-contaminated during any step of the food production process, especially if workers handle both raw and cooked products (Li *et al*., 2005). In Slovenia in a plant for beef-slaughtering 250 smears from the surface of beef carcasses *S. aureus* was isolated on the thorax in 78 % (39/50) of the specimens, 62 % (31/50) on the front legs, 58 % (29/50) on the abdomen wall, 14 % (7/50) on the thigh and 10 % (5/50) on the neck. The established contamination of workers' hands was 50 % of specimens before the beginning of the work and 58.33 % of specimens taken after the handling of five slaughtered carcasses (Podpečan *et al*., 2007).

Large outbreaks of Staphylococcal food poisoning (SFP) are relatively rare in developed countries. In Finland, France, Japan, Korea, and the Netherlands, *Staphylococci* account for an estimated 10%, 30%, 5%, 15%, and 5% of total food borne disease outbreaks, respectively (Tremolieres, 1996*)*. In a study of food poisoning in England milk products (8%) such as cream, cheese, and custards were the most frequent products contaminated. In France among the staphylococcal food poisonings reported in a two-year period (1999- 2000), among the cases in which the food involved had been identified, milk products and especially cheeses were responsible for 32% of the cases (Haeghebaert *et al.,* 2002*)*. In the United States, among the staphylococcal food poisoning cases reported between 1975 and 1982, 1.4% were from milk products and seafoods. Thus, the origins of staphylococcal food poisoning differ widely among countries; this may be due to differences in the consumption and food habits in each of the countries. In France, for example, the consumption of raw milk cheeses is much higher than in Anglo-Saxon countries. This may explain the relative importance of milk products involved in Staphylococcal food poisoning in France (Bhatia and Zahoor, 2007).

2.2.2.1. Risk factors and sources of the infection

Staphylococci have been isolated sporadically from a wide variety of environmental sources such as soil, beach sand, seawater, fresh water, plant surfaces and products, feeds, meat and poultry, dairy products, and on the surfaces of cooking ware, utensils, furniture, clothing, blankets, carpets, linens, paper currency, and dust and air in various inhabited areas (Götz *et al*., 2006). *S. aureus* dissemination on different farms may be due to their closeness and to the community status of the milk refrigerating tank *S. aureus* can be isolated from different body parts of an animal as well as from the environment. However an infected udder quarter remains the main reservoir of the bacteria. In many occasions *S. aureus* was isolated from swabs taken from the cows head, skin swabs, legs and nasal mucosa. Furthermore *S. aureus* was found on the milkers hands as well as on the nasal mucous membrane of the humans working at the dairy farms, in bedding and the drinkers. Although *S. aureus* is transmitted mostly during the milking time, heifers are often carriers in spite of the fact they never milked (Roberson *et al.,* 1994).

The two most important sources to foods and water contamination are nasal carriers and individuals whose hands and arms are inflicted with boils and carbuncles and are permitted to handle foods (Acco *et al*., 2003; Smith and Hogan, 2001).Inadequate hygienic condition of dairy, environment, poor milking procedure, poor animal health service and lack of proper attention to health of the mammary gland were important for the contamination of milk and milk products in the dairy farms (Mekibib *et al*., 2010).

Meat is a good source of animal protein and the expectation of all consumers is to purchase meat that is safe and wholesome (Govindarajan, 1990). Meat produced in an unhygienic condition could pose threat to the health of the consumers as well as impair the keeping quality of such meat. Contamination of meat can result from contaminated working surfaces, equipments and the workers" hands used in the processing (Lues *et al*., 2007). The quality of water used in meat processing at the butcher shops also play a major role in reducing or

increasing meat contamination, as water is used in washing the working surfaces, carcasses, blood of meat, equipment and workers" hands. Bacterial contamination of meat products is unavoidable consequence of meat processing (Jones *et al*., 2008). Hygienic and quality control methods of meat and meat products, especially in food catering have been recommended in many countries. Without proper hygienic control, the environment in Abattoir's area can act as important sources of bacterial contamination (Tavakoli*et al.*, 2008).

2.2.2.2.Foods involved in Staphylococcal poisoning

Many different foods can be a good growth medium for *S. aureus*, and have been implicated in staphylococcal food poisoning, including milk and cream, cream-filled pastries, butter, ham, cheeses, sausages, canned meat, salads, cooked meals and sandwich fillings(Bergdoll, 1989). In one case, cheese was involved in an outbreak because it had been made from milk contaminated after pasteurization and before inoculation with lactic starter culture. In this particular case, the starter culture did not grow properly, resulting in a fermentation accident that allowed the *S. aureus* strain to develop and produce SE. In 1985, chocolate milk was the origin of a staphylococcal food poisoning in the Kentucky, USA. This chocolate milk was contaminated and stored at too high a temperature for 4 to 5 h, before pasteurization. Pasteurization killed the staphylococci but had no effect on the SEs. In the case of canned foods that have been correctly processed, bacteria and SEs are usually destroyed. Nevertheless, some cases of staphylococcal food poisoning involving canned mushrooms that were correctly processed were reported in the USA (Bennet, 1992).

The foods that are most often involved in staphylococcal food poisoning differ widely from one country to another. In the United Kingdom, for example, 53% of the staphylococcal food poisonings reported between 1969 and 1990 were due to meat products, meat-based dishes, and especially ham; 22% of the cases were due to poultry, and poultry-based meals, 8% were due to milk products, 7% to fish and shellfish and 3.5% to eggs (Wieneke *et al*., 1993). In France, things are different. Among the staphylococcal food poisonings reported in a two-

year period (1999-2000), among the cases in which the food involved had been identified, milk products and especially cheeses were responsible for 32% of the cases, meats for 22%, sausages and pies for 15%, fish and seafood for 11%, eggs and egg products for 11% and poultry for 9.5% (Haeghebaert *et al*., 2002). In the United States, among the staphylococcal food poisoning cases reported between 1975 and 1982, 36% were due to red meat, 12.3% to salads, 11.3% to poultry, 5.1% to pastries and only 1.4% to milk products and seafoods. In 17.1% of the cases, the food involved was unknown (Larsen *et al.,* 2000).

2.2.2.3.Transmission of Staphylococci infections

Staphylococci are 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. They can be carried on the hands of healthcare personnel and food preparers. Contaminated surfaces and medical equipments are also possible sources of staphylococci and SFP is the result of the consumption of a heat stable preformed (produced in the food) protein enterotoxins that are produced by certain strains of *Staphylococci species* (Foster, 1991; Aycicek *et al*., 2005; Bania *et al*., 2006).

Staphylococci may be expected to exist at least in low numbers, in any or all food products that are of animal origin or in those that are handled directly by humans, unless heat processing steps are applied to effect their destruction (Hobbs and Gilbert, 1981). In all cases of SFP, the foodstuff or one of the ingredients, was supposed to be contaminated with SEs producing staphylococci strain and was exposed, at least for a while, to temperatures that allow *Staphylococci* growth (Jay, 2000; Baron, 2007).

It has been found that large numbers of staphylococci must be present in foods to cause SFP. Staphylococci may grow to large and dangerous numbers in foods without causing changes in the odor, taste or physical appearance, thereby providing no warning signal to the consumer of such foods (Hagstad and Hubbert, 1986; Soomro *et al*., 2003; Bendahou *et al*., 2008). Disease transmission by staphylococci species can occur in the following settings:

gastrointestinal, skin and hair infections, systemic infections and nosocomial infections (Foster, 1991; Salyers and Shah, 2002; Todar, 2008).

2.3. Pathogenesis and clinical features

S. aureus is a pyogenic pathogen known for its capacity to induce abscess formation at sites of both local and metastatic infections. This classic pathologic response to *S. aureus* defines the framework within which the infection will progress. The bacteria elicit an inflammatory response characterized by an initial intense polymorphonuclear leukocyte (PMN) response and the subsequent infiltration of macrophages and fibroblasts. Either the host cellular response (including the deposition of fibrin and collagen) contains the infection, or infection spreads to the adjoining tissue or the bloodstream. In toxin-mediated staphylococcal disease, infection is not invariably present. For example, once toxin has been elaborated into food, staphylococcal food poisoning can develop in the absence of viable bacteria. In staphylococcal toxic shock syndrome (TSS), conditions allowing toxin elaboration at colonization sites (e.g., the presence of a superabsorbent tampon) suffice for initiation of clinical illness (Lowy, 2010).

Mastitis caused by *S. aureus* can be expressed by wide spectrum of clinical signs, from mild cases without clinical signs to extreme cases with lethal exit. Per acute form of infection is often seen as a gangrenous mastitis with lethal exit. Acute and sub acute cases resemble mastitis caused by other pathogens. These cases often lead to chronic forms of infections. Chronic and sub acute cases are the most common forms and from the herd health point of view they are the most important. In many occasions their clinical feature is characterized by elevation of somatic cells only (Benić *et al*., 2011).

Staphylococcal food poisoning is caused by the SEs in the contaminated food. The incubation period of illness ranges from 30 minutes to 8 hours, but usually 2-4 hours (CDC,

2009). The onset of symptoms depends on susceptibility to the SEs, the amount of contaminated food eaten, the amount of toxin in the food ingested and the general health of the patients. The main symptoms are nausea, vomiting, retching, abdominal cramping and prostration, often accompanied by diarrhea and sometimes fever. In severe cases, patients may present with headache, muscle cramping, severe fluid and electrolytes loss with weakness and low blood pressure or shock. Patients usually recover within two days, but can take longer in severe cases that may require hospitalization. Death following a case of staphylococcal food poisoning is very rare and may occur among the elderly, infants, and severely debilitated persons (CHP, 2011).

2.4. Principles of detection of *Staphylococci* and assays for enterotoxins

2.4.1. Principles of detection of Staphylococci

The isolation and enumeration of *Staphylococci* from clinical specimens are routine operations in the hospital and veterinary clinical laboratory. Depending upon the type of infection and contamination present, an appropriate sample is obtained accordingly and sent to the laboratory for definitive identification by using biochemical or enzyme-based tests (Götz *et al*., 2006). No specific test may be useful in every case to isolate the staphylococci from the wide variety of foods in which they are found. As a result, attempts have been made to find a combination of selective and enrichment media that will support the growth of the *Staphylococci* and at the same time suppress the growth of other micro flora present that tend to overgrow the *Staphylococci* (Bergdoll and Lee Won, 2006).

Staphylococci from a variety of sample are usually isolated in primary culture on blood agar and in a fluid medium such as thioglycolate broth. Every specimen should be plated onto blood agar (preferably sheep blood agar) and other media as indicated. On blood agar, abundant growth of most staphylococcal species occurs within 18–24 h. Since most species

cannot be distinguished from one another during this time period. A Gram stain is first performed to guide the way, which should show typical gram-positive bacteria, cocci, in clusters. Secondly, culture the organism in mannitol salt agar, which is a selective medium with 7–9% NaCl that allows *Staphylococcus (eg. S. aureus)* to grow producing yellow-colored colonies as a result of mannitol fermentation and subsequent drop in the medium's pH. Furthermore, for differentiation on the species level, catalase (positive for all "*Staphylococcus*" species), coagulase (fibrin clot formation), DNAse (zone of clearance on nutrient agar), lipase (a yellow color and rancid odor smell), and phosphatase (a pink color) tests are all done. For Staphylococcal food poisoning, phage typing can be performed to determine if the *Staphylococci* recovered from the food to determine the source of infection (Götz *et al*., 2006).

In the laboratory routine, rapid distinction of *Staphylococci* from *Micrococci* can be made by demonstrating the susceptibility of *Staphylococci* to 200 µg of lysostaphin per ml and resistance to erythromycin at 0.04µg per ml, plus the production of acid from glycerol or, alternatively, demonstrating susceptibility of *Staphylococci* to a 100 µg furazolidone disk and resistance to a 0.04-unit bacitracin disk. Furthermore, *Staphylococci*, with the exceptions of *S. lentus*, *S. sciuri* and *S. vitulus* exhibit a negative reaction with the rapid modified oxidase test, whereas micrococci are positive for this test (Baker, 1984). Rapid Diagnosis and Typing Diagnostic microbiology laboratories and reference laboratories are key for identifying outbreaks and new strains of *"Staphylococcus"*. Recent genetic advances have enabled reliable and rapid techniques for the identification and characterization of clinical isolates of " *Staphylococcus* " in real-time. 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 (Götz *et al*., 2006).

Conventional methods

Conventional methods for the determination of phenotypic characters at the cellular and population levels were developed first and then examined for their correlation to DNA

relatedness (reviewed by Kloos and Schleifer, 1986; Schleifer, 1986). Key characters now used for species and subspecies identification include the following: colony morphology (fig. 1), oxygen requirements, coagulase, clumping factor, heat-stable nuclease (thermonuclease), hemolysins, catalase, oxidase, alkaline phosphatase, urease, ornithine decarboxylase, pyrrolidonyl arylamidase, β-galactosidase, acetoin production, nitrate reduction, esculin hydrolysis, aerobic acid production from a variety of carbohydrates including D-trehalose, Dmannitol, D-mannose, D-turanose, D-xylose, D-cellobiose, L-arabinose, maltose, α-lactose, sucrose, and raffinose, and intrinsic resistance to novobiocin and polymyxin B (reviewed by Kloos and Bannerman, 1999). Some conventional methods may require up to three to five days before a final result can be obtained, while others only require several hours for interpretation. They are usually quite reliable and have served as a reference for more recent studies aimed at simplifying and expediting character analyses(Annex 1) (Götz, 2006).

2.4.2. Assays for enterotoxins

There are several procedures like animal assays, immunological, molecular biological, biosensors *etc* to detect staphylococcal enterotoxins. The production of enterotoxin needs long incubation time (20hours). Some factors, which affect the incubation period, are the pH, the water activity and the used substrates. Numerous methods are based on the evidence of the enterotoxins directly in the food (ELISA, reversal passive latex agglutination and others), with a possibility to detect nanogram amounts of enterotoxins in one gram or in one milliliter of food (Strachan, 1997).

The advantage of these methods is that enterotoxins are detected even if the producer *S. aureus* should not be identified by the classical bacteriological procedure, because it is usually devitalized by temperature. DNA amplification methods (PCR) can show the presence of enterotoxigenic strains of *S. aureus* before the expression of enterotoxins on the base of specific gene sequences and in this way detect the potential source of contamination. The advantage of the PCR methods is that it is able to detect genes which code the

production of staphylococcal enterotoxins also from heat treatment of food, because the DNA remains unchanged (Tsen and Chen, 1992). Several ELISA-based diagnostic kits for detection of staphylococcal enterotoxins are commercially available. Slight variations between the kits exist with some capable of differentiating between the enterotoxin serotypes, whereas others not having this capability. An alternative diagnostic kit that is not ELISA-based is the reversed-passive latex agglutination assay (RPLA). It uses latex particles coated with enterotoxin antibodies that agglutinate in the presence of Staphylococcal enterotoxins (Bhatia and Zahoor, 2007).

2.5. *Staphylococci* antimicrobial resistance

Antibiotic resistant *Staphylococcus* are major public health concern since the bacteria can be easily circulating in the environment. Infections due to methicillin resistant *S.aureus* (MRSA) have increased worldwide during the past twenty years (Deresinski, 2005). Multiple drug-resistance *S.aureus* have been frequently recovered from foodstuffs (Normanno *et al.,* 2007a), nasal mucosa of humans (Acco *et al.,* 2003), clinical case (Stefani and Goglio, 2010) and life stock (Wulf and Voss, 2008). As regard CNS, methacillin-resistant *S.epidermididis* (MRSE) has been increasingly found to be associated with noscomial infection. Multiple antibiotic resistant CNS was also recovered from food and potable water (Normanno *et al.,* 2007a).

Penicillin resistance is probably the most well known antibiotic resistance of *S. aureus*. Figures for *S. aureus* in the UK suggest that around 60% of strains are penicillin resistant (β-lactamases) but that resistance to other agents is uncommon (Muhammad *et al.,* 1993). The prevalence of penicillin resistance among *S. aureus* appears to have decreased in the United States in recent years (Makovec and Ruegg, 2003); by contrast, an increase in penicillin resistance was reported in Finland (Myllys *et al*., 1998).

Reported resistance levels differ considerably between countries, ranging from 20–30% for Denmark and Norway, to more than 85% for small isolate collections from Ireland (De Oliveira *et al*., 2000) and Brazil (Costa *et al.,* 2000). Antimicrobial resistance is generally least marked in Sweden and high in countries like Brazil where control over antimicrobial use is less stringent. In Portugal study conducted by Soares *et al.* (2011) the overall antimicrobial susceptibility profiles revealed that the highest percentage of resistance was detected for penicillin (30.5%). Even within countries, estimates of resistance prevalence may vary widely. For example, estimates of the prevalence of penicillin-resistance in bovine *S. aureus* from the United States range from just over 30% to more than 70% (De Oliveira *et al*., 2000).

The infection of man with bacteria resistant to antimicrobials is a cause of increasing concern. Some of these antimicrobial resistant bacteria reach man in food and particularly in food of animal origin such as milk and meat to cause disease directly or act as potential sources of antimicrobial resistance for human pathogens (Haveri *et al.,* 2005). *S. aureus* may be present in milk and milk products as a result of milk collected from the animal suffering from disease condition and excreting *S. aureus* in milk or due to unhygienic conditions during production, processing, storage and handling of milk products, which are the main causes of food borne diseases. Unpasteurized milk contains a wide range of bacteria, principally those causing mastitis, but also the faecal flora arising from udder contamination. The main carriers of antimicrobial resistance in milk are the S*taphylococci.* Some of this resistance is transferable. Consumption of raw milk and raw milk products and meat carries a risk of food poisoning, but also a potential risk of acquiring antimicrobial-resistant milk and meat flora (Jones, 1987). The presence of up to 50,000 bacteria per ml in milk even at the most recent EU quality standards means that unpasteurized milk used for any purpose must be considered as a source of antimicrobial resistance for the consumer. This antimicrobial resistance has been documented by a number of workers (Jousimies-Somer *et al.,* 1996; Haveri *et al.,* 2005).

Of the gram-positive bacteria, CNS had the highest prevalence of antimicrobial resistance. Coagulase-negative staphylococci were more often resistant to penicillin, methicillin, macrolides and lincosamides than *S. aureus* described in monitoring of antimicrobial resistance and antibiotic usage in animals in The Netherlands (MARAN, 2007). These results were similar to what was found in Finland and Germany. Most studies analyzed the development of resistance in CNS with phenotypic tests only and without species differentiation, or in CNS differentiated with commercial phenotypic tests of unknown quality (Lüthje and Schwarz, 2006; MARAN, 2007). Knowledge of resistance of CNS (species) is therefore limited. Of 170 isolates from milk samples of mastitis cases, 51 (30%) CNS was phenol typically resistant to penicillin (Sampimon *et al*., 2009).

Multiple resistances (resistance to at least two antibiotics), 30 (28.6%) isolates are multi-resistant with strains of *S. equorum* and *S. saprophyticus* reaching up to six antibiotic resistances per strain, whereas, *S. epidermidis* (26.7%) was resistant to a maximum of three antibiotics. Multiple resistant strains even included the strains of *S. aureus, S. sciuri* and *S. warneri* (Soares *et al.,* 2011). A *S. aureus* isolated from a total of 25 (21.73%) of the 115 samples milk samples from cattle and tested using Kirby-Bauer disk diffusion method for their antimicrobial susceptibility to 15 different antimicrobial drugs revealed almost 80-90% of the isolates were showed multiple drug resistance to majority of the antimicrobial agents tested (Sharma *et al.,* 2011). Resistant bacteria may transfer resistance genes to other bacteria and become important in the spread of antibiotic resistance. Indiscriminate use of antimicrobial agents and antibiotic sale behavior (for example, sale of antibiotics without prescription, sale of under dose and substituting brands) enhances the development of drug resistance (Ombui *et al*., 2000).

The uncontrolled application of antimicrobials in the environment is leading to a constant increase in the rate of antimicrobial resistance among community-acquired *Staphylococci* (Harakeh, 2006; Harakeh *et al*., 2005). *Staphylococci* species can rapidly acquire resistance to a broad range of antimicrobials, thereby posing a major concern in the treatment of

Staphylococcal infections (Bozdogan *et al*., 2004). Studying antimicrobial resistance in humans and animals is important for detecting changing patterns of resistance, implementing control measures on the use of antimicrobial agents and preventing the spread of multidrug-resistant strains of bacteria (van Duijkeren *et al*., 2003).

2.6. Management, prevention and control

There is no significant growth of *Staphylococci* at temperature below 4.40C and the organisms are destroyed when kept at 77^0C for 20 minutes (Ash, 2008). Storing foods at temperature less than 4.4^0C or greater than 60^0C effectively prevents replication of staphylococcal organisms and significant toxin production (Hocking and Doyle, 1997; Salyers and Whitt, 2002; Ash, 2008).

2.6.1. Treatment of Staphylococcal infections

The objective of treatment in human patients is to replace fluids, salt, and minerals that are lost by vomiting or diarrhoea (Foster, 1991; Sandel and McKillip, 2004). The choice of antimicrobial agents to treat both coagulase positive staphylococcal and CNS infections has become increasingly problematic because of the prevalence of multidrug-resistant strains. Staphylococcal resistance has increased to most antibiotic families, including lactams, amino glycosides, fluoroquinolones, and (to a lesser extent) glycopeptides (Lowy, 2010).

Some strains of *Staphylococcus* have acquired genes making them resistant to multiple antimicrobial agents. Both plasmid(s) and the chromosome have been found to confer resistance in *S. aureus* (Hui *et al.,* 2001).These organisms are uniformly resistant to penicillins and cephalosporins. Penicillinase resistant penicillins such as oxacillin and flucloxacillin are used for serious infections. First or second generation cephalosporins such as cephalothin, cephalexin and cefuroxime are usually safe in patients who are hypersensitive

to penicillins. Vancomycin is usually effective for methicillin-resistant staphylococci. Erythromycin and its newer relatives are used in milder infections. The infections can also be treated with combination therapy using sulfa drugs and minocycline or rifampin (Kloos and Bannerman, 1994; Rho and Schaffner, 2007).

2.6.2. Prevention and control of Staphylococcus infection and intoxication

The *Staphylococci* are ubiquitous organisms that cannot be eliminated from our environment. At least 30–50 % of individuals carry these organisms in their nasal passages or throats, or on their hands. Any time a food is exposed to human handling; there is the possibility that the food will be contaminated with *Staphylococci.* Not all of these may be enterotoxin producers, but 30–50 % may well be. Heating of the food after handling will normally assure against food poisoning unless the food has been held unrefrigerated for several hours before the heating; if enterotoxin has formed in the food, the heating might not be sufficient to destroy it. The course of action recommended is to keep susceptible foods refrigerated at all times except when being prepared and while being served. Refrigeration should be carried out in such a manner as to facilitate quick cooling of the entire food mass. If it is impossible to guarantee that a susceptible food will be kept refrigerated, special care should be taken in its preparation to avoid contamination if at all possible *(*Bergdoll1 and Lee Wong et al., 2006).

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. Study area

The study was conducted in central Ethiopia: Assela, Adama, Bishoftu, Addis Ababa and Holeta.

Bishoftu: Bishoftu is located in Oromia National Regional State about 45 km South-east of Addis Ababa, just on the escarpment of the Great Rift Valley and the geography of the area is marked by creator lakes. It is found at 9^0N latitude and 40^0E longitude and at an altitude of 1850 meters above sea level in the central high lands of Ethiopia. It has a human population of about 95,000. It experiences a bimodal pattern of rainfall with the main rainy season extending from June to September (of which 84% of rain is expected) and a short rainy season from March to May with an average annual rainfall of 800mm. The mean annual minimum and maximum temperatures are 12.3 ^{0}C and 27.7 ^{0}C, respectively, with an overall average of 18.7 ^{0}C. The highest temperatures are recorded in May and the mean relative humidity is 61.3%. Bishoftu is the center of Ada'a Liben Woreda and it has a total land area of about 1610.56 Km2 and is divided in to three agro-ecological zones namely midland (94%) highland (3%) and lowland (3%) (CSA, 2003).

Assela: Assella town, the capital of Arsi zone, is located Oromia region, South Eastern Ethiopia, at about 175 km Southeast of Addis Ababa at 6° 59' to 8° 49' N latitudes and 38° 41' to 40° 44' E longitudes with an altitude of the area ranges from 2500 to 3000metre above sea level. Agricultural production system of the study area is of mixed crop and livestock production. Dairy farming using improved breeds is a common practice in urban and peri-urban areas (KARC, 2008). The farming system is semi-intensive that run small to medium sized with up to 100 milking cows, most of which are cross- breed among Holstein-Friesian, Jersey and local Arsi breed introduced by the artificial insemination program and exotic

breeds, since the establishment of CADU (Chilalo Agricultural Development Unit) in the mid- 1960s by the Swedish- funded integrated rural development in Africa (Halderman, 2004).

Addis Ababa: Addis Ababa is the capital city and administration center for the Federal Democratic Republic of Ethiopia. Currently, there are 10 subcities "*Kifle Ketemas*" in Addis Ababa city administration delineated on the basis geographical set up, population density, asset and service providers' distribution and convenience for administration (AACA, 2004). Addis Ababa is situated at latitude of 9°3'North and 38°43'East (ILCA, 1994). It lies in the central highlands of Ethiopia at an altitude of 2500 m.a.s.l. It has an average rainfall of 1800 mm per annum. The annual average maximum and minimum temperature is 26°C and 11°C, respectively; with an overall average of 18.7°C. Highest temperatures are reached in May. The main rainy season extends from June to September. Addis Ababa has a relative humidity varying from 70% to 80% during the rainy season and 40% to 50% during the dry season. The human population is estimated at about 3 million inhabitants (NMSA, 1999).

Adama: Adama district located in the Rift Valley, about 95 Km southeast of Addis Ababa (8.33°N and 39.17°E) with an altitude of 1622m above sea level. It receives an annual rainfall ranging from 400 to 800mm. The temperature range is 13.9 to 27.7°C (NMSA, 2006). Adama is one of the most populous townships in the country with a significant number of households engaged in smallholder dairying (Mekonnen and Tesfaye, 2010).

Holota: Holeta is located at 40km west of Addis Ababa and at an elevation of 2400m.a.s.l in the central Ethiopia ($9^0$3'N and $38^0$30'E). The area is characterized by mild subtropical weather, with average minimum and maximum annual temperatures of 6.3^{o}C and22.1^{o}C, respectively. The area also experience bimodal rain fall pattern with a long rainy season extending from July to September while the short rainy season extends from March to April (CSA, 2004).

3.2. Type and origin of samples

Udder milk, raw bovine, bulk buckets milk of farms, swab samples of carcass from abattoir, from milk container (bucket and farm tank), knife, slaughter line, hand and nasal of butcher and milker's were collected prior to milking in this study.

3.3. Study populations and sample size

The study animals for this study represent cattle and cattle derived foods. The breeds include Holiestin Frisian (HF), cross, Borana and zebu breed lactating dairy cows managed under intensive and semi intensive. Moreover, dairy personnel and milking utensil (milking bucket and milk storage in farm), meat, slaughter equipment (knife and slaughter line) and abattoir workers were also included.

The sample size for this study is determined by the following formula given by Thrusfield (2007).

$$n = \frac{1.96^2 \times P_{exp}(1-P_{exp})}{d^2},$$

Where 1.96 = the value of Z at 95% confidence interval, d =desired absolute precision, n=required sample size, and P_{exp}=expected prevalence

Therefore, by using the above formula and taking in to account 95% confidence interval, desired absolute precision of 5% and an expected prevalence as shown in table 2.

Table 2: Expected prevalence and sample size of the study.

	Items to be sampled	Known prevalence	Reference	Sample size
1	Dairy Cattle	43.6%*	[1]	378
2	Abattoir	28%	[2]	310

*The average prevalence synthesized from different authors.

[1] = Lakew *et al*. (2009), Mekonnen and Tesfaye(2010), Alehegn (2008) and Abunna *et al*. (2013), Mekibib *et al*. (2010);

[2] = Mekonnen *et al*. (2013);

Using the above formula, the study involved 1,001 samples.

3.4. Study design

A cross- sectional study was conducted from October 2013 to May 2014 which was used to determine the bacteriological analysis of *Staphylococcus* in cattle, in foods of cattle origin, equipments, and humans. Sampling was carried out regularly from abattoir and samples of raw milk are pooled at the farm on cow basis. Pooled raw milk samples were taken from the udder of each farm and farm milking bucket.

3.5. Sampling method

The five districts in this study were selected purposively due to concentration of dairy farms, food chains and their cosmopolitan nature of the districts in human settlement. Using simple random sampling technique the fifty-three study dairy farms and five abattoirs from each study area were incorporated in the study. On the average five cows from each respective herd/farm was included in the study population. The study involved total of 1,001sample from both abattoir (beef-meat) and farms (dairy cows) of which 297 udder milk, 50 tank milk of the selected dairy farms, 50 milking bucket swab, 50 farm bucket swab, 67 hand and nasal swab of milkers, 361 carcass swab, 37 knife swab, 37 slaughter line swab, 52 hand and nasal swab of butchers. Besides sample collection for bacteriology, a structured questionnaire survey was employed on 53 farms and 108 individuals on dairy cow owners to collect epidemiological information.

3.6. Data collection and transportation

3.6.1. Questionnaire survey

In this study basic farm data, information on personnel, educational status, farm animal population structure, management factors of the farms, data on milk handling hygiene and storage were collected through a structured questionnaire. A structured questioner was

prepared and pre-tested. The collect data was tabulated, classified and further categorized for systematic and suitable statistical analysis (Annex-1).

3.6.2. Collection of sample from farm

During data collection breed, age, sex, parity level, stage of lactation, husbandry system, udder health status and previous history of mastitis were recorded. Age of the study animals were determined from birth records or from owner and categorized according to Mekibib *et al.(*2010) as young (3 to 6 years), adults (7 to 10 years), and old (>10 years). Parity was also categorized as < 4 calves, 4-7 calves and >7 calves. Stage of lactation, early (one month), middle (two to four months), late (over five months).

Raw milk samples were collected from two critical control points (directly from the cows' udder at farm level, from the milking bucket at farm level) together with milkers' and container swab that were considered to be associated with the hazard, when a measurement can be conducted and when control measures can be taken in order to reduce the hazard to an acceptable level. The swab samples were collected before milk sampling by using sterile swabs.

Raw milk samples were collected aseptically from individual udder milk and milking buckets of the farm. In those herds, milk samples were collected from all four udder quarters of each cow. Udders and teats were cleaned with warm water, left to dry, and wiped with cotton buds soaked in 70% alcohol, paying particular attention to the teat orifices. Pooled raw milk samples were collected aseptically after thoroughly mixing the milk. Pooled swab of milker hands, milker nasal and milking container samples were collected aseptically before milking. Primarily, farmers were informed as samples would be collected, and so as to get their normal pre-milking preparations, but just after they completed preparations all the required samples were collected. At the end of the milking procedure, bulk tank milk samples (farm milking bucket) (100 mL) were collected in sterile screw capped bottles (NMC, 1990; Quinn *et al*., 2002).

3.6.3. Collection of sample from abattoir

The abattoir samples were collected repeatedly and pooled carcasses swab samples comprising neck, foreleg; thoracic, abdomen and hind leg swab were collected from the slaughter house. In addition, samples from equipments of the slaughter house were collected comprising surface swabs taken from the surface of meat-cutting equipment such as knives, from slaughter line and from human nasal and hand swab of butcher.

For every swab sample, a sterile test tube filled with 4ml of buffered peptone water (BPW) was used. Prior to sampling, swab tips were moistened in the BPW and for swab sampling the swab were rotated and rubbed against the sampled surface several times. After completion of swabbing, the swab was put inside into a test tube containing BPW.

Accordingly, 514 samples from farm (347raw-milk and 167 swab of milking containers and milker hands and nasal) and 487 samples from abattoir (126 equipment samples from the slaughter house and hand and nasal swab of butcher and 361carcasses swab) were collectd. All animal (food), equipment (swab) and utensile (swab) and human (swabs) samples were aseptically collected in a sterile container using disposable gloves to avoid contamination. Each sample was labeled with necessary information, including date of sampling, type of sample, source of samples (farm and abattoir) and identification of the animal.. At all levels of sampling, the sampling bottles were capped, labeled with a permanent marker and stored in an ice packed cool box and transported to the Microbiology laboratory at the College of Veterinary Medicine and Agriculture, Addis Ababa University, Debre Zeit and Assela regional laboratory for bacteriological analysis.

3.7. Isolation and identification of *Staphylococci*

Final identification of *Staphylococci* organisms and species was done based on culture characteristics, Gram staining, series of biochemical tests for example catalase test, coagulase test and sugar fermentation as described by Quinn *et al*. (2002).

3.7.1. Culturing and colony appearance

All the samples was directly streaked onto 7% sheep blood agar and incubated aerobically at 37^0C for 24–48 hours. The bacteriological media used was prepared according to the manufacturer's recommendations. The plates were examined for the presence of *Staphylococcus* colonies. Isolates supposed to belong to *Staphylococcus* species on the basis of their morphological aspects (round, smooth and white or yellow colonies) and haemolytic pattern on the surface of BAP were collected. Presumed staphylococcal colonies were then sub-cultured on nutrient agar plates (NAP) and incubated at 37^0C for 24-48 hours to get a pure culture (clone of cells derived from a single cell). After growth of presumptive colonies were identified by using conventional bacteriological techniques on the basis of colony characteristics, pigment production and hemolysis. Final identification of the organisms and species was done based on Gram staining, catalase test, O-F glucose test, oxidase test, sugar fermentation and coagulase test (by using rabbit plasma). Pure cultures of a single colony type from the NAP were inoculated into nutrient slants and incubated at 37^0C for 24-48 hours under aerobic culture conditions. The pure isolates in the nutrient slant were preserved and maintained at 4^0C for further need (Quinn *et al*., 2002).

3.7.2. Gram's staining

All suspected cultures of *Staphylococci* species were subjected to Gram's stain and observed under a light microscope for Gram's reaction, size, shape and cell arrangements. The Gram stained smears from typical colonies that showed Gram-positive cocci occurring in bunched, grapelike irregular clusters were taken as presumptive *Staphylococcus* species Quinn *et al*. (2002).

3.7.3. Catalase test

The center of an 18/24hour pure colony of the isolates were picked using a sterile loop form the nutrient agar plate and mixed with a drop of 3% H2O2 on a clean glass slide. If the

organism positive, bubbles of oxygen liberated within a few seconds and the catalase negative isolates did not produce bubbles. The catalase positive cocci were considered as *Staphylococci* (Quinn *et al*., 2002).

3.7.4. Mannitol salt agar

The colonies that were identified by Gram-staining reaction, O-F glucose, oxidase and catalase test as *Staphylococcus* were streaked on MSA plates and incubated at 37^0C 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 discolouration 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 (Quinn *et al*., 2002).

3.7.5. 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 37^0C for 24 hours to 0.5 ml of fresh rabbit plasma. After mixing by gentle rotation, the tubes were incubated at 37^0C 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(Quinn *et al*., 2002).

3.7.6. 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 (a deeper purple) around the colonies(Quinn *et al*., 2002).

3.8. Antimicrobial susceptibility testing

Positively identified *Staphylococci* strains were tested for their susceptibility to different antimicrobials using the disk diffusion method with incubation at 37 °C overnight (Wikler, 2008). Fifteen different antimicrobials drugs (Oxoid, Hampshire, England) were used: Amoxycillin (AML-25 µg), Cefoxitin (FOX-30µg), Chloramphenicol (C-30µg), Ciprofloxacin (CIP-5µg), Cloxacillin(OB-5µg), Erythromycin (E-15µg), Gentamicin (CN-10µg), Kanamycin(K-30µg), Nalidixic acid (NA-3030µg), Nitrofurantoin(F-50µg), Penicillin G (P-10U), Streptomycin (S-10µg), Sulphamethoxazole Trimethoprim(SXT-25µg), Tetracycline (TE- 30µg) and Vancomycin(VA-30 µg). Well isolated colonies of the same morphological type were selected from a non selective agar plate and suspension was made in sterile saline. The turbidity of the suspension was adjusted by comparison with a 0.5 McFarland turbidity standard. A sterile swab was dipped into the standardized suspension of bacteria and excess fluid was expressed by pressing and rotating the swab firmly against the inside of the tube. The swab was streaked in three directions and continuously brushed over the Mueller hinton agar and inoculated plates were allowed to stand for 3-5 minutes. The discs were placed onto the agar surface using sterile forceps and gently pressed with the point of a sterile forceps to ensure complete contact with the agar surface and the plates were incubated aerobically at 37°C for 16hour –18hour for all disc except for Vancomycin 24hour

(CLSI, 2007; CLSI, 2012; Oxoid, Hampshire, England). Inhibition zone diameters were measured and values obtained from the Clinical laboratory standard institute (CLSI, 2012; CLSI, 2013) and manual of the manufacturer (Oxoid) were also used to interpret the results obtained. The isolates were then classified as resistant, intermediate or susceptible to a particular antibiotic based on the cut-off value as indicated in Table 3. Multiple antibiotic resistant (MAR) phenotypes were recorded for isolates showing resistance to three and more antibiotics (Rota, 1996).

Table 3: Zone diameter interpretive standards for *Staphylococci species.*

Antimicrobial agent	Disc code	Potency	Zone diameter nearest whole mm		
			S	I	R
Amoxicillin	AML	25µg	≥20		≤19
Cefoxitin	FOX	30µg	≥22*	-	≤21*
			≥25**		≥24**
Chloramphenicol	C	30µg	≥18	13-17	≤12
Ciprofloxacin	CIP	5µg	≥21	16-20	≤15
Cloxacillin	OB	5µg	13**	11-12	10**
			18*		17*
Erythromycin	E	15µg	≥23	14-22	≤13
Gentamicin	CN	10µg	≥15	13-14	≤12
Kanamycin	K	30µg	≥18	14-17	≤13
Nalidixic acid	NA	30µg	≥19	14-18	≤13
Nitrofurantoin	F	50µg	≥17	15-16	≤14
Penicillin G	P	10UNITS	≥29	-	≤28
Streptomycin	S	10µg	≥15	12-14	≤11
Sulphamethoxazole trimethoprim	SXT	25µg	≥16	11-15	≤10
Tetracycline	TE	30µg	≥19	15-18	≤14
Vancomycin	VA	30µg	≥15		≤15***

For *S. aureus* and *S.lugdunensis*. *For CNS except *S.lugdunensis*. * All *Staphylococci* for which Vancomycin 14mm or less should be tested by reference MIC method (CLIS, 2007).

S=susceptible, I=intermediate, R=resistance

3.9. Data management and analysis

The data collected through questionnaire survey and laboratory results of the collected samples were entered into Excel databases and analyze using Epi-info version 3.5.1 and SPSS version 20 software programs (for fisher's exact). Descriptive statistics such as percentages and frequency distribution was used to describe the nature and the characteristics of the data (to describe/present bacterial isolates and antimicrobial susceptibility which was expressed as percent/percentile of resistant, intermediate and susceptible). In addition, the proportion of bacteria resistant to at least one of the fifteen antibiotics and resistant two or more were calculated. Moreover, comparison between each geographical study area, sample source, sample type, study subject against antimicrobial susceptibility was done by fisher's exact or Chi-square (χ^2). Logistic regression was used to reveal the strength of the association of the potential risk factors with positivity of sample. In this line, the degree of association between risk factors and the prevalence of *Staphylococcus* was analyzed using test odds ratio (OR). In all the analysis, the level of significance was set at 5% and at the 95% confidence interval.

4. RESULTS

4.1. Overall prevalence and distribution of *Staphylococcus* isolates in the farm, abattoir and humans using different sample types of central Ethiopia

Presence of *Staphylococci* was detected in 428 out of 1,001 analyzed samples. The contamination rate of the sample from farm was significantly higher than that of the sample from abattoirs (P=0.02) having prevalence 45.9% (236/514) (with 95%CI of 41.6, 50.3) and 39.4% (192/487) **(**with CI 35.1, 43.9), respectively (Table 4).

The results of biochemical characterization of these isolates showed that *S. aureus* was the most frequently isolated species among different samples types accounting for 172 (17.2%) the population studied. The other *Staphylococci* isolates were distributed as *S. hyicus* 82 (8.2%), *S. intermedius* 74 (7.4%) and CNS 96 (9.6%) (Table 4).

Table 4: Prevalence and distribution of S*taphylococcus* isolated from cattle, food, human and material swab of central Ethiopia

Sample source &type	***S.aureus***			***S.intermidise***			***S.hycus***			**CNS**			**Total**		
	N	N	P%	CI %	n	P%	CI %	n	P%	CI %	n	P%	CI %	n	P%	CI %
Farm	**514**	**115**	**22.4**	**18.9,26.3**	**39**	**7.6**	**5.5,10.3**	**39**	**8**	**5.5,10.3**	**41**	**8**	**5.9,10.8**	**236**	**45.9**	**41.6,50.3**
UM	297	67	22.6	17.9,27.7	22	7.4	4.7,11	23	8	5,11.4	27	9.1	6.1,13	140	47.1	41.3,53
TM	50	14	28	16.2,42.5	4	8	2.2,19.2	8	16	7.2,29.1	3	6	1.3,16.5	29	58	43.2,71.8
TS	50	10	20	10,33.7	3	6	1.3,16.5	1	2	0.1,10.6	4	8	2.2,19.2	19	38	24.7,52.8
BS	50	10	20	10,33.7	3	6	1.3,16.5	1	2	0.1,10.6	3	6	1.3,16.5	17	34	21.2,48.8
FNS	17	4	23.5	6.8,49.9	3	17.6	3.8,43.4	3	19	4,45.6	2	11.8	1.5,36.4	12	70.6	44,89.7
FHS	50	10	20	10,33.7	4	8	2.2,19.2	3	6	1.3,16.5	2	4	0.5,13.7	19	38	24.7,52.8
Abattoir	**487**	**57**	**11.7**	**9.1,15**	**35**	**7.2**	**5.1,9.9**	**43**	**9**	**6.5,11.8**	**55**	**11.3**	**8.7,14.5**	**192**	**39.4**	**35.1,43.9**
CS	361	38	10.5	7.6,14.3	29	8	5.5,11.5	33	9	6.5,12.7	38	10.5	7.6,14.3	139	38.5	33.5,43.8
KS	37	5	13.5	4.5,28.8	2	5.4	0.7,18.2	5	14	4.5,28.8	2	5.4	0.7,18.2	14	37.8	22.5,55.2
SLS	37	7	18.9	8,35.2	1	2.7	0.1,14.2	2	5	0.7,18.2	7	18.9	8,35.2	18	48.6	31.9,65.6
ANS	15	0	0	0,21.8	2	13.3	1.7,40.5	2	13	1.7,40.5	3	20	4.3,48.1	7	46.7	21.3,73.4
AHS	37	7	18.9	8,35.2	1	2.7	0.1,14.2	1	3	0.1,14.2	5	13.5	4.5,28.8	14	37.8	22.5,55.2
Over all	**1001**	**172**	**17.2**	**14.9,19.7**	**74**	**7.4**	**5.9,9.2**	**82**	**8.2**	**6.6,10.1**	**96**	**9.6**	**7.9,11.6**	**428**	**42.8**	**39.7,45.9**

Key: UM=udder milk, TM=tank milk, BS=bucket swab, TS=tank swab, FHS=farm hand swab, FNS=farm nasal swab, AAH=abattoir hand swab, ANS=abattoir nasal swab, CS=Carcass swab, KS=Knife swab, SLS=slaughter line swab, N=sample size, n=number of positive, p%=prevalence in percentage

4.2. Area and bacteria species specific prevalence and distribution of *Staphylococci* isolated from in the farm, abattoir and humans using different sample types

The prevalence of *Staphylococci* has no statically significant difference among geographic region of the area sampled. Out of 253 samples in Bishoftu, 103 (40.7%) were *positiv*e for *Staphylococci* (Table 5). The 103 *Staphylococci* isolates were comprised of 41.75% (43/103) *S. aureus,* 19.4% (20/103) *S. intermedius*, 15.5% (16/103) *S. hycius* and 19.4% (20/103) CNS. The identification results showed a predominance of CPS (i.e. *S. aureus, intermedius* and *hycius*) with a total of 76.7% (79/103) isolates (Data not shown).

In Holeta: Out of 194 analyzed samples 78 (40.2%) were positive for *Staphylococcus* bacteria. In the area *S. aureus* was more prevalent than other *Staphylococci species*. Tanks milk was the most contaminated with *Staphylococci* with a prevalence of 60% (6/10) followed by Slaughter line swab and udder milk with 50% (4/8) and 46.7% (28/60) prevalence, respectively (Table 5).

In Adama**:** Out of the total 180 samples, 67 (37.2%) were positive for *Staphylococcus* bacteria (Table 5). *S.aureus* was the leading in predominance with a total of 16.3% (33/67) isolates, comprised of 37.2% (67/108)of the total isolates of *Staphylococci species* followed by *S. intermidius* 20.9% (14/67), CNS 17.9% (12/67)and *S. hicus* of 11.9% (8/67) (data not shown).

Assela**:** Amongst the 181 samples from Abattoir, Farm and Human *Staphylococci* were found in 88 of samples. There by, the proportion of positive samples ranged from 2.2 % (2/9) in milker hand swab to 88.9% (8/9) in milker nasal swab (Table 5).

In Addis Ababa: out of the total 193 samples taken, 92 (47.7%) proved to be positive (Table 5). Out of 92 bacteria isolated were *S. aureus* occupy 33.7% (31/92), *S. intermidus* 22.8% (21/92) (10.9), *S. hyicus17.4% (*16/92)and 26.1% (24/92)(data not shown).

Table 5: Prevalence and distribution of S*taphylococci* isolated from food, human and material swab of Holeta, Addis Ababa, Bushoftu, Adama and Assela

sample & type source	**Holota**			A.A			Bishoftu			Adama			Assela		
	N	P(n(%))	CI	N	P(n(%))	CI	N	P(n(%))	CI	N	P n(%)	CI	N	P(n(%))	CI
FA	100	43(43)	33.1,53.3	72	36(50)	38,62	166	82(49.4)	41.6,57.3	89	37(41.6)	31.2,52.5	87	38(43.7)	33.1,54.7
UM	60	28(46.7)	33.7,60	40	20(50)	33.8,66.2	106	51(48.1)	38.3,58	48	22(44.9)	30.7,59.8	42	19(45.2)	29.8,61.3
TM	10	6(60)	26.2,87.8	8	4(50)	15.7,84.3	15	12(80)	51.9,95.7	8	4(50)	15.7,84.3	9	3(33.3)	7.5,70.1
TS	10	3(30)	6.7,65.2	8	4(50)	15.7,84.3	15	8(53.3)	26.6,78.7	8	1(12.5)	0.3,52.7	9	3(33.3)	7.5,70.1
BS	10	3(30)	6.7,65.2	8	3(37.5)	8.5,75.5	15	4(26.7)	7.8,55.1	8	4(50)	15.7,84.3	9	3(33.3)	7.5,70.1
NS	0	0	0	0	0	0	0	0	0	8	4(50)	15.7,84.3	9	8(88.9)	18.4,90.1
HS	10	3(30)	6.7,65.2	8	5(62.5)	24.5,91.5	15	7(46.7)	21.3,73.4	8	2(25)	3.2,65.1	9	2(2.2)	2.8,60
AA	94	35(37.2)	27.5,47.8	121	56(46.3)	37.2,55.6	87	21(24.1)	15.6,34.5	91	30(33)	23.5,43.6	94	50(53.2)	42.6,63.6
CS	69	24(34.8)	23.7,47.2	103	45(43.7)	33.9,53.8	54	11(20.4)	10.6,33.5	69	24(34.8)	23.7,47.2	66	35(53)	40.3,65.4
KS	8	3(37.5)	8.5,75.5	6	5(83.3)	35.9,99.6	11	0	0,28.5	5	1(20)	0.5,71.6	7	5(71.4)	29,96.3
SLS	8	4(50)	15.7,84.3	6	5(83.3)	35.9,99.6	11	5(45.5)	16.7,76.6	5	1(20)	0.5,71.6	7	3(42.9)	9.9,81.6
NS	1	1(100)	100,100	0	0	0	0	0	0	7	2(28.6)	3.7,71	7	4(57.1)	51.8,99.7
HS	8	3(37.5)	8.5,75.5	6	1(16.7)	0.4,64.1	11	5(45.5)	16.7,76.6	5	2(40)	5.3,85.3	7	3(42.9)	9.9,81.6
OA	**194**	**78(40.2)**	**33.2,47.5**	**193**	**92(47.7)**	**40.4,55**	**253**	**103(40.7)**	34.6,47	**180**	**67(37.2)**	**30.1,44.7**	**181**	**88(48.6)**	**41.1,56.1**

Key: UM=udder milk, TM=tank milk, BS=bucket swab, TS=tank swab, FHS=farm hand swab, FNS=farm nasal swab, AAH=abattoir hand swab, ANS=abattoir nasal swab, CS=Carcass swab, KS=Knife swab, SLS=slaughter line swab, N=sample size, n=number of positive, p%=prevalence in percentage, FA=farm over all, AA=abattoir over all, OA=over all

4.3. Association between some of the risk factors with occurrence of *Staphylococci* in the central Ethiopia

Prevalence of *Staphylococci* were significantly higher ($p<0.02$) in farms with prevalence 45.9% than abattoirs (39.4%). Sample types also influences significantly the prevalence of *Staphylococci* in central Ethiopia and had no statistical significance association with the geographical area from where sample were collected. Similarly sample source and sample type had statistically significant association with the prevalence of *S. aureus in central Ethiopia* ($P<0.05$). Whereas area has no statistical association with the prevalence of *S. aureus* ($p>0.05$) (Table 6).

Table 6: Association between some of the factors with occurrence of *Staphylococci* in dairy farm, abattoir and human in the central Ethiopia

Risk factors		N	Over all *Staphylococci species*							*S.aureus*						
			Prevalence	X^2	df	P-value	OR	95%CI	P-val	Prevalence	X^2	df	P-value	OR	CI%	P-val
Sample source	Abattoir	487	92(39.4)	4	1	0.02	1	ref		57(11.7)	19.3	1	0	1	ref	
	Farm	514	236(45.9)				1.3	1.,1.7	0.04	115(22.4)				2.17	1.5,3	0
Sample type	AHS	37	14(37.8)	18	10	0.04	1	ref		7(18.9)	26.3	10	0	1	ref	
	ANS	15	7(46.7)				1.44	0.4,4.8	0.56	0				0	0	0.96
	BS	50	17(34.4)				0.85	0.3,4.8	0.71	10(20)				1.07	0.4,3.1	0.9
	FHS	50	19(38)				1.01	0.4,2.4	0.99	10(20)				1.07	0.4,3.1	0.9
	FNS	17	12(70.6)				3.94	1.1,13	0.03	4(23.5)				1.32	0.3,5.3	0.7
	KS	37	14(37.8)				1	0.4,2.5	1	5(13.5)				0.67	0.2,2.3	0.53
	MS	361	139(38.5)				1.03	0.5,2.1	0.94	38(10.5)				0.5	0.2,1.2	0.13
	SLS	37	18(48.6)				1.56	0.6,3.9	0.35	7(18.9)				1	0.3,3.2	1
	TM	50	29(58)				2.27	0.9,5.4	0.06	14(28)				1.67	0.6,4.7	0.33
	TS	50	19(38)				1.01	0.4,2.4	0.99	10(20)				1.07	0.4,3.4	0.9
	UM	297	140(47.1)				1.47	0.7,2.9	0.29	67(22.6)				1.25	0.5,2.9	0.62
Area	A.A	193	92(47.7)	7.6	4	0.1	1	ref		31(16.1)	0.55	4	0.97	1	ref	
	Adama	180	67(37.2)				0.65	0.4,0.9	0.04	33(18.3)				1.17	0.7,2	0.56
	Assela	181	88(48.6)				1.03	0.7,0.9	0.85	33(18.2)				1.16	0.7,1.9	0.58
	Bushoftu	253	103(40.7)				0.75	0.5,1.1	0.14	43(17)				1.07	0.6,1.8	0.79
	Holeta	194	78(40.2)				0.74	0.5,1.1	0.14	32(16.5)				1.03	0.6,1.8	0.91

Key: UM=udder milk, TM=tank milk, BS=bucket swab, TS=tank swab, FHS=farm hand swab, FNS=farm nasal swab, AAH=abattoir hand swab, ANS=abattoir nasal swab, CS=Carcass swab, KS=Knife swab, SLS=slaughter line swab, P-val= P-value

4.4. Association between some of the risk factors with occurrence of *Staphylococci* in udder milk in the central Ethiopia

Association of different potential risk factors with *Staphylococcus* prevalence in udder milk was checked by chi-square test. The results of association of risk factors with *Staphylococci* were indicated in Table 7.

Prevalence of *Staphylococcus* related to specific risk factors were determined as the proportion of affected udder milk out of the total examined. As indicated in Table 7 family size of farm and income level of the farm were found to behaving statistically significant difference on the prevalence of *Staphylococcus* in udder milk ($P<0.05$). Accordingly, the likelihood of the isolation of *Staphylococcus* from udder milk cows was 3.197 times higher in family size having > 10 than < 5 family members. Udder milk from HF had higher risk of isolation with *Staphylococcus* (59.7%) than cross (43.8%) and Arsi breed (33.3%) which had significant difference on prevalence of *Staphylococcus* ($P< 0.05$). Herd size of the farm and lactation stage was also assessed in relation to the *Staphylococcus* prevalence. There were Higher *Staphylococcus* rate (54.2%) was recorded during the early lactation stage as compared to mid lactation stage that accounted for 40.8% and for late lactation 55.7% also farm having greater than 50 herd size were more affected than those with fewer and moderate herd size (Table 7).

Table 7: Association between some of the factors with occurrence of *Staphylococci* in udder milk in the central Ethiopia

Risk factors		N	+ve	%	x2	df	p-value	OR	CI (%)	P-Value
Area	A.A	40	20	50	0.34	4	0.987	1	ref	
	Adama	49	22	44.9				0.814	0.35,1.88	0.631
	Assela	42	19	45.2				0.826	0.35,1.97	0.666
	Bushoftu	106	51	48.1				0.927	0.45,1.92	0.839
	Holeta	60	28	46.7				0.875	0.39,1.95	0.743
Family size	1-4	128	54	42.2	9.9	2	0.007	1	ref	
	5-10	129	58	45				1.119	0.68,1.83	0.654
	>10	40	28	70				3.197	1.49, 6.85	0.003
Ethnicity of owner	Oromo	119	49	41.2	7	4	0.134	1	ref	
	Amhara	63	28	44.4				1.1429	0.61,2.11	0.671
	Mixed	99	55	55.6				1.785	1.04, 3.06	0.035
	Foreign	7	5	71.4				3.571	0.66,19.1	0.137
	SNNP	9	3	33.3				0.714	0.17,2.99	0.645
Age of owner (year)	<26	14	8	57.1	4.55	3	0.208	1	ref	
	26-40	41	23	56.1				0.958	0.28,3.26	0.946
	>40	115	46	40				0.5	0.16,1.53	0.226
	All age	127	63	49.6				0.738	0.24,2.25	0.593
Sex of owner	Female	56	25	44.6	1.56	2	0.458	1	ref	
	male	164	74	45.1				1.019	0.55,1.87	0.950
	mixed	77	41	53.2				1.412	0.71,2.82	0.32
Education Level	illiterate	17	11	64.7	11	6	0.087	1	ref	
	<7	41	18	43.9				0.427	0.13,1.37	0.154
	7-8	37	11	29.7				0.231	0.07,0.78	0.018
	9-10	50	29	58				0.753	0.24,2.36	0.626
	11-12	35	13	38.1				0.322	0.09,1.08	0.066
	>12	54	28	51.9				0.587	0.19,1.82	0.356
	All level	63	30	47.6				0.496	0.16,1.5	0.216
Income level(birr)	1-2000	42	19	45.2	8.1	2	0.017	1	ref	
	2001-5000	65	21	32.3				0.578	0.26,1.28	0.178
	>5000	190	100	52.6				1.345	0.68,2.63	0.386
Hospitalization history	no	260	122	46.9	0	1	0.491	1	ref	
	yes	37	18	48.6				1.072	0.54,2.13	0.844
Hand washing	no	7	2	28.6	0.38	1	0.274	1	ref	
	yes	290	138	47.6				2.27	0.43,11.9	0.332
Number of cattle	1-20	149	59	39.6	6.99	2	0.03	1	ref	
	21-50	57	30	52.6				1.695	0.92,3.13	0.093
	>50	91	51	56				1.945	1.15,3.3	0.014

Table 7: Association between some of the factors with occurrence of *Staphylococci* in udder milk in the central Ethiopia (continued)

Risk factors		N	+ve	%	x2	df	p-value	OR	CI(%)	P-Value
presence of sheep	no	202	93	46	0.11	1	0.37	1	ref	
	yes	92	45	48.9				1.122	0.68,1.83	0.647
presence of goat	no	274	127	46.4	0.52	1	0.235	1	ref	
	yes	23	13	56.5				1.504	0.64,3.55	0.350
presence chicken	no	249	122	49	1.7	1	0.096	1	ref	
	yes	48	118	37.5			0.624	0.33,1.17		0.146
Presence of equine	no	240	114	47.5	0.01	1	0.457	1	ref	
	yes	57	57	45.6				0.927	0.51,1.65	0.797
Breed of cow	HF	72	43	59.7	8.44	3	0.038	1	ref	
	Cross	219	96	43.8				0.526	0.3,0.90	0.02
	Borana	3	0	0				0	0	0.958
	Arsi	3	1	33.3				0.337	0.02,3.89	0.383
Age of cow (years)	3-6	149	67	45	1.48	2	0.477	1	ref	
	7-10	119	61	51.3				1.287	0.79,2.08	0.305
	>10	29	12	41.4				0.863	0.38,1.93	0.722
Parity level of cow(calve)	<3	233	108	46.4	1.01	2	0.605	1	ref	
	4-7	58	28	48.3				1.080	0.6,1.92	0.792
	>7	6	4	66.7				2.315	0.41,12.88	0.337
Lactation stage (month)	1	22	12	54.5	6.27	2	0.043	1	ref	
	2-5	169	69	40.8				0.575	0.23,1.4	0.224
	>5	106	59	55.7				1.046	0.41,2.63	0.923
Average of daily milk yield	<5L	37	15	40.5	0.76	2	0.684	1	ref	
	5-10L	134	65	48.5				1.3816	0.66,2.89	0.391
	>10L	126	60	47.6				1.3333	0.63,2.8	0.448
Udder health	infected	6	2	33.3	2.08	3	0.557	1	ref	
	health	276	131	47.5				1.807	0.325,10.02	0.499
	injured	6	4	66.7				4	0.363,44.1	0.258
	soiled	9	3	33.3				1	0.112,8.95	1
Udder tick infestation	no	294	140	47.6	1.13	1	0.146	1	ref	
	yes	3	0	0				0	0	0.959
Mastitis history	no	284	131	46.1	1.82	1	0.089	1	ref	
	yes	13	9	69.2				2.628	0.79,8.73	0.114
Barn hygiene	poor	78	37	47.4	3.57	2	0.167	1	ref	
	moderate	78	30	38.5				0.693	0.37,1.31	0.258
	good	141	73	51.8				1.19	0.68,2.07	0.539

Table 7: Association between some of the factors with occurrence of *Staphylococci* in udder milk in the central Ethiopia (continued)

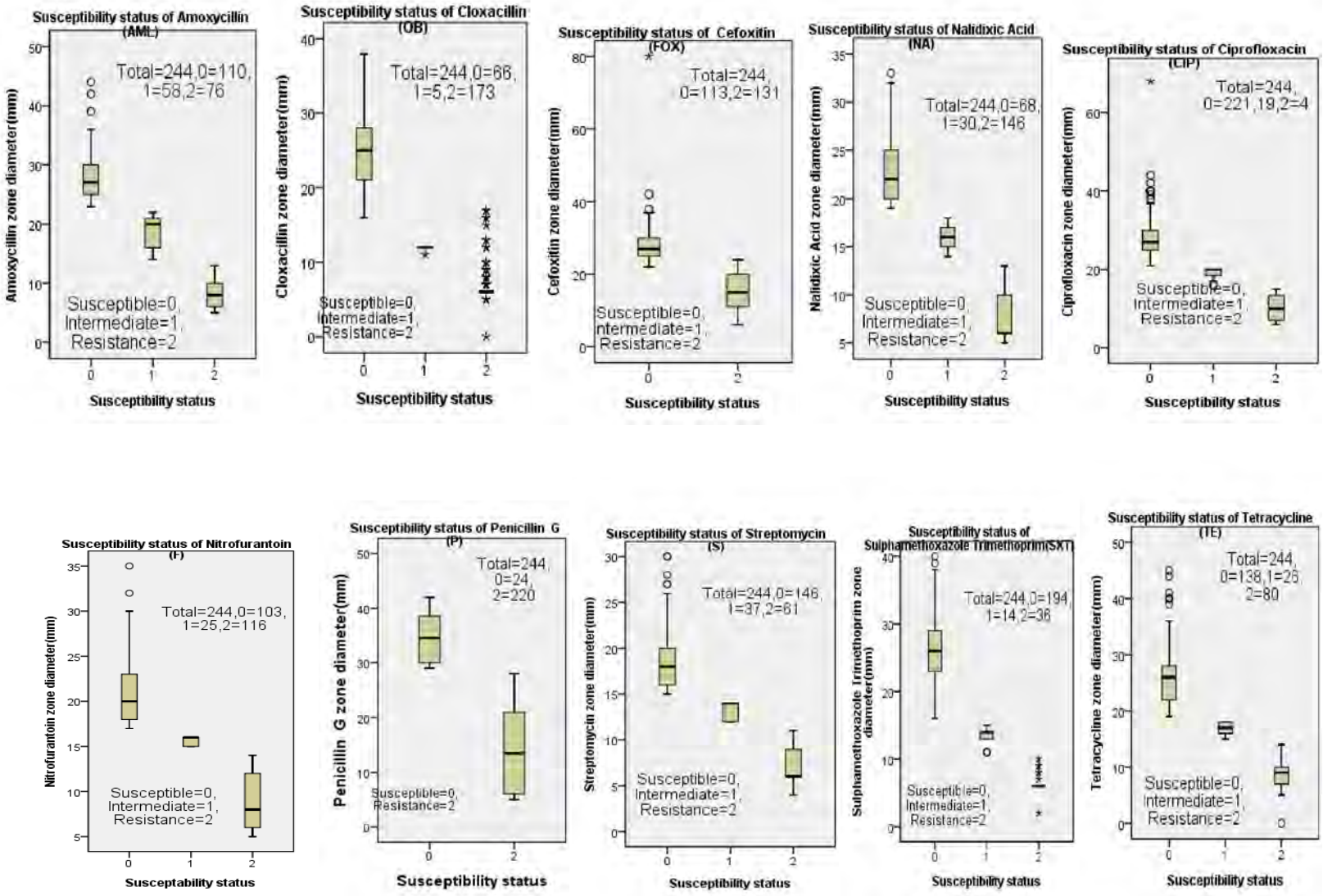
Risk factors		N	+ve	%	x2	df	p-value	OR	CI (%)	P-Value
Manure handling	poor	59	24	40.7	2.08	2	0.354	1	ref	
	moderate	66	29	43.9				1.143	0.56,2.33	0.712
	good	172	87	50.6				1.493	0.82,2.72	0.19
Species mixing	no	153	79	51.6	2.2	1	0.069	1	ref	
	yes	144	61	42.4				0.688	0.43,1.09	0.11
Age mixing	no	24	15	62.5	1.85	1	0.087	1	ref	
	yes	273	125	45.8				0.507	0.21,1.19	0.121
source of H_2O	tap	286	135	47.2	4.4	2	0.11	1	ref	
	lake	5	4	80				4.474	0.49,40.52	0.183
	river	6	1	16.7				0.223	0.03,1.94	0.174
Date of antibiotic Rx	no Rx	193	85	44	1.78	1	0.091	1	ref	
	≤1week	0	0	0				0	0	
	>1week	104	55	52.9				1.42	0.88,2.3	0.146
Date of Antihelmintic Rx	No Rx	117	55	47	0.73	2	0.693	1	ref	
	≤28days	9	3	33.3				0.564	0.13,2.36	0.433
	>28days	171	82	48				1.039	0.65,1.66	0.874

4.5. Antibiotic susceptibilities of *Staphylococci* isolated from food, human and material swab of central Ethiopia

In the antimicrobial resistance trials, out of 428 *Staphylococci* species isolates, 244 (50.9%) were subjected to antibiotic susceptibility tests. Of 244 tested isolates 97.5% (238) isolates showed antimicrobial resistance properties to at least one of the antibiotics tested. A large proportion of the isolates of this study area were resistant to Penicillin G (10units) (90.2%), Cloxacillin (5µg) (70.9%), Erythromycin (15µg) (70.9%), Nalidixic Acid (30µg) (59.8%), Cefoxitin(30µg) (53.7%), Vancomycin(30µg) (52.9%) and Nitrifurantoin (50µg) (47.5%). Only a small proportion of the isolates from total sample were resistant to Ciprofloxacin (5µg) (1.6%) and Gentamycin(10µg) (2%) (Figure 1).

A summary of the diameter zone of inhibition in mm (y-axis), percentile (y-axis) and number of isolates (in the body) with resistant, intermediate and susceptible phenotypes (x-axis) of the total 244 *Staphylococci* isolates studied for the 15 different antibiotics was indicated in Figure 1. Similarly, for isolates originated from the 5 study areas their antibiotic susceptibility profile was shown in Figure 2.

The line in the middle of the box plot indicates the 50% of the isolates with the corresponding zone diameter. Figure 1 and 2 of the box plot shows the five descriptive statistics: minimum, first quartile, median, third quartile, and maximum. For example, for Penicillin G resistance box plot:-the dark line in the middle of the boxes is the median at 14mm of zone diameter. Half of isolates have the zone diameters greater than the median value (14mm), and half of them have a value lower than 14mm. The bottom of the rectangular box indicates the 25th percentile (6mm) indicating 25% of the isolates have a zone diameter below 6mm. The top of the box represents the 75^{th} percentile (21mm). Twenty-five percent of zone diameters have values above the 75^{th} percentile (21mm).



Figures 1: Drug susceptibility status of *Staphylococcus* in central Ethiopia

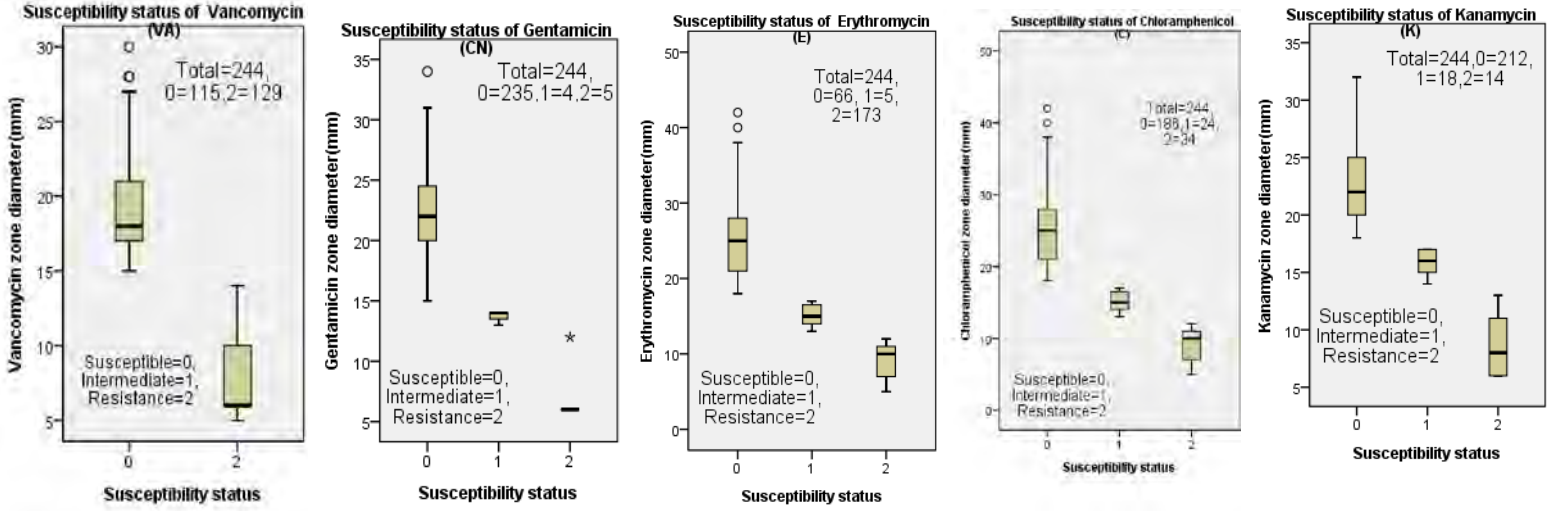
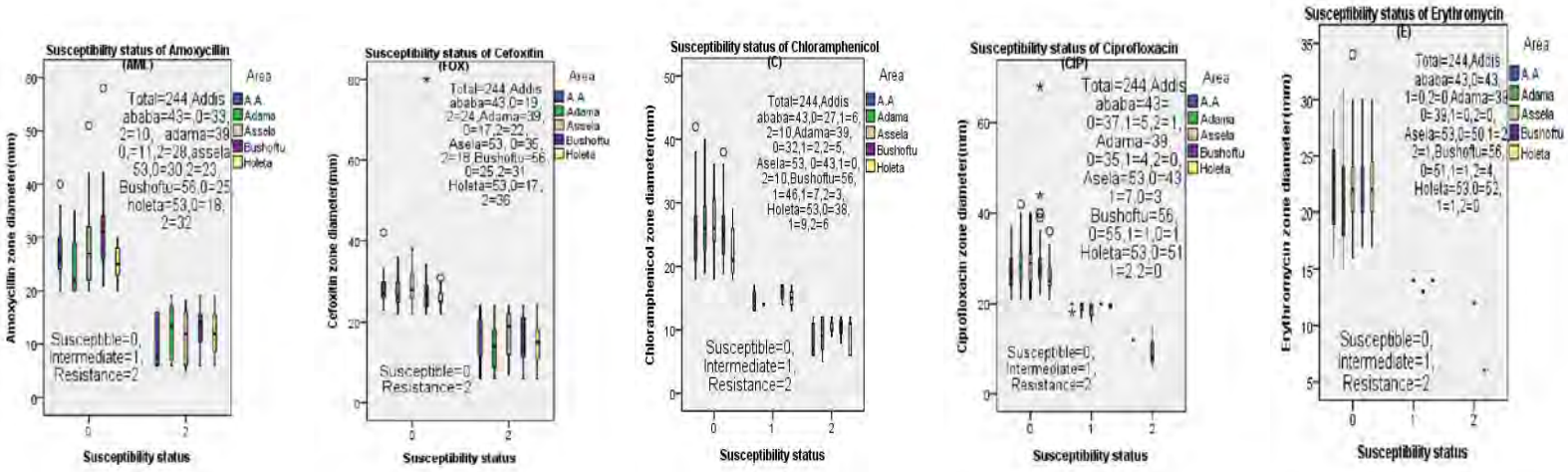


Figure 1: Drug susceptibility status of *Staphylococcus* in central Ethiopia (Continued)



Figures 2: Drug susceptibility status of *Staphylococcus* in five study area

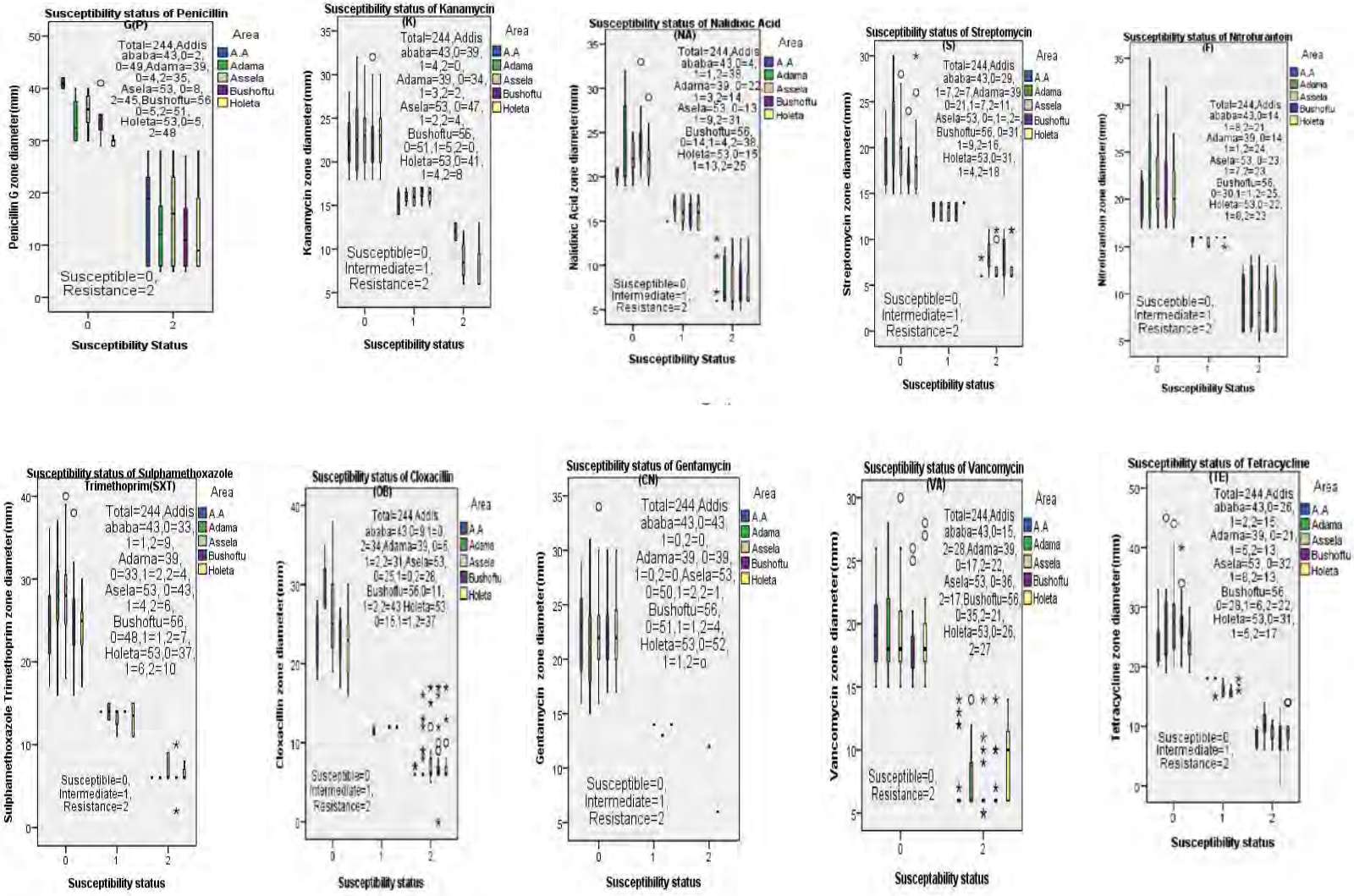


Figure 2: Drug susceptibility status of *Staphylococcus* in five study area (Continued)

4.6.Antibiotic resistant of *Staphylococci* in different sample types isolated from central Ethiopia

The *Staphylococcus* isolates from dairy farm and milkers showed antimicrobial resistance similar to the isolates from abattoir and abattoir butcher with no statically significant difference between them except for Amoxycillin, Tetracycline, Streptomycin and Vancomycin, Isolates from swab of human hand, slaughter line, and farm tank milk demonstrated a higher resistance pattern to Amoxycillin (83.3%) Vancomycin (73.3%), Streptomycin (56.3%) and Tetracycline (62.5%) respectively (Table 8).

Table 8: Antibiotic resistant of *Staphylococci* in different sample types isolated from central Ethiopia

Drug	Sample types (n(%))														
	AHS	**ANS**	**BS**	**FHS**	**FNS**	**KS**	**MS**	**SLS**	**TM**	**TS**	**UM**	**TOTAL**	**X2**	**df**	**P-value**
AML	3(33.3)	(0)0	6(54.5)	10(83.3)	4(66.7)	1(11.1)	35(43.2)	6(40)	12(75)	8(71.7)	42(60)	127(52)	27.338	10	0.0023
FOX	4(44.4)	1(25)	3(27.3)	8(66.7)	2(33.3)	3(33.30	43(53.1)	8(53.3)	12(75)	8(72.7)	39(55.7)	131(53.7)	12.69	10	0.24
C	1(11.1)	0(0)	0(0)	2(16.7)	2(33.3)	1(11.1)	12(14.8)	3(20)	4(25)	1(9.1)	8(11.4)	34(13.9)	19.67	20	0.48
CIP	0(0)	0(0)	0(0)	1(8.3)	0(0)	1(11.1)	1(1.2)	0(0)	0(0)	0(0)	1(1.4)	4(1.6)	17.5	20	0.62
OB	5(55.6)	3(75)	7(63.6)	8(66.7)	3(50)	5(55.6)	56(69.1)	13(86.7)	14(87.5)	11(100)	48(68.6)	173(70.9)	30.91	20	0.06
E	2(22.2)	1(25)	4(36.4)	4(33.3)	1(16.7)	2(22.2)	22(27.2)	6(40)	8(50)	4(36.4)	22(31.4)	76(31.1)	16.319	20	0.6966
CN	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	2(12.5)	0(0)	3(4.3)	5(2)	29.111	20	0.0856
K	0(0)	0(0)	1(9.1)	0(0)	1(16.7)	1(11.1)	3(3.7)	2(13.3)	1(6.3)	0(0)	5(7.1)	14(5.7)	24.321	20	0.2287
NA	7(77.8)	1(25)	4(36.4)	6(50)	1(16.7)	9(100)	55(67.9)	11(73.3)	7(43.8)	6(54.5)	39(55.7)	146(59.8)	30.425	20	0.0633
F	3(33.3)	2(50)	4(36.4)	5(41.7)	3(50)	2(22.2)	35(43.2)	8(53.3)	12(75)	5(45.5)	37(52.9)	116(45.5)	18.241	20	0.5715
P	8(88.9)	4(100)	10(90.9)	12(100)	466.7)	7(77.8)	71(87.7)	14(93.3)	15(93.8)	11(100)	64(91.4)	220(90.2)	9.3645	10	0.4979
S	0(0)	0(0)	6(54.5)	6(50)	1(16.7)	1(11.1)	16(19.8)	1(6.7)	9(56.3)	3(27.3)	18(25.7)	61(25)	50.336	20	0.0002
SXT	0(0)	0(0)	2(18.2)	3(25)	2(33.3)	1(11.1)	8(9.9)	2(13.3)	3(18.8)	1(9.1)	14(20)	36(14.8)	17.108	20	0.646
TE	1(11.1)	1(25)	2(18.2)	7(58.3)	2(33.3)	1(11.1)	18(22.2)	4(26.7)	10(62.5)	2(18.2)	32(45.7)	80(32.8)	44.496	20	0.0013
VA	2(22.2)	1(25)	1(9.1)	8(66.7)	2(33.3)	3(33.3)	39(48.1)	11(73.3)	10(62.5)	4(36.4)	34(48.6)	115(47.1)	18.653	10	0.0449
TOTAL	9	4	11	12	6	9	81	15	16	11	70	244			

Key: UM=udder milk, TM=tank milk, BS=bucket swab, TS=tank swab, FHS=farm hand swab, FNS=farm nasal swab, AAH=abattoir hand swab, ANS=abattoir nasal swab, CS=Carcass swab, KS=Knife swab, SLS=slaughter line swab, n=number of resistant

4.7. Multiple drug resistance of *S.aureus* and other *Staphylococci species* isolated from farm, abattoir and human of central Ethiopia

Almost 89.3% of the isolates were observed with multiple drug resistance to majority of the antimicrobial agents tested. *S. aureus* showed resistance to all antimicrobials tested. Penicillin G, Cloxacillin and Amoxicillin were the drugs to which a large proportion of the isolates were resistant to *S.aureus*. However, very small proportions of *S. aureus* were resistance to Ciprofloxacin, Gentamicin and Kanamycin. In the present observation, 85/92 (92.4%) *S. aureus* strains showed multidrug resistance (Table 9).

Table 9: Multiple drug resistance of *S.aureus* and other *Staphylococci* species isolated from food, human and material swab of central Ethiopia

Over all Multiple drug resistance					Multiple drug resistance for *S.aureus*			
No. of drug	Frequency	%	95%CI (%)		frequency	%	95%CI (%)	
			lower	upper			lower	upper
0	6	2.5	0.9	5.3	0	0:00	0:00	0:00
1	6	2.5	0.9	5.3	1	1.1	0	5.9
2	20	8.2	5.1	12	6	6.5	2.4	13.7
3	17	7	4.1	11	8	8.7	3.8	16.4
4	44	18	13.4	23	17	19	11.1	27.9
5	36	14.8	10.6	20	16	17	10.3	26.7
6	35	14.3	10.2	19	15	16	9.4	25.5
7	23	9.4	6.1	14	10	11	5.3	19.1
8	23	9.4	6.1	14	9	9.8	4.6	17.8
9	14	5.7	3.2	9.4	3	3.3	0.7	9.2
10	8	3.3	1.4	6.4	3	3.3	0.7	9.2
11	6	2.5	0.9	5.3	2	2.2	0.3	7.6
12	3	1.2	0.3	3.6	2	2.2	0.3	7.6
13	3	1.2	0.3	3.6	0	0:00	0:00	0:00
Total	244	100			92	100		

5. DISSCUSSION

5.1. Over all *Staphylococci* prevalence

In the present study, the presence of *Staphylococci* was detected in 428 out of 1,001 analyzed samples. The detection was made in pooled udder milk, pooled bucket milk, cow bucket swab, farm bucket swab, hand and nasal swab of milkers, carcass swab, knife swab, slaughter line swab, hand and nasal swab of butchers. From a total of 428 *Staphylococci* isolates, 236(55.1%) originated from the farm raw milk (udder and farm tank), container and human swab; and 192(44.9%) from meat, equipments and human swab in abattoir. The contamination rate of the sample from farm (45.9%) was significantly higher than that of the sample from abattoirs (39.4%). Coagulase-posative *Staphylococci* isolates 32.8% (328/1001) dominated in prevalence than the coagulase negative *Staphylococci* with prevalence of 9.6% (96/1001). This is similar with the finding of Siraj (2012) who report in Ambo that coagulase negative *Staphylococci* (CNS) is the second most prevalent pathogens next to coagulase positive *Staphylococcus*. However, it is contrary to the findings of Bendahou *et al.* (2008) who reported coagulase-negative *Staphylococcus* (54 %) is the predominantly prevailing isolates. The differences in prevalence reports of *Staphylococcus species* in the present study and other reports could be attributable to difference in sample type, differences in the origin of the samples or by geographical differences, differences in study methods and materials employed by the investigators.

5.2. Prevalence of *Staphylococci* in abattoir

In this study, the overall prevalence of *Staphylococcus* in abattoir was 39.4%. This finding is higher than that of Endale and Hailay (2013) 28% in Mekele, Haimanot *et al*. (2010) 12% and Anbessa (2013) 2.28% in Jimma. The type of sample specific prevalence of *Staphylococcus* were found to be 38.5%, 37.8%, 48.6%, 46.7% and 37.8% from swab of

carcass, knife, slaughter line, nasal and hand swabs of butchers in the abattoir samples, respectively. This contamination rate is comparable with the findings in Italy on several kinds of foodstuffs that revealed a total prevalence of coagulase positive *Staphylococci* of 17.3%, with contamination rates ranging from 17.1% to 48.1% in meat products (Normanno *et al.,* 2005). The contamination rate of carcass swab 38.5% (139/361), butchers hand 37.8% (14/37) and equipment 43.2% (32/74) higher than that of Pompeian (2007) who finding 6.42%, 5% and 50% in swab taken from carcass, equipment and workers, respectively. It has been suggested that variation in abattoir prevalence of *Staphylococci* species in different reports is directly related with the variation in the level organized farm to abattoir, standard sanitary operational procedures practiced by the abattoir personnel that includes poor personnel hygiene and type of slaughter used.

5.3. Prevalence of *Staphylococci* in dairy farms

The overall prevalence of *Staphylococcus* in dairy farms was 45.9%. This is in line with the findings of Mathios *et al.* (2009) who reported 41.4% in dairy farms in Assela and Alehegn (2008) 44% in Debre zeit and Siraj (2012) 48.98% in Ambo. Similarly, it was comparable with the findings of Tadesse and Chanie (2012) who reported 57.67% in Addia Ababa, Birhanu *et al.* (2013) 58.6% in Assela and Mekonnen and Tesfaye (2010) who reported 35.8% in dairy farm in Adama. However, the prevalence reported in this study is lower than that of Mekibib *et al*. (2010)) who reported 77.1% in Holota, but higher than Abunna *et al*. and Zeryehun *et al*. (2013), who reported 21% and 28.7% in Addis Ababa, respectively. The present finding is also higher than Bedane (2012) who reported 29.2% in dairy cows.

The products specific prevalence of *Staphylococcus* were found to be 47.1%, 58%, 38% 34%, 70.6% and 38% from udders milk, farm tanks milk, farm tanks swab, buckets swab, nasal and hand swab of milkers samples, respectively. A high prevalence of *Staphylococcus* was recorded in farm raw tank milk than in farm tanks swab and buckets swab. This finding

comparable with finding of Mekonnen *et al.* (2011) and Alehegn (2008) who reported 33% and 29.5% *Staphylococcus* prevalence in tank milk, respectively, in Debrezeit. Factors that could be hypothesized to be causes of contamination of milk in this study include insufficient pre-milking udder preparation, insufficient cleaning of milkers' hands, milking buckets and storage containers. In the present study the high prevalence of *Staphylococcus* was seen in udder milk than farm tank milk. Farm milk tanks were made up of plastics in the current study area. Plastic containers have characteristics that make them unsuitable for milking and milk handling. According to Soomro *et al*. (2003) plastic containers scratch easily and provide hiding places for bacteria during cleaning and sanitization and plastic containers are poor conductor of heat and hence will hinder effective sanitization by heat. Additionally, the number of personnel working at farm was higher which might have contributed to milk contamination.

5.4. Prevalence of *Staphylococci* by species

Characterization of 428 isolates showed that *S. aureus* was the most frequently isolated species among different samples accounting for 40.2% (172/428) the population studied. The other *Staphylococci* isolates were distributed as, *S. hyicus* 19.2% (82/428); *S. intermedius* 17.2% (74/428); CNS 22.4% (96/428).

In this study, the overall prevalence of *S. aureus* was 17.2% with a statistically significant higher prevalence in dairy farms (22.4%) and dairy products (28% tank milk and 22.6% udder milk) followed by farm nasal swab (23.5%). Abattoir contamination rate revealed 10.5%, 13.5%, 18.9%, 0% and 18.9% from swab of carcass, knife, slaughter line, nasal and hand of butchers with over all prevalence of *S. aureus* (11.7%). In this study the prevalence of meat contamination (10.5%) with *S. aureus* is comparable with finding of Normanno *et al.* (2007b). They reported that the contamination of *S. aureus* from meat product samples (10%) and milk and dairy samples (17%) with over all prevalence 12.8%. It is also consistent with the findings of Mokonnen *et al*. (2013) who reported an overall prevalence of *S. aureus*

21.2% in Mekele. The overall prevalence *S.aureus (*17.2%) also similar with the 17.2% observed in Egypt (Seedy *et al.* (2010), 15.5% Addis Ababa (Mekuria *et al*., 2013) and with the 19.5% by Jakee *et al.* (2008) that isolated *S. aureus* strains from human and animal sources. However, it is lower than those of Jørgensen *et al*. (2005). Their *S. aureus* from bovine bulk milk report was 75% while it was 37.8%in raw milk products in Norway. Meanwhile, it is higher than Fagundes *et al.(* 2010) who report *S. aureus* strains in 18 (7.3%) milk samples: 14 (6.7%) from samples of individual cows, and 4 (10.8%) from bulk tank milk in São Paulo, Brazil.

In the present study, milk from lactating dairy cows showed 67 (22.6%) positive for S. *aureus*. This is in line with the findings of Bitaw *et al.* (2010) who found 20.3% in raw milk of dairy farms in Bahir Dar town and its environs. Similarly, it was comparable with the findings of Mekonnen *et al.* (2011) and Alehegn (2008) that isolate from dairy farms in Debre Zeit 22.6% and 15.8%, respectively. However, the present findings are lower than that of Workineh *et al.* (2002), Dego and Tareke (2003) who reported 39.2 and 40.3% *S. aureus* isolates at Addis Ababa and Southern Ethiopia, respectively, but higher than Bishi (1998) and Hussein *et al.* (1997) who reported 9 and 10% prevalence in Addis Ababa, respectively. The present finding is also in contrast with findings of Lakew *et al*. (2009), Ndegwa *et al.* (2000) and Bedada and Hiko (2011) who reported 41.1, 43.3, and 39.1% in dairy cows, respectively. The possible explanation for this might be that *S. aureus* is a contagious pathogen transmitted from one cow to another or individual by contact with animals during unhygienic milking procedures (Rowe, 1999).

In dairy farm *S. intermidius* was 22 (7.4%), 8 (4%), 3 (6%), 3 (6%), 3 (17.6%) and 4 (8%) in udder milk, farm tank milk, tank swab, bucket swab, nasal and hand swab respectively with an overall prevalence of 7.6%. Similarly in abattoir *S. intermidius* was 29 (8%), 2 (5.4%), 1 (2.7%), 2 (13.3%) and 1 (2.7%) in swab of carcass, knife, slaughter line, nasal and hand of butchers respectively with an overall prevalence of 7.6%. The prevalence of *S. intermidius* in the present work is lower than that reported by Alehegn (2008) of 27.3% of udder milk

sampled. *S. hicus* was isolated in 39 (8%), 23 (8%), 8 (16%), 1 (%), 1 (%), 3 (19%) and 3 (6%) of the udder milk, farm tank milk, tank swab, bucket swab with an overall prevalence of 8%. The results showed that CNS species frequently occurred in raw milk (8.6%) which was lower than the results of Tsegmed (2006) who reported CNS in 54% of raw milk of cattle in Lamprell *et al.* (2004) of 29% in 1036 samples. The different rates of *S. intermidius,* and *S.hycus,* investigation found in these reports could be explained by the different techniques used in these studies, differences in the origin of the samples or by geographical differences. The prevalence of CNS in dairy farm and abattoir was found to be 41 (8%) and 55 (11.3%), respectively, with an overall prevalence of 96 (9.6%). The high number of CNS isolated in the current study may be due to lack of hygienic measures and unhygienic milking. Because CNS are a part of the normal teat skin flora and mucosa of humans and animals, some species are also found free-living in the environment (Kloos and Bannerman, 1994; Lourdes *et al*., 2004). Therefore, they are common cause of contamination of milk and milk products. In addition, unpasteurized milk may contain CNS if the cow suffers from mastitis of CNS (Jones, 1998; Kaloreu *et al*., 2007). In the past, CNS was often regarded as skin flora opportunists but recent data now indicate that they are associated with several subclinical and clinical infections (Shah, 2003). In present dairy farm CNS species were detected in 27 (9.1%), 3(6%), 4 (8%), 3 (6%), 2 (11.8%) and 2 (4%) in udder milk, farm tank milk, tank swab, bucket swab, nasal and hand swab, respectively, with an overall prevalence of 41(8%). Coagulase production was described as one of the most reliable criteria for the identification of pathogenic *Staphylococcus* species. *Staphylococci* producing coagulase are usually pathogenic (Quinn *et al.,* 2002; Lamprell *et al*., 2004; Morrison, 2008).

5.5. Risk factors for S*taphylococci* infection

Based on observations made throughout the study period in the farms improper hygiene and poor farm management practices contributed to the presence of *Staphylococcus* especially in those from the small holder private farms. *S. aureus* incidence is at a considerable higher percentage which indicates that the alarming situation for dairy farms

and for public health as well. Statistical the present observation showed high prevalence of *Staphylococcus* was more likely to occur in cows that were in late lactation stage and high productive. Low income level of the owners and large family size of the farm increases the probability of isolation of *Staphylococcus* from dairy cows. No statistical significant association was observed in this study among age, mastitis history, antibiotic use and educational status for *Staphylococcus* infection of lactating dairy cows. These findings is similar with other finding (Mekuria *et al*., 2013; Denis *et al*., 2008 and Grace *et al*., 2009).

Galton *et al*. (1986) and Anderson and Pritchard (2008) reported that pre milking udder preparations play an important part in the contamination of milk during milking. Most of the dairy owners did not use towel and a few dairy owners used a single towel for all cows commonly to dry the udders. The reuse of towel for cleaning and sanitizing may result in recontamination of the udder. Since drying was not or insufficiently practiced, contamination level of milk was becoming higher. Furthermore, milkers wash their hands at the beginning of milking but did not dry their hands and not repeat washings between milkings and some of the milkers used milk to moisten the teats when they became dry in between milkings, which could be additional sources of contamination for milk. Handling of small quantities of milk with a big container is subjected to a high rate of contamination with a small milk volume to container ratio (Bonfoh, 2003). This also contributes to the high level of contamination of milk.

It is very difficult to separate potential effects of confounding risk factors for disease occurrence from the effect of management changes that have been adopted by dairy farm in the production systems. Many risk factors that are not specific to dairy farms have influence on the occurrence of the disease. The confounding differences have influenced almost every study that has attempted to compare disease rates between dairy cows and dairy farm workers. Therefore, it is hasty to draw overly broad conclusions about this issue.

5.6. Drug susceptibility test

5.6.1. Over all Staphylococci drug susceptibility status

In the antimicrobial resistance trials, out of 428 *Staphylococci* species isolates, 244 (50.9%) were subjected to antibiotic susceptibility tests. Of which 244 tested isolates 97.5% (238) isolates showed antimicrobial resistance patterns to at least one of the antibiotics tested. This study revealed that milk, meat, container used in the farm and material used in abattoir may be contaminated with multiple drug resistant *Staphylococcus* species. *Staphylococcus* species isolated from human also have multiple drug resistant to different drugs. The high frequency of resistance observed with Penicillin G (90.2%), Cloxacillin (70.9%), Erythromycin (70.9%), Nalidixic Acid (59.8%), Cefoxitin (53.7%), Vancomycin (52.9%) and Nitrifurantoin (47.5%) could be attributed to their use in the treatment of diseases in animals and humans. Only a small proportion of the isolates from total sample were resistant to Ciprofloxacin (1.6%) and Gentamycin (2%). Almost 89.3% of the isolates were observed with multiple drug resistance to the majority of tested antimicrobial agents . The current finding was slightly higher than Sharma (2011) who reported 60-70% of the isolates were observed with multiple drug resistance. Indiscriminate use of antimicrobial agents and antibiotic sale behavior (for example, sale of antibiotics without prescription, sale of under dose and substituting brands) enhances the development of drug resistance (Indalo, 1997).

In present study the resistance of tetracycline is not in an agreement with Mekuria *et al*. (2013) (66.7%) and preliminary finding conducted by Bayhun (2008) (55.3%). Similarly, apparent difference was observed in the report of Tariku *et al.* (2011) (0%). In addition, the present observation is similar to the study result of Nwankwo and Nasiru (2011) who reported the resistance of penicillin G (92.9%) and Gentamycin (7.6%) but his finding is not in an agreement with the present finding in the case of resistance to Chloroamphenicol (48.1%), tetracycline (68.9%), sulphamethoxazole-trimethoprim (84.5%) and vancomycin (0%).

5.6.2. Drug susceptibility status of Staphylococci by study Area

In the current study, although the prevalence of *Staphylococcus* species have no statistically significant difference among geographic region of the study area, antimicrobial resistance to the isolates had statistically significant difference except for Penicillin G, Tetracycline, Streptomycin, Gentamycin and Sulphameth oxazole-trimethoprim. Isolates from Addis Ababa dairy farm and abattoirs (23.3%), (46.5%) and (88.4%), had higher resistance to Chloramphenicol, Erythromycin and Nalidixic acid, respectively, than other study areas. Isolate originated from Adama dairy farm and Abattoirs (71.8%), (61.5%) and 79.5% showed higher resistance to Amoxycillin, Nitrofurantoin and Cloxacillin, respectively, than other study sites. Similarly isolates from Holeta showed higher resistance to Cefoxitin (67.9%) and Kanamycin (15.1%), while isolation from Assela showed higher resistant to Ciprofloxacin (5.7%) than other study sites. One of the main reasons for the increase environmental multi-drug resistant bacteria is the indiscriminate use of antimicrobials during animal husbandry. Because of the emergence of such bacteria, antimicrobials used in fighting infections are less effective. It is important to note that *S. aureus* can easily acquire resistance to most antimicrobials (Bozdogan *et al*, 2004).

5.6.3. Drug susceptibility status of Staphylococci in sample source and sample type

The *Staphylococcus* isolates from dairy farm and milkers showed antimicrobial resistance similar to the isolates from abattoir and abattoir butcher with no statistically significant difference between them except for Amoxycillin, Tetracycline, Streptomycin and Vancomycin. *Staphylococcus* isolated from slaughter line swab showing high antimicrobial resistance to Vancomycin (73.3%) than isolate from raw milk, container swab, meat swab, knife swab and nasal and hand swab of human have statistically significant difference between them. Similarly, Amoxycillin for isolates from human hand swab, Streptomycin and Tetracycline for isolates from farm tank milk statistically demonstrated a higher resistance pattern with prevalence 83.3%, 56.3% and 62.5%, respectively. This is in agreement with previous reports in Addis Ababa by Mekuria *et al*. (2013) and in Taiwan by Ma *et al. (*2006).

The fact that resistance is high in environmental isolates is mainly because antimicrobials are frequently prescribed by veterinarians as treatment for bacterial infections on farms and veterinary Clinic. Thus, the indiscriminate use of those antimicrobial agents might account, at least in part, for such a high resistance.

Bacterial infection of human beings through consumption of contaminated food is a common finding worldwide. If care is not taken during milk processing or cooking of meat so as to destroy all the bacteria present in the products, resistant *Staphylococcus* especially *S. aureus* in these products may find their way to the human body where they can cause disease that may prove difficult to treat. In developed countries, the main reservoirs for antimicrobial drug resistance in enteric bacteria have been attributed to farm animals like cattle (Spika, 1987; CDC, 1996; Holmberg, 1984; Tacket, 1985; Ryan, 1987). Contact with these animals or consumption of food products from them has been the main route of dissemination of resistance in to the human populations. Therefore, transmission of drug resistant bacteria from farms into the community and subsequently to patients in hospital may occur through food (Spika, 1987; Holmberg, 1984; Tacket, 1985). This demonstrates how resistant bacteria arising from indiscriminate use of antibiotics in animals may impact on human health. Isolation of the antibiotic resistant bacteria has been reported from pasteurized milk (Ryan, 1987; Ombui, 2000).

The resistance profile *Staphyloccocus* to Amoxicillin (60%), Cloxacillin (68.6%) and Vancomycin (48.6%) in dairy cows was found to be high. This might indicate transfer of resistant strain among environment, livestock and human since this antimicrobials are not used in veterinary practice (Martil *et al.,* 2004). *Staphylococci* are frequently isolated from bovine mastitis which is one of the most common causes for the use of antimicrobial in lactating dairy cows. Similarly, the present investigation indicated that the resistance pattern of penicillin was found to be 94.6% which is similar to the finding made by Tariku *et al*. (2011) (87.2%), Daka (2012) 67.9% and Mekuria *et al*. (2013) 96.7% 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 penicillin G in West India.

In this study, a higher resistance to penicillin was determined in dairy farm workers, dairy cows, and container swab, abattoir and abattoir workers and penicillin resistance was found to be no significantly different in all sample sources and sample type. Similarly, the result of the present investigation showed high percentage of resistance to Cloxacillin in dairy farm workers, dairy cows, and container swab, abattoir and abattoir workers. This may pose risk to consumers or individuals who have contact with animals especially for immune compromised ones. In the present study, the prevalence of Cloxacillin-resistant *S. aureus* was not variable with sample source and type of samples.

5.6.4. Antibiotic susceptibility status of of S.aureus

The observations made in the present study unequivocally proved that *S. aureus* showed resistance to all antimicrobials tested. These indicate that the problem is highly distributed and disseminated. Penicillin G, Cloxacillin and Amoxycillin were the drugs to which a large proportion of the *S.aureus* isolates were resistant to. However, very small proportions of *S. aureus* were resistance to Ciprofloxacin (1.1%), Gentamicin(1.1%) and Kanamycin(8.7%). The resistance status of *S. aureus* to Sulphamethoxazole-Trimethoprim in line with the reports of 15.7% to 19% in South Africa (Ateba, 2010). Contrarily, a study reported that small percentage (7.7%) of *S. aureus* isolated was resistant to Sulphamethoxazole Trimethoprim (25µg) (Daka *et al.,* 2012).

Particularly, the present study has demonstrated the existence of alarming level of resistance of *S. aureus* to commonly used antimicrobials (Penicillin G (94.6%) and tetracycline(31.5%) in the study farms for dairy cows. The results were in accordance with reports from earlier studies in Ethiopia Addis Ababa (Mekuria *et al*., 2013), Awasa (Daka *et*

al., 2012) and in other countries (Jakee *et al.,* 2008; Edward *et al.,* 2002; Gentilini *et al.,* 2002) suggesting a possible development of resistance from prolonged and indiscriminate usage of some antimicrobials. This is in contrast with the report of Ma *et al*. (2006) on his report with respect to pencillin and tetracycline in Taiwan. This is not surprising because Penicillin G and tetracycline are the most commonly used antimicrobials for the treatment of infection or mastitis in veterinary practice in Ethiopia. Moreover, penicillin resistance is plasmatic and, it spread out very quickly to several other strains. Pereira *et al.* (2009) showed that 70 to 73% of *S. aureus* strains isolated from various foods were resistant to β-lactam such as Penicillin and Ampicillin. However, it was found out that about 90% of *S. aureus* isolated from patients was found resistant in 1980s.

In this study, the resistance of *S. aureus* from human sources to some antimicrobials (Amoxacillin, Tetracycline and Vancomycin) was generally greater than that from bovine sources (udder milk). From this, an association has been suggested between the use of antimicrobials in animal dairy farms and an increased risk of humans contracting resistant strains which is similar to studies done by Mekuria *et al*. (2013), Witte (1998), Cruchaga *et al*. (2001) and Kidd *et al.* (2002). However, apparent difference was also noted by Erskine *et al.* (2002) and Iversen *et al*. (2004), who have shown that food-producing animals play an negligible role in the transmission of resistant strains.

5.6.5. Multidrug resistant Staphylococci

The present investigation showed that the overall 218 (89.3%) multidrug resistant *Staphylococcus* species isolated from dairy farm samples: milk of dairy cows, container swab, nasal and hand swab of milker, and from abattoir samples: meat, knife, slaughter line and human hand and nasal swab. Almost 87.1% (27/31) of the isolates from human were observed with multiple drug resistance to all of the antimicrobial agents tested. Moreover, the present investigation showed 20(47.6%) multidrug resistant *S. aureus* isolated from milk of dairy cows. *S. aureus* strains have developed multidrug resistance worldwide with broad diversity in prevalence rate in different regions (Normanno *et al*., 2007b). In

the present observation, 85/92(92.4%) *S. aureus* strains showed multidrug resistance. This is higher than the findings of Normanno *et al.* (2007b) who reported that 9.6 and 4% of *S. aureus* strains had resistance to three and four of the tested antimicrobials, respectively. Although study was conducted at hospital level where more resistant strains were found than food source isolates Nihal *et al.* (2011), Barena and Fetene (2003) from Jimma Hospital and Chao *et al.* (2007) reported nearly similar rate of multi-drug resistant *S. aureus* (80%) and (79%) with the present investigation respectively.

The present study illustrated that *Staphylococci* is not only prevalent problem in human. But can also occur in dairy farms and abattoir with higher prevalence which demonstrates that animal should not be ignored as reservoir for human infection or colonization. The occurrence of multidrug resistance *Staphylococci* specie particularly CPS should be under consideration during selection of antimicrobials for the treatment of animals especially if the possibility exists in the transfer of resistance in or between microbial species. Moreover, *Staphylococci* are common human commensal, and multidrug resistant CPS may present without clinical illness. However, when they cause infection they are extremely serious. Furthermore, dairy cows and beef cattle become infected with multidrug resistant CPS, therefore diagnosis of CPS does not have implication for treatment only but also it indicates zoonotic transmission since it becomes reservoir for human infection.

Finally, this study provides some important base line data about the contamination status of dairy and beef based food products in central Ethiopia and the patterns of resistance of *Staphylococcu*s species towards commonly used antimicrobials. The presence of multidrug resistance strains is alarming because such strains are considered a serious danger to public health. Additional research is required to better define the ecology and evolution of bacterial resistance to antimicrobial agent in the environment as a whole. Further research and subsequent management of these problems is vital to help ensure that the emergence of drug resistant bacteria is limited and that antimicrobial agents remain effective.

6. CONCLUSION AND RECOMMENDATIONS

This study highlighted the high prevalence and drug resistance of *Staphylococci* species isolated from dairy farms and abattoirs. According to the findings from this study the hands and nasal of human, dairy cow, container used for milking and equipment used in abattoir are a source of contamination of animal milk and meat with bacteria *Staphylococcus* and their contamination were indicators for poor hygienic practice. The contamination of the carcasses, the hand of butchers, equipment with *Staphylococcus* can be the indicator of work hygiene among workers and equipment on the slaughtering line. The indifferent distribution of the prevalence of *Staphylococcus* over different geographical area is indicators for lack of proper personal, environmental hygiene and sanitation; and absence of difference in animal husbandry practice in the study area. Most Staphylococci *species* isolates were multiply resistant to various antimicrobial agents. Milk, meat, equipment and hand and nasal of human contain resistant *Staphylococci* species posing a potential risk to consumers. The indiscriminate use of those antimicrobial agents might account, at least in part, for such a high resistance. Based on the findings of the present study the following recommendations are made:

- Abattoir workers and meat handlers should be educated and advocated on the adverse effects of lack of proper personal, environmental hygiene and sanitation; safeguard the public against the risks of food borne bacterial infections, practicing good sanitation and meat handling techniques in the abattoir
- Awareness creation is required for dairy cow owners and milkers, especially to those of small holder dairy farm, regarding the importance of regular health checkups of dairy cattle, adequate udder preparation, use of clean dairy equipment and utensils, hygienic milking technique, and milkers hands should be practiced.
- Multiple drug resistant *Staphylococci* species *(S. aureus*) have a wide distribution in milk and meat and therefore care should be taken in to account during processing to destroy the micro-organisms to avoid the risk of human infection.
- The occurrence of multidrug resistance *Staphylococcus* particularly CPS should be under consideration during selection of antimicrobials for the treatment of beef and dairy cattle.

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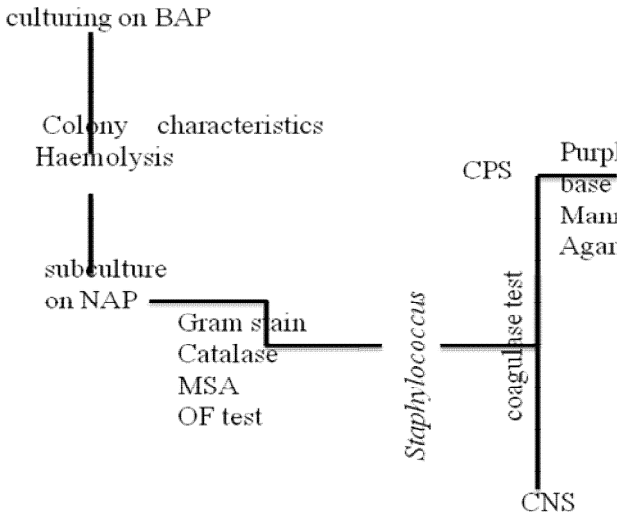
8. APPENDIX

Annex 1: Variables to explain *Staphylococcus* occurrence

Variables	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
1. Date of sample collection					
2. Name of the owner					
3. District					
4. Locality					
5. Family size of the owner					
6. Ethinicity of the owner					
7. Age of sampled owner					
8. Sex of sampled owner					
9. Education level of sampled owner					
10. Income level monthly of sampled owner					
11. Recent hospitalization history					
12. Is hand washing practiced before & after milking					
13. Number of Cattle owned					
14. Number of sheep owned					
15. Number of goats owned					
16. Number of chicken owned					
17. Number of equines owned					
18. Breed of the sampled cattle					
19. Age of the sampled cattle					
20. Sex of the sampled cattle					
21. Parity level of the sampled cattle					
22. Lactation stage of the sampled cattle					
23. Daily milk yield of the sampled cattle					
24. Udder health status of the sampled cattle					
25. Udder teat infestation					
26. Previous mastitis infected history					
27. Barn hygiene status of the sampled cattle					
28. Manure handling status in the farm					
29. Is mixed species farming practiced?					
30. Is mixed age farming practiced?					
31. Source of water for the farm animals					
32. Date of previous antibiotic treatment					
33. Date of previous antihelmintic treatment					

Annex 2: Flow chart of the laboratory analysis protocol



BAP= Blood agar plates, MSA= *Staphylococcus* , CPS= Coagulas plates , PAB= Purple agar base

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

Serial number	Type of sample	Sample code	Colony characteristics on primary culture media					Haemolysis	Appearance in gram stain		Catalase test	Oxidase	O-F glucose test	Coagulase reaction	Growth on MSA	Mannitol fermentation(MSA)	Maltose fermentation (PAB)	*staphylococcus*
			Shape	size	pigmentatio	opacity	surface		Gram	Arrangeme								

Annex 4: The main differentiating characteristics of the Gram-positive cocci

Bacteria	Haemolysis	Catalase	Oxidase	Coagulase	O-F Glucose
Pathogenic *Staphylococci*	+(-)	+	-	+	F
Non-Pathogenic *Staphylococci*	-(+)	+	-	-	F
Enterococci	(+)	-	-	-	F
Streptococci	(+)	-	-	-	F
Micrococci	-(+)	+	+	-	O

(+)=some strain positive, (-) =some strain negative, F=fermentative, O=oxidative, R=resistant, S=susceptible, +=90% or more strains are positive, -=90% or more strains are negative

Annex 5: Record sheet for identification of coagulase positive *Staphylococci* and coagulase negative *Staphylococci*

serial number	*Staphylococci* species	Haemolysis	Pigment production	Coagulase test	Fermentation		Polymyxin B 300 unit disc
					MSA	PAB	
1	*S.aureus*	+	+	+	+	+	R
2	*S.intermidius*	+	-	+	±	±	S
3	*S.hicus*	-	-	+	-	-	R
4	CNS	-	-	-	-	-	

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

Annex 6: Sample collection sheet for bacteriological analysis

serial number	Date of collection	Type of sample	Source	Sample code	Area