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Prevalence and antibiotic susceptibility of *Staphylococcus aureus* from lactating cow's milk in Bahir Dar dairy farms

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Staphylococcus aureus **is competitive in milk and dairy environments; pathogenic strains have been found to cause disease in their host throughout the world. Therefore, this study was designed to assess the prevalence of *Staphylococcus aureus* and determine their antibiotic susceptibility from lactating cow milk. A cross-sectional study was conducted in Bahir Dar dairy farms from October 2012 to March 2013. A total of 218 raw milk samples from lactating cows were collected from dairy farms in Bahir Dar, North-West Ethiopia. The *S. aureus* bacteria were isolated on Mannitol Salt agar (Becton, Dickinson) where yellow colonies were selected and counted and then maintained for antibiotic susceptibility tests. Susceptibilities of the isolates were tested against 9 antibiotics using the Kirby-Bauer disc diffusion method. Overall, 98 milk samples (45%) were found to be contaminated with S. aureus with average count varying between 3.3×10^2 to 7.2×10^4 CFU/ mL. *S. aureus* prevalence showed significant variation among cows of different hygienic conditions ($p < 0.05$). *S. aureus* isolates were highly susceptible to ciprofloxacin (100%) followed by gentamycin (96%), chloramphenicol (74%), erythromycin (68%), trimethoprimsulfamethoxazole (66%) and tetracycline (60%). In contrast, isolates were highly resistant to penicillin (94%) and cephoxitin (62%). Most of the isolates (96%) were resistant to one or more antibiotics. In general, the results of the present study revealed that milk provided to the consumers in the city was found to be less hygienic. Thus, farmers should ensure strict personal hygiene and that of animals, and general sanitary condition of the farms should be improved and maintained.**

Key words: Antibiotics, Bahir Dar, milk, *Staphylococcus aureus*, susceptibility.

INTRODUCTION

Most foods contain viable bacteria unless thoroughly heated or pasteurized. Otherwise, food serves as an important vehicle for transmission of pathogenic organisms to consumers. Contamination of food products with pathogenic organisms may influence considerably their harmlessness, endanger the health of consumers

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and decrease shelf life, resulting in food borne infections, intoxications and economic losses from food spoilage (HPSC, 2012).

Milk is one of the most important foods for human beings and also universally recognized as a complete diet due to its essential components (Javaid et al., 2009). However, health risk to consumers can be associated with milk, due to the presence of zoonotic pathogens and antimicrobial drug residues (Vyletělova et al., 2011). Milk is an excellent bacteriological medium for a large number of microorganisms, including *Staphylococcus aureus*. When milk is drawn from the udder of a healthy animal, it contains organisms that have entered the teat canal through its opening. *S. aureus* bacteria are mechanically flushed out during milking. The number ranges during milking between several hundred to several thousand per milliliter (Farzana et al., 2004).

Milk can be contaminated by *S. aureus* when there is infection of the mammary gland or by bad hygiene habits, such as coughing or sneezing and not washing hands when handling milk storage equipment, during or after milking, and in some cases, human activity is responsible for the contamination, as this bacteria colonizes the nasal pathways in human beings (Fagundes et al., 2010). The micro biota found on the hands and on the uniform of food handlers, especially of milk, is a reflection of hygiene habits as the single most important factor in the contamination of milk (Lingathurai and Vellathurai, 2011). *S. aureus* is competitive in milk and dairy environment. Pathogenic strains are usually coagulase-positive and have been found to cause disease in their hosts throughout the world. The presence of *S. aureus* shows up unsanitary conditions in the cattle herd and counts above 10^3 CFU/mL in milk increase the risk of staphylococcal toxin production more resistant to the heat processes of pasteurization (Tortora et al., 2005). Diseases in cattle caused by *S. aureus* are ranging from simple abscesses and mastitis to the more severe toxic shock syndrome (Tesfaye et al., 2010).

The growth of *S. aureus* and potential production of heat-stable enterotoxins with respect to the food matrices and conditions of food preparation represent a potential, even actual threat of a public health problem residing in food poisoning outbreaks. That is why the control of *S. aureus* growth during the fermentation of young raw milk cheese means prevention against staphylococcal enterotoxin production is recommended (Charlier et al., 2009).

S. aureus in raw milk comes from cows with mastitis, from handlers or from deficient hygiene (Fagundes et al., 2010). When found in milk, high levels of contamination can be reached quickly under favorable conditions. Its presence in foods can be a risk to human health, causing a public health problem, as these bacteria produces toxins that can cause toxic food infections (Quintana and Carneiro, 2006). The capacity to coagulate plasma, the principal characteristic of the *S. aureus*, is highly correlated to the capacity to produce enterotoxins harmful to the tissues of the contaminated host (Murray et al., 2006).

S. aureus mastitis is a serious problem in dairy production and infected animals may contaminate bulk milk. *S. aureus* is still an important cause of food borne intoxications worldwide (Ertas et al., 2010). The ability of *S. aureus* to grow and produce staphylococcal enterotoxins (SEs) under a wide range of conditions is evident from the variety of foods implicated in staphylococcal food poisoning (SFP) (Le Loir et al., 2003). SFP is suspected when the symptoms including nausea, violent vomiting, abdominal cramps and diarrhea affect the patients between 1 and 8 h after food consumption (Balaban and Rasooly, 2001). In addition, the prevalence of mastitis and its associated pathogens in animals can be reduced by improving on the farm management techniques within the dairy industry (Pitkälä et al., 2004). It is thus of paramount importance to ensure that proper hygienic practices are enforced in both the area where the animals are kept and the milking environment (Lingathurai and Vellathurai, 2011).

Although it is difficult to control mastitis caused by *S. aureus* with antibiotics only, various antimicrobial agents antifungals are constantly being used to treat this disease in cattle. This practice results in the development of antibiotic-resistant strains (Thaker et al., 2013). The usage of antibiotics correlates with the emergence and maintenance of antibiotic-resistant traits within pathogenic strains (Shitandi and Sternesjö, 2004). These traits are coded for by particular genes that may be carried on by the bacterial chromosome, plasmids and transposons or on gene cassettes that are incorporated into integrons **(**Rychlik et al., 2006), thus are easily transferred among isolates. Multiple antibiotic resistant *S. aureus* strains have been isolated from milk obtained from cattle, beef and human samples in many parts of the world (Pesavento et al., 2007). The prevalence of antibiotic resistance usually varies between isolates from the different sampled stations and even between isolates from different herds on the same farm (Waage et al., 2002). Consequently, the good quality milk is a challenge that can be overcome provided that basic care is taken at the source of production (Alves, 2006). The importance of microorganisms in the milk means that their microbial contamination index can be used to judge the quality, as well as the sanitary conditions of its production and the health of the herd (Guerreiro et al., 2005).

Significance of the study

Antibiotic-resistant *S. aureus* isolates poses a severe challenge to both veterinary and health professions and dairy cattle producers because of their negative impact on therapy. Therefore, determination of levels of *S. aureus* and an evaluation of the antibiotic-resistant

phenotypes of the isolates could serve as a tool for determining the hygiene standards implemented during milking. Data on antibiotic resistance could also be used to characterize these opportunistic pathogens, which may further limit the risks associated with the consumption of contaminated milk and its products. There is few published data about bovine mastitis in dairy farms of Bahir Dar and its surrounding by Bitew et al. (2010) and Almaw et al. (2008). However, there is no report about the current status of the prevalence and susceptibility assay specifically of *S. aureus* from healthy lactating cow's milk in dairy farms of Bahir Dar.

Objectives of the study

General objective

To determine the prevalence and antibiotic susceptibility of *S. aureus* from lactating cow milk in Bahir Dar dairy farms

Specific objectives

1. To determine the prevalence of *S. aureus* in milk of cow in Bahir Dar Dairies
2. To evaluate the antibiotic susceptibility patterns of *S. aureus* isolates
3. To assess some associated risk factors for contamination of cow milk by *S. aureus* and
4. To assess the time course of growth of *S. aureus*

MATERIALS AND METHODS

The study area

The study was conducted in dairy farms of Bahir Dar. Bahir Dar is the capital of Amhara National regional state and is located at about 578 km North-West of Addis Ababa which is the capital of Ethiopia. It has a total population of 256,999 (CSA, 2011). The area of the town is 160 km^2.. Geographically the region is located between 9°20' and 14°20' latitude North and 30°20' and 40°20' longitude East. It has a summer rainfall; the highest rainfall is between June and September and a winter dry season (December to March) with mean annual rainfall of 1200 to 1600 mm, mean temperature 10 to 20°C and an altitude at 1500 to 2300 m above sea level (Bureau of Agriculture, 2006).

Study design

Cross sectional study was conducted. The study was conducted on dairy farms found in Bahir Dar, North-West Ethiopia between October 2012 and March 2013. The study populations were all lactating Holstein crossbred cows. The laboratory study was done in Microbiology Laboratory, Bahir Dar University.

Sample size determination

The desired sample size was calculated according to the formula given by Thrusfield (2005) cited in Tariku et al. (2011). About 218 lactating cows were considered to be as a sample.

Sample collection

Aseptic procedures were followed for milk sample collection, handling, and transportation to the Microbiology laboratory, Bahir Dar University. Samples of approximately 10 ml of fresh milk were collected from dairy farms by using sterilized test tubes and ice box. Two hundred and eighteen (218) observational check list copies were prepared and then observation was implemented to identify potential risk factors and evaluate their effects on the quality of the milk. Data on each farmer's herd has been collected in a properly designed data collection manner.

Isolation of *S. aureus* from milk samples

The samples were processed immediately upon arrival using aseptic techniques. Ten-fold serial dilutions (10^{-1} to 10^{-3}) were performed using sterile saline solution and 1 ml from each dilution was taken aseptically and pour plated in to three plates of mannitol salt agar (Oxoid, England). The plates were incubated aerobically at 37°C for 18 to 24 h (Quinn et al., 2004). After growth of organisms, plates with yellow colonies with bright yellow zones were counted. The mean number of *S. aureus* cfu/ml of milk for each original sample was calculated by taking the average number of bacteria per milliliter from the three dilutions. The number of cfu/ml was then calculated.

A colony of all Staphylococci positive samples was sub-cultured on mannitol salt agar (MSA) and incubated for 24 h at 37°C and the isolates were retained for antibiotic susceptibility test as slants on nutrient agar.

Antibiotic susceptibility testing

Antibiotic susceptibility testing was performed by the Kirby-Bauer disc diffusion method. Antibiotic susceptibility tests were performed on 50 *S. aureus* isolates to determine their antibiotic-resistance profiles (Kirby et al., 1966). *S. aureus* from the agar slants were inoculated in tryptose soy broth and incubated at 37°C. Fresh overnight cultures were used for antibiotic sensitivity tests. An aliquot from each isolate suspension was spread plated by sterilized swab on Mueller Hinton agar (Oxoid, England). Antibiotic discs were gently pressed onto the inoculated Mueller Hinton agar (Oxoid, England) to ensure intimate contact with the surface and the plates were incubated aerobically at 37°C for 18 to 24 h (CLSI, 2011). Antimicrobial susceptibility test was conducted using 9 antibiotics. The antibiotics used were erythromycin (15 µg), penicillin G (10 IU), gentamicin (10 µg), trimethoprim-sulfamethoxazole (25 µg), chloramphenicol (30 µg), vancomycin (30 µg), tetracycline (30 µg), cephoxitin (30 µg) and ciprofloxacin (5 µg). The susceptibility of the *S. aureus* isolates (Inhibition zone diameters) to each antibiotic agent were measured and the results were categorized as either susceptible, intermediate or resistant based upon interpretive criteria developed by the Clinical and Laboratory Standards Institute (CLSI) to antimicrobials (CLSI, 2011).

Growth pattern of *S. aureus* bacteria

The selected *S. aureus* isolate was evaluated for growth pattern in nutrient broth. To assess the growth pattern of the *S. aureus* isolate, exponentially growth culture was inoculated into liquid nutrient broth and incubated. Growth was determined

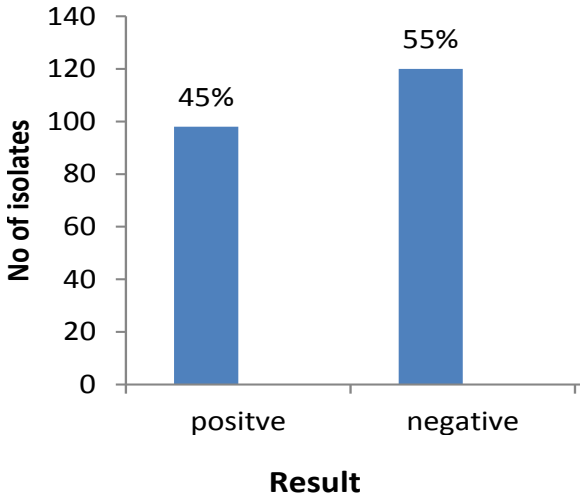

Figure 1. Prevalence of *S. aureus* in Bahir Dar dairy farms (n=218). Positive: *S. aureus* contaminated samples; Negative: Samples free from *S. aureus.*

Table 1. Viable count of *S. aureus* in lactating row cow milk in Bahir Dar Dairy farms.

Number of contaminated samples	Corresponding viable count (CFU/ml)
16	10^2
21	10^3
61	10^4

turbidimetrically after interval of 6 h for three days by measuring optical density (at 540 nm wave length) using spectrophotometer (Appendix Table 1).

Data analysis

The data was analyzed using SPSS 16 software. A *p* value less than 0.05 were considered as statistically significant. Descriptive statistics such as percentages and frequency distributions were used to describe the nature and the characteristics of the data. The association between prevalence of *S. aureus* and the associated risk factors were compared by using Chi square test.

Ethical clearance

This study has been ethically cleared by Bahir Dar University Biology Department. The objectives of this study were well explained to all participating smallholder dairy farmers who all expressed their consent to participating in the study.

RESULTS AND DISCUSSION

Prevalence of *S. aureus*

Out of 218 milk samples (from a total 218 cows) analyzed, 98 of them were found to be contaminated by *S. aureus*, corresponding to 45% of samples and 120 (55%) of them were free from *S. aureus* (Figure 1), with an average viable count varying between 3.3×10^2 to 7.2×10^4 CFU/ mL. A total of 98 potential isolates were sub-cultured and further analyzed for antibiotics susceptibility. The results demonstrated the presence of *S. aureus* and the levels of contamination with *S. aureus* were high in milk samples. The frequency of *Staphylococcal* counts varied between different herds of farmers. Out of the contaminated samples of raw milk, 16 had levels of *S. aureus* corresponding to 10^2 CFU/mL; 21 had levels of 10^3 CFU/mL and 61 samples had count of 10^4 CFU/mL (Table 1).

Counts of *S. aureus* above 10^3 increase the probability of production of staphylococcal toxins that are resistant to boiling carried out in the homes when buying raw milk, and to the pasteurization processes (Tebaldi et al., 2008). Considering this, most of the samples had numbers of *S. aureus* above 10^3 CFU/mL, thus such milk consumed in Bahir Dar has a serious risk to the health of the population.

The presence of *S. aureus* also shows deficient sanitary conditions of the cattle herd. The presence of *S. aureus* in milk results from Bahir Dar dairy farms are similar to those of Oliveira et al. (2011), where out of 50 samples of raw milk in Brazil, 68% samples were contaminated with *S. aureus*. Similarly, Daka et al. (2012) analyzed a total of 160 milk samples and found 78 samples positive for *S. aureus* (49% of total isolates) in

Table 2. Risk factors for the prevalence of *S. aureus* in milk of lactating cows, Bahir Dar dairy farms.

Associated risk factors	Negative No. (%)	Positive No. (%)	Total (%)	χ2 (p-value)
Washing teats and udder before milking				
Yes	117 (53.7)	52 (23.85)	169(77.5)	61.137(0.000)
No	3 (1.4)	46 (21.1)	49(22.5)	
Milkers wash their hands after milking each cow				
Yes	38 (17.43)	6 (2.75)	44 (20.18)	21.85(0.000)
No	82(37.6)	92 (42.2)	174 (79.8)	
Milkers use any agent to clean their hands				
Yes	5 (2.3)	0 (0)	5 (2.3)	4.179 (0.049)
No	115(52.75)	98 (44.95)	213 (97.7)	
Floor hygiene				
Good	37 (17)	2 (0.9)	39(17.9)	30.444(0.000)
Poor	83 (38)	96 (44)	179 (82)	
Hair				
Covered	41 (19)	14(6)	55 (25)	11.303(0.001)
Not covered	79(36.2)	84 (38.5)	163 (74.77)	
Milking utensils cleaned				
Yes	33 (15)	6 (3)	39 (18)	16.783(0.000)
No	87 (40)	92 (42)	179 (82)	

+Total number of cows observed =218, while milking by 41 milkers; Positive: *S. aureus* contaminated samples; Negative:- Samples free from *S. aureus.*

Hawasa, Ethiopia. Moreover, Mekonnen et al. (2011) observed that 39.5% of milk samples in total of 200 milk samples were contaminated with *Staphylococcus* species in Debre Zeit, Ethiopia.

In contrast, our study had very low count of *S. aureus* compared to the previous study conducted by Ateba et al. (2010) in South Africa who reported 100% prevalence of *S. aureus* in the milk samples analyzed. Quintana and Carneiro (2006) analyzed raw milk in Morrinhos, in the state of Goias and found that 28.5% of the samples had *S. aureus* above 10^4 CFU/mL. Similarly, Wani and Bhat (2003) examined 100 milk samples and recovered 95 bacterial isolates. Out of these bacterial isolates 45% were *S. aureus.* These results are similar with the results obtained in this study.

Contamination in milk was also detected in other countries, with similar results. In Palestine, 48 (36.9%) of samples were positive for *S. aureus* out of 130 samples (Farhan and Salk, 2007). In Turkey, Ekici et al. (2004) found 18.18% of samples contaminated of the total samples studied. In the north of Morocco, Bendahon et al. (2008) isolated 40% of *S. aureus* in raw milk from samples.

In India, 61.7% *S. aureus* were detected in 60 samples of raw milk (Lingathurai and Vellathurai, 2011) and D'Amico and Donnelly (2010) found 29% samples positive for *S. aureus* in Vermont, in the United States.

Associated risk factors for the contamination of milk by *S. aureus*

The main source of the infection is the udder of infected cows which is transferred via milker's hands, utensils, towels and the environment in which the cows are kept (Radostitis et al., 1994). *S. aureus* has adapted to survive in the udder and establish chronic and subclinical infections. From there it is shed into the milk, which serves as a source of infection for healthy cows during the milking process. The high prevalence of *S. aureus* can most likely be attributed to the wide distribution of the organism inside mammary glands and on the skin of teats and udders (Radostitis et al., 1994). The mammary gland is more susceptible to new infection during the early and late dry period, which may be due to the absence of udder washing and teat dipping, which in turn may have increased the presence of potential pathogens on the skin of the teat (Radostits et al., 2000). The associated risk factors for the contamination of milk in this study are indicated in Table 2. As Table 2 indicated, the majority of the milkers (97.7%) did not use any kind of agent soap, detergents and disinfectants) to clean their

Table 3. Antibiotics used in this study to test for resistance/sensitivity.

Antibiotic	Abbreviation	Antibiotic disc conc. (µg)	Inhibition zone diameter (mm)		
			R	I	S
Cephoxitin	CX	30	≤21	_	≥22
Chloramphenicol	C	30	**≤12**	**13-17**	**≥18**
Ciprofloxacin	CIP	5	≤15	16-20	≥21
Erythromycin	E	15	≤13	14-22	≥23
Gentamycin	GEN	10	**≤12**	**13-14**	**≥15**
Penicillin	P	10	**≤28**	_	**≥29**
Tetracycline	TE	30	≤14	15-18	≥19
Trimethoprim-sulfamethoxazole	SXT	25	≤10	11-15	≥16
Vancomycin	VA	30	≤14	_	≥15

Source: The inhibition zone measurements were according to the Clinical and Laboratory Standards Institute 2011. The abbreviations are as they appeared on the antibiotic discs. R: - Resistance, I: - Intermediate and S: - Susceptible

hands. However, 2.3% of them used detergent to wash their hands.

Cross-contamination can be avoided if hand of milkers, utensils or equipment is washed with detergents and water in between using it after milking each cow. Hand washing is an essential component of infection control (Larson et al., 2003). During this study, it was observed that, majority of milkers (79.8%) did not wash their hands after milking each cow, but all of them washed their hands at the beginning. While 82% of milking utensils for each cow were not cleaned. Observations showed that some utensils were not cleaned properly, which could result in milk contamination due to microbes (*S. aureus*). The utensils in which the food is displayed for sale must be kept clean, covered and protected as they easily become contaminated if left dirty or unprotected (FAO, 2001).

Further observations revealed that most of the milkers had washed teats and udder before milking, but washing quality varied significantly. From food hygiene point of view, the quality of the working environment depends on the facilities or equipment provided like disposal of waste products. Based on observation, 82% of the lactating cows are housed in poor hygienic conditions.

Antibiotic susceptibility of *S. aureus*

The *S. aureus* isolates were tested for antibiotic susceptibility against nine antibiotic agents and they were classified depending on their inhibition zone diameter (Table 3).

Out of 98 isolates of *S. aureus*, antibiotic susceptibility tests were performed on 50 isolates. In this study, *S. aureus* isolates were found to be highly susceptible to ciprofloxacin (100%) followed by gentamycin (96%), vancomycin (82%), chloramphenicol (74%), erythromycin (68%), trimethoprim-sulfamethoxazole (66%), tetracycline (60%), cephoxitin (38%) and penicillin (6%). However, resistance to penicillin was most common (94%), followed by cephoxitin (62%), tetracycline (34%), trimethoprim-sulfamethoxazole (30%), chloramphenicol and erythromycin (20%), vancomycin (18%) and gentamicin (4%). All isolates tested for antibiotic sensitivity were susceptible to ciprofloxacin. The resistance pattern of *S. aureus* isolates to nine antibiotics tested in this study is shown in Table 4.

The highest resistance observed against penicillin in the present study is similar to that of Thaker et al. (2013) in India, which indicated as the overall (100%) *S. aureus* isolates were resistant to Penicillin-G. In Jimma Town, Ethiopia; Tariku et al. (2011) reported that out of 86 isolates of *S. aureus* which were isolated from Dairy farms, 87.2% were Penicillin resistant. Furthermore**,** Abera et al. (2010) in Adama Town, Ethiopia, showed that 94.4% of *S. aureus* isolates were resistant to penicillin.

The present study demonstrated that the resistant isolates may have been transferred to cow then to milk, which can be the reason of infection in human beings consuming raw milk. This contamination can be evaded by improving hygienic conditions and careful handling of cow during milking.

In this study, most of the isolates (96%; n=48) were resistant to one or more antibiotic agent. Five isolates (10.4%) were resistant to single antibiotic and 15 isolates (31.3%) showed resistance to 2 antibiotics. Multiple resistances to 3 or more antibiotics were found in 28 (58.3%) of *S. aureus* isolates (Table 5). However, 2 (4%) of isolates were sensitive to all antibiotic agents tested. The highest multiple drug resistance in *S. aureus* isolates were seen against three common antibiotics; Penicillin, Cephoxitin and Trimethoprim- sulfamethoxazole.

The overall susceptibility of *S. aureus* to all antimicrobial agents tested is resulted 288 (64%) susceptible, 14 (3%) intermediate resistant and 148 (33%) resistant (Figure 2).

The overall susceptibility (64%) of *S. aureus* isolates to

Table 4. Antibiotic sensitivity pattern of *S. aureus* (n=50) from lactating cow milk samples in Bahir Dar dairy farms, number (%).

Antimicrobial agent	Susceptible	Intermediate	Resistant
Erythromycin	34 (68)	6 (12)	10 (20)
Penicillin	3 (6)	-	47 (94)
Gentamicin	48 (96)	-	2 (4)
Trimethoprim- sulfamethoxazole	33 (66)	2 (4)	15 (30)
Chloramphenical	37 (74)	3 (6)	10 (20)
Vancomycin	41 (82)	-	9 (18)
Tetracycline	30 (60)	3 (6)	17 (34)
Cephoxitin	19 (38)	-	31 (62)
Ciprofloxacin	50 (100)	0 (0)	0 (0)

Table 5. Pattern of antibiotic resistance in bacterial isolates from lactating cow milk.

Number of antibiotics	Number of resistant isolates (%)	Resistance pattern
1	5 (10.4)	P (4) CX(1)
2	15 (31.3)	VA, P (2) TE, P (3) SXT, P (2) CX,P (6) E,P (1) P, CX (1)
3	15(31.3)	SXT,P, CX (7) TE, P, CX (2) C, TE, P (1) TE, VA, P (1)
4	6 (12.5)	P, E, CX (3) TE, P, CX (1) C, TE, P, CX (1) C, TE, VA, P (1) C, SXT, P, E (1) SXT,VA,P,CX(1) TE, SXT, P, E(1)
5	6 (12.5)	TE, SXT, P,CX (1) C, TE, VA, P, CX (2) C, TE, SXT, P, CX (2) C, VA, P, E, CX (1) TE, VA, P, GEN,CX(1)
6	1 (2)	C,VA, P, GEN, E, CX (1)

Total number of isolates = 48; values in parenthesis indicate percentage of the total isolates; C, chloramphenicol; CX, cephoxitin; E, erythromycin; GEN, gentamycin; P, penicillin; TE, tetracycline; SXT, trimethoprim- sulfamethoxazole; VA, vancomycin

all antibiotics tested in this study is in agreement with the reports of Mekonnen et al. (2005) in Ethiopia which shows isolates are 62.7% .The overall susceptibility in the present study is higher compared to that of Sasidharan et al. (2011) in Malaysia which is 6% to all antibiotics used. The present study has demonstrated the existence of high levels of resistance of *S. aureus* to commonly used antibiotics and the results are in accordance with reports from earlier studies in other countries which suggests that a possible development of resistance due to prolonged

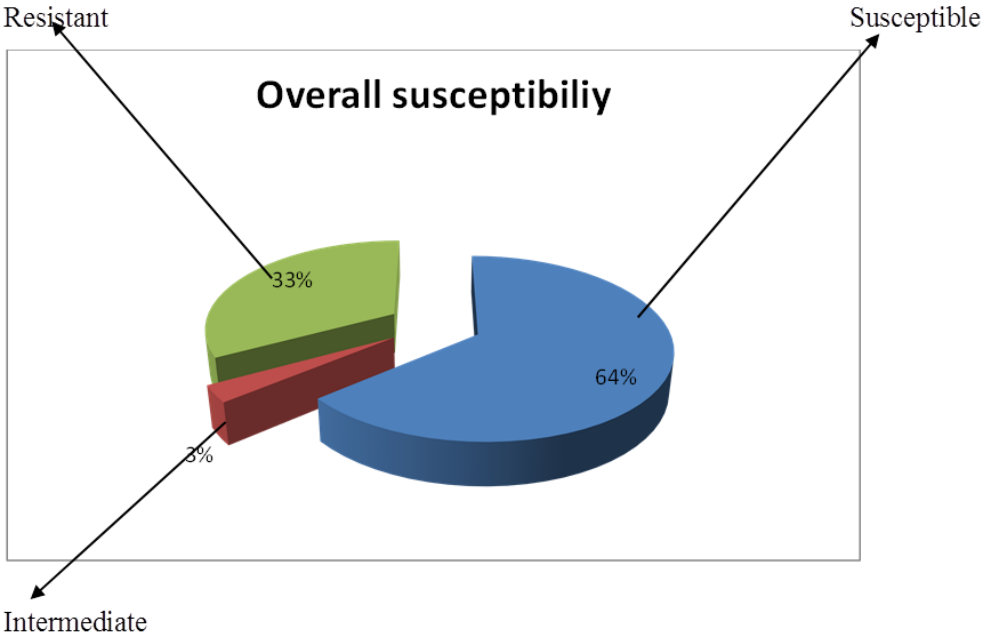

Figure 2. The overall susceptibility of *S. aureus* to all antimicrobial agents.

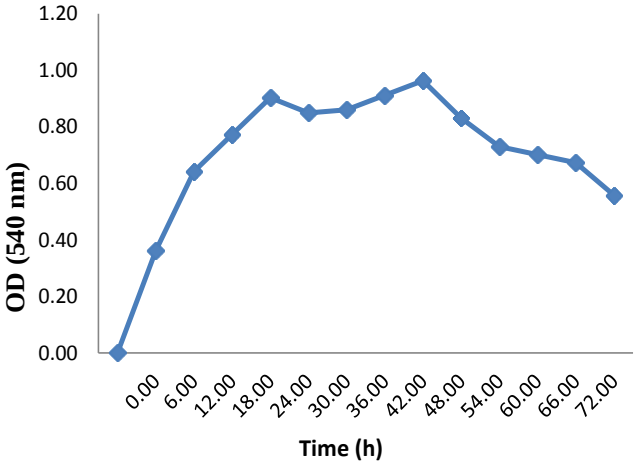

Figure 3. Growth curve of the most resistant *S. aureus* isolate.

and indiscriminate usage of antibiotics (Edward et al., 2002; Gentilini, 2000).

Growth pattern of *S. aureus* bacteria

The most resistant *S. aureus* isolate was used to assess the growth pattern in liquid medium (nutrient broth). Growth curve of the bacterial isolate indicated that *S. aureus* population increases linearly and sustains longer in fluctuating stationary phase (Figure 3).

S. aureus is a major pathogen of increasing importance due to the rise in antibiotic resistance. The growth and survival of *S. aureus* is dependent on the cells ability to adapt to environmental changes. *S. aureus* has evolved many mechanisms to overcome such changes, particularly in an infection (Lowy, 1998). A growth curve of *S. aureus* grown under ideal conditions can be divided into three phases: Lag, exponential, and stationary, as shown in Figure 3. During exponential phase, bacterium metabolism is rapid and efficiently to ensure constant growth. As the bacteria age and stop growing (post-exponential), cellular metabolism is re-organized for long-term survival under stationary conditions.

Conclusion

Results of the present study showed that lactating cow milk is highly contaminated by *S. aureus.* The study also revealed that *S. aureus* is still prevalent in small holder dairy farms in the study area. A large proportion of the isolates obtained in this study were resistant to three or more antibiotics. The presence of resistant *Staphylococci* in milk production poses a risk of spreading the pathogens to other animals, humans involved in animal care and milk processing, and consequently to the general population. The contamination of milk with *S. aureus* was associated with several risk factors. In general, the results of the present study revealed that milk provided to the consumers in the city was found less hygienic. Lack of general hygiene of milk handlers, personal hygiene, and environmental hygiene were identified as the major sanitary deficiencies. Therefore, the probability of milk contamination in these farms was high.

Recommendations

Based on the results of present study the following recommendations are forwarded:

1. Information on health hazards associated with contaminated raw milk should be extended to the public, so that consumption of untreated/improperly treated raw milk could be avoided.
2. Farmers should ensure strict personal hygiene and that of animals, and general sanitary condition of the farms should be improved and maintained.
3. Monitoring antimicrobial susceptibility in pathogenic bacteria in animals is recommended.
4. Further research is recommended about impacts and dynamics of genetic antibiotic determinants of *S. aureus*.
5. Proper risk assessment should be conducted to further clarify the possible health hazard for consumers related to the presence of *S. aureus* in milk.

Conflict of Interests

The authors have not declared any conflict of interests.

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APPENDIX

Observational check list

1. Description of the farm
Name _____
2. Herd composition
Species _____
3. Quality of milking environment
I) Floor hygiene: (a) relatively good_____
(b) Poor_____
4. Pre- milking hygienic condition of cows:
(I) Are teats and udder washed before milking? (a) Yes
(b) No
(II) Do you use any agent (detergent or disinfectant) for cleaning? a) Yes b) No
(III) Udder hygiene (a) Free of dirt_____
(b) Slightly dirty_____
5. Hand washing habit
I) Do milkers wash their hands before milking? (a) Yes
(b) No
II) Do milkers use detergent or disinfectant to clean their hands? a) Yes
(b) No
III) Have you drying your hands? (a) Yes
(b) No
IV) Do milkers wash their hands after milking each cow? (a) Yes
(b) No
6. Hair of milkers: Covered_____
Not covered_____
7. Milking utensils and environment
I) are milking utensils cleaned? (a) Yes
(b) No
II) Cleaning quality of milking utensils: (a) good_____
(b) Poor_____

Appendix Table 1. Time course growth pattern of *S. aureus* bacteria by measuring optical density.

Time (h)	Optical density (at 540 nm wave length)
0	0.361
6	0.642
12	0.7725
18	0.903
24	0.849
30	0.860
36	0.9115
42	0.963
48	0.831
54	0.730
60	0.7015
66	0.673
72	0.558