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Identification and antimicrobial susceptibility of *Staphylococcus aureus* isolated from milk samples of dairy cows and nasal swabs of farm workers in selected dairy farms around Addis Ababa, Ethiopia

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Staphylococcus aureus **is a major problem of public health which causes a number of human and animal diseases. In order to isolate and identify *S. aureus* from milk of dairy cows and nasal swabs of farm workers of different farm settings, a cross sectional study was conducted on a total of 260 lactating dairy cows milk and 68 nasal swabs of farm workers in selected dairy farms around Addis Ababa, Ethiopia. The collected nasal swabs and milk (California Mastitis Test Screened) were cultured on sheep blood agar. Presumptive Staphylococci colonies were sub-cultured on mannitol salt agar and confirmed by BiOLOG Identification system. Antimicrobial susceptibility pattern of *S. aureus* isolates was done by Kirby-Bauer disk diffusion method using twelve antimicrobials. The prevalence of *S. aureus* was found to be 51 (15.5%) out of the total samples examined. In addition, the prevalence of *S. aureus* was 42 (16.2%) from milk of 260 lactating dairy cows and 9 (13.2%) from nasal swabs of 68 farm workers. The prevalence of *S. aureus* ranges from 8 (8.9%) to 13 (36.1%) in selected dairy farms where poorly managed farms showed high prevalence. *S. aureus* was more likely to occur in cows that were poorly managed and treated frequently with antimicrobials. Antimicrobial susceptibility test revealed the resistance of S*. aureus* to the tested antimicrobials. Thus, out of a total of 51 isolates, high resistance rate was observed primarily to penicillin G 47 (92.2%) followed by tetracycline 34 (66.7%), amoxicillin-clavulinic acid 19 (37.3%), oxacillin 17 (33.3%), cephalothin 14 (27.5%) and low level of resistance to chloroamphenicol 12 (23.5%), erythromycin 12 (23.5%), sulphamethoxazole-trimethoprim 11 (21.6%), gentamicin 10 (19.6%), clindamycin 9 (17.6%), vancomycin 2 (3.9%) and rifampicin 1 (2%). There was no statistically significant difference (p>0.05) between type of samples in determining resistance pattern to each antimicrobial except for pencillin G and tetracycline. Multidrug resistance was also observed in 23 (45.1%) of the total isolates and most of them were from milk samples, 20 (47.6%) with no statistically significant difference (P>0.05) between dairy farm workers and dairy cows. S*. aureus* became almost resistant to β-lactams and tetracycline. Hence, antimicrobial susceptibility should be conducted before treating dairy cows. Consequently, reduction in transfer of resistant S*. aureus* strains between humans and animals could possibly be made.**

Key words: *Staphylococcus aureus,* dairy cow milk, farm workers, antimicrobial susceptibility test, Ethiopia.

INTRODUCTION

Staphylococcus aureus is a versatile pathogen of humans and animals that causes a wide variety of diseases.

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In human, it causes diseases that range from mild skin infection to more severe diseases such as pneumonia and septicaemia (Ateba et al., 2010). In animals, it is commonly associated with mastitis leading to contamination of milk and dairy products (Oliver et al., 2005). In the last few decades, Staphylococcal food poisoning has been reported as third cause of food-borne illnesses in the world. Among the foods implicated in Staphylococcal food poisoning, milk, dairy products and meats, particularly handled foods, play a vital role since enterotoxigenic strains of *S. aureus* have been commonly isolated in them (Ateba et al., 2010). *S. aureus* is present in a variety of locations on the dairy farms, and several studies suggested that transfer of *S. aureus* between humans and cows is possible (Zadoks et al., 2000). Multidrug-resistant staphylococcal isolates such as Methicillin-Resistant *S. aureus* (MRSA) were isolated primarily from human samples, but such isolates were detected in animal samples (Lee et al., 2004; Asao et al., 2003). Thus, the transfer of *S. aureus* between humans and cows may result in serious problems.

Antimicrobial resistance is a main public health worry worldwide. Public hazards associated with the consumption of antibiotic contaminated milk could be allergic responses, changes in intestinal flora and development of antibiotic resistant pathogenic bacteria (Thirapatsakun, 1999). The expansion of resistance both in human and animal bacterial pathogens has been allied with the widespread remedial use of antimicrobials or with their administration as growth promoters in animals. Further transfer of antimicrobial resistant bacteria to humans via the food chain has been reported (Angulo et al., 2004). In this context, *Staphylococcus* present in milk may serve as a reservoir for human infections, thus allowing these microorganisms to persist and spread in the community. Up to now, many researchers have focused on the spread of resistant *S. aureus* in clinical setting (Ateba et al., 2010; De silva ciombra et al., 2003). However limited number of investigations has been studied with regards to the presence of antimicrobial resistance in food animals in Ethiopia (Mekonnen et al., 2005; Hundra et al., 2005) and no data were published yet on individuals who have contact with animals. In this view, the study was designed to isolate *S. aureus* from milk of lactating dairy cows and nasal swabs of dairy farm workers around Addis Ababa, Ethiopia. Moreover, the study aimed to evaluate antimicrobial resistance of pattern of *S. aureus*. Therefore, the data presented should provide a good estimate of magnitude and antimicrobial susceptibility of *S. aureus* for both human and animal.

MATERIALS AND METHODS

Study design and area of investigation

A cross-sectional study was conducted from November 2011 to April 2012 in selected dairy farms located around Addis Ababa with a target to supply milk for consumers in Addis Ababa. The study area was selected based on the presence of potential dairy farms. Accordingly, dairy farms located at Holeta and Sebeta towns around Addis Ababa were selected.

Sample size and sampling technique

A total of 328 samples were considered from different farm settings selected around Addis Ababa, Ethiopia. The sample size of dairy cows (n = 260) was determined according to the formula given by Thrusfield (2005) by taking prevalence of *S. aureus*, 19.6% isolated from mastitic dairy cows conducted by Abera et al. (2010) at Adama, Ethiopia. Accordingly, the calculated value for sample size is equal to 236. But to increase precision, 10% contingency was added. Thus, the sample size was determined to be 260 for dairy cows. However, the numbers of dairy farm workers were obviously lower than the numbers dairy cows in selected dairy farms. Hence, all farm workers were considered in selected dairy farms and then the sample size was 68. Therefore, a total of 328 samples were considered for this study. In sampling of dairy cows and dairy farm workers convient non-probability sampling technique was applied. Consequently, all lactating dairy cows' milk were collected until sample size was achieved. Whereas, all dairy farm workers present in the four selected farms were included in the study. But those dairy cows and dairy farm workers that were treated with antimicrobials within one month were excluded.

Moreover, on site, questionnaires were administered to elicit basic information on each farm, the number of cows, the kinds of antimicrobials used, treatment and disease records of the dairy cows, and basic information on the workers (for example, age, gender, type of occupation, physical conditions, educational level and history of antibiotic use) were requested. Then, consent forms were filled by dairy farm workers before collecting samples.

Sample collection, transport and handling

Milk samples were collected according to the National Mastitis Council Guideline (1990) by principal investigator. Then, the collected samples were transported using icebox to Microbiology Laboratory of Institute Biodiversity Conservation, Addis Ababa, where they were immediately screened with California Mastitis Test (CMT) to identify sub-clinical mastitic cows in case it was not identified clinically. Milk samples from mastitic cows were immediately cultured or stored at 4°C for a maximum of 24 h until it was cultured on blood agar media.

Nasal swabs sample were collected using sterile cotton swabs from the anterior nares of consented dairy farm workers (n = 68) in selected dairy farms. Each sterile cotton swab was dipped into sterile distilled water prior to collection. Then, samples for culture were obtained by firmly rotating new pre-moistened cotton-tipped swabs on both nares of volunteer dairy farm workers. After a pair of swabs was taken, subsequently, it was put into a single screw capped tube containing nutrient broth with 10% fetal bovine serum. Then, samples for culture were placed in racks for easy handling and held in an icebox, properly packed and kept cold. Finally, it was transported to Microbiology laboratory of Institute of Biodiversity Conservation to be processed immediately after arrival (CLSI, 2008).

Culture and identification

Milk samples

Bacteriological examination was done according to the National Mastitis Council Guideline (1990). Finally, pure colony was taken from secondary culture of MSA and sub-cultured on BUG (BiOLOG Universal Growth Media) at 37°C for 18 to 24 h as a primary and

Table 1. Over all prevalence of *S. aureus* isolated from milk samples of lactating dairy cow and nasal swabs of dairy farm workers among selected dairy farms around Addis Ababa.

Farm name	*S. aureus* Number (%)
HARCF1 (n=116)	18 (15.5)
HCIF (n=89)	8 (8.9)
HARCF2 (n=87)	12 (13.8)
BOCF (n=36)	13 (36.1)
Total (n=328)	51 (15.5)

P-value = 0.02 (*S. aureus* ssp. *aureus*) HARCF1: Agricultural Research Center Farm (Holeta branch), HARCF2: Agricultural Research Center Farm (Adaberga branch), HCIF: Holeta Cattle Improvement Farm, BOCF: Bethel Orphan Center Farm.

secondary culture. Well-isolated fresh colonies from BUG (Biolog, USA) media are inoculated into 18 to 20 inoculation fluid to have bacterial suspension with turbidity equivalent to 20% transmittance as measured by turbidity meter. This suspension was poured into Micro plates with multi-channel pipettes. The micro plates were loaded into Omnilog tray to be incubated, analyzed and interpreted for 18 to 24 h as per guidelines of BiOLOG Users Guideline (2008) and finally identified *S. aureus* was obtained.

Nasal swabs samples were streaked on blood agar (Oxoid, UK) containing 7% sheep blood for isolation of Staphylococci after getting incubated at 37°C. All suspected colonies of *Staphylococcus* species were sub-cultured on mannitol salt agar and incubated at 37°C and examined after 24 to 48 h for growth and change in the color of the medium. The presence of growth and change of pH in the media as red to yellow color were regarded as salt tolerant staphylococci. For further identification, pure colony was taken from secondary culture of MSA and sub-cultured on BUG media as a primary and secondary culture. Finally, pure colony was taken by sterile swab from secondary culture of BUG and suspension was made as per BiOLOG Users Guideline (2008) and *S. aureus* was identified finally by OmniLog Identification System.

Antimicrobial susceptibility test was performed for all *S. aureus* isolates according to the criteria of the Clinical and Laboratory Standards Institute (CLSI, 2008). For susceptibility test, one antimicrobial from each subclass of antimicrobials which were commonly used for treatment of bovine mastitis or considered as important antimicrobial agents for human were selected for antibiogram based on the criteria of Clinical and Laboratory Standards Institute (CLSI, 2008).. Thus, antimicrobials used for treatment of bovine mastitis included in this study were erythromycin (E/15 µg), cephalothin (KF/30 µg), penicillin-G (10 unit), sulphoxazole-trimethoprim (SXT/25 µg), amoxicillin-clavulinic acid (AMC/30 µg), chloroamphenicol (C/30 mg),(Oxoid), tetracycline (TE/30 µg) and gentamicin (CN/10 µg)(Biomerioux). Antimicrobials not used for treatment of bovine mastitis but important for human was oxacillin (OX/1 µg), vancomycin (VA/30 µg), clindamycin (DA/10 µg) and rifampicin(RD/5 µg) (Oxoid).

Finally, the diameters of the zone of inhibition around the disks were measured to the nearest millimeter using rulers, and the isolates were classified as susceptible, intermediate and resistant according to the interpretative standards of Clinical and Laboratory Standards Institute (CLSI, 2008). Moreover, isolates showing resistance to three or more antimicrobial subclass were considered as multidrug resistant.

Quality control

Confidence in the reliability of test results is increased by adequate quality assurance procedures, and the routine use of control strains. Thus, *S. aureus* ATCC25923 as a positive control and *E. coli* ATCC-25922 as a negative control (for culture on MSA) were taken as an important part of quality control for culture, BiOLOG identification and antimicrobial susceptibility through this study. Thus, quality control microorganisms yielded values within the established ranges, indicating that the test was performed in a satisfactory manner.

Variables

Independent variables such as type of farm, type of occupation, level of education, previous history of diseases and use of antimicrobials for humans, and type of farm, breed type, age, stage of lactation, pregnancy status, type of mastitis, history of antimicrobials use, milking method and disinfectant use habit for animals were interpreted against dependent variable of *S. aureus* colonization or infection. In addition, types of the farm and study subjects were considered as independent variable for interpreting against dependent variable of antimicrobial sensitivity pattern.

Statistical analysis

The collected data were entered into EPI data version 3.1 and exported to SPSS version 17 computer software then the data were analyzed. Accordingly, descriptive statistics such as percentages and frequency distribution was used to describe/present bacterial isolates and antimicrobial susceptibility which was expressed as percent of resistant, intermediate and susceptible. In addition, the proportion of bacteria resistant to at least one of the twelve antibiotics and resistant two or more were calculated. Moreover, comparison between each dairy farm, study subject against antimicrobial susceptibility was done by fisher's exact or Chi-square. Bivariate logistic regression was used to see the association of the potential risk factors with occurrence *S. aureus*. The degree of association between risk factors and the occurrence *S .aureus* were analyzed using odds ratio (OR). In all analysis, associations were considered to be significant when $P<0.05$.

RESULTS AND DISCUSSION

In the present study, the overall prevalence of *S. aureus* was found to be 51 (15.5%) (Table 1). This finding is in accordance with the findings observed in Egypt (17.2%) by Seedy et al. (2010) and 19.5% by Jakee et al. (2008) who isolated *S. aureus* strains from human and animal sources.

Microbiological examination of milk from lactating dairy cows showed 42 (16.2%) (Table 2) positive for *S. aureus* in the present study. This is in line with the findings of Bitaw et al. (2010) who found 20.3% in dairy farms in Bahir Dar town and its environs. Similarly, it was closely comparable with the findings of Bishi (1998) and Hussein et al. (1997) who reported 9 and 10% prevalence in Addis Ababa, 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. 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 and 43.3, 39.1% in dairy cows, respectively. The possible explanation for this might be that *S. aureus* is a contagious patho-

Table 2. Number and percentage of *S. aureus* isolated from dairy cows at four different farms in and around Addis Ababa.

Name of farm	*S. aureus* ssp. *Aureus* Number (%)
HARCF1 (n=96)	15 (15.6)
HCIF (n=72)	8(11.1)
HARCF2 (n=72)	10(13.9)
BOCF (n=20)	9(45.0)
Total (n=260)	42(16.2)

P-value = 0.00 (*S .aureus* ssp. *aureus*), HARCF1: Agricultural Research Center Farm (Holeta branch), HARCF2: Agricultural Research Center Farm(Adaberga branch), HCIF: Holeta Cattle Improvement farm, BOCF: Bethel Orphan Center Farm

Table 3. Number and percentage *S. aureus* isolated from dairy farm workers at four different farms around Addis Ababa.

Farm name	*S. aureus* ssp. *Aureus* Number (%)
HARCF1 (n=20)	3(15)
HCIF (n=17)	0(0.0)
HARCF2 (n=15)	2(13.3)
BOCF (n=16)	4(25.0)
Total (n=68)	9(13.2)

P-value: 0.01 (*S. aureus*), HARCF1: Agricultural Research Center Farm(Holeta branch), HARCF2: Agricultural Research Center Farm(Adaberga branch), HCIF: Holeta Cattle Improvement Farm, BOCF: Bethel Orphan Center Farm.

pathogen transmitted from one cow to another or individual by contact with animals during unhygienic milking procedures (Rowe, 1999).

In the present investigation, 13.2% of *S. aureus* was isolated from dairy farm workers (Table 3). Similar finding has been reported by Jakee et al. (2008) who reported 20% from nasal swabs diseased human. This is slightly lower for the report of Kluytmans and Struelens (2009) who stated approximately 20% of healthy human is persistent *S. aureus* carriers, about 30% are intermittent carriers and around 50% are never colonized with *S. aureus*. Because nasal carriage rates vary according to the population studied, for example, being higher in children than adults (Armstrong-Esther and Smith, 1976). Even in this study, poorly managed farm showed higher prevalence than persistent *S. aureus* carriers' healthy individuals.

In this study, the isolation and identification of *S. aureus* obtained from four different dairy farms setting vary; its prevalence range from 8.9 to 36.1% (Table 1). Various studies have been conducted to evaluate the prevalence of *S. aureus* from communal and commercial farms. The results reported in our study are similar when compared to those formerly documented (Shitandi and Sternesjo, 2004; Gundogan et al., 2006; Ateba et al., 2010). Based on observations made throughout this study, therefore we report that improper hygiene and poor farm management practices contributed to the presence of *S. aureus* especially in those from the small holder private farms. The *S. aureus* incidence at a considerable high percentage indicates the alarming situation both for dairy farms and for public health as well.

The present observation showed high prevalence of *S. aureus* was more likely to occur in cows that were poorly managed and frequently treated (Table 4). No statistical significant association was observed in this study among breeds, age, stage of lactation, pregnancy status, type of mastitis and milking methods for *S. aureus* infection of lactating dairy cows. These finding is similar with finding of Denis et al. (2008) and Grace et al. (2009).

Moreover, high prevalence of *S. aureus* in dairy farm workers more likely occurs in individuals with disease history in the farm (Table 5). No statistical significant association was observed in these studied different farm types, type of occupation, educational status and workers who used or did not use antimicrobial previously for treatment of a disease. This findings agrees with findings of Christiane et al. (2009) who stated individuals who have contact with animal are more likely to be colonized with *S. aureus* and he also observed antibiotic usage prior to sampling has no risk with respect to colonization. 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.

The observations made in the present study unequivocally proved that *S. aureus* showed resistance to all antimicrobials tested except for rifampicin and vancomycin (in some dairy farms) (Table 6). These indicate that the problem is highly distributed and disseminated. The *S. aureus* isolates from dairy farm workers showed antimicrobial resistance similar to the isolates from bovine sources with no statically significant difference between them except for pencillin G and tetracycline, and this is in agreement with previous reports in Taiwan by Ma et al. (2006). Moreover, the overall resistance of *S. aureus* isolates, to vancomycin, rifampicin, clindamycin and gentamycin showed less than 25% of resistance and this is similar with the report of Ma et al. (2006) from the dairy farm in Taiwan.

However, the present study has demonstrated the existence of alarming level of resistance of *S. aureus* to commonly used antimicrobials (pencillin G and tetracycline) in the study farms for dairy cows. The results were in accordance with reports from earlier studies in other countries (Jakee et al., 2008; Edward et al., 2002; Gentilini et al.,

Table 4. Number and percentages of dairy cows infected with *S. aureus* and bivariate predictors of infection rate.

Risk factor	Infected (n = 42) Number (%)	Non-infected (n = 218) Number (%)	Bivariate OR (95%CI)	P-value
Farm type				
HARCF1 (n;96)	15(35.7)	81(37.2)	5.1(1.7-12.5)	
HARCF2 (n=72)	10(23.8)	62(28.4)	6.5(2.1-20.6)	0.00
HCIF (n=72)	8(19.0)	64(29.4)	1	
BOCF (n=20	9(21.4)	11(5.0)	4.4(1.6-12.5)	
Breed				
Holstein(n=95)	17(40.5)	78(35)	0.88(0.41-1.89)	0.7
Jersey(n=72)	10(23.8)	62(28.4)	1	
Cross(BxH)(n=93)	15(35.7)	78(35.8)	1.2(0.5-2.8)	
Age				
<4 years(n=108)	18(42.9)	90(41.3)	4.04(0.88-18.4)	
4-7 years(n=72)	16(38.1)	56(25.7)	1.2(0.4-3.6)	0.07
7-10 years(n=54)	3(7.1)	51	(23.4) 1	
>10 years(n=26)	5(11.9)	21(9.6)	0.8(0.4-3.6)	
Stage of lactation				
Early(n=77)	12(28.6)	65(29.8)	1.8(0.82-3.98)	
Mid(n=119)	16(38.1)	103(47.2)	3.5(0.64-36)	0.141
Late(n=64)	14(33.3)	50(22.9)	1	
Pregnancy status				
Yes(n=109)	21(50)	88(40.4)	1.47(0.762-2.86)	0.248
No(n=151)	21(50)	130(59.6)	1	
Type of Mastitis				
Clinical (n=49)	14(33.3)	35(16.1)	0.05(0.22-1.22)	0.09
Sub-clinical(n=64)	28(66.7)	36(16.5)	1	
Antibiotic use				
Yes (n=106)	26(61.9)	82(37.6)	2.69(1.36-5.32)	0.004
No(n=152)	16(38.1)	136(89.5)	1	
Milking method				
Machine(n=95)	15(35.7)	80(36.7)	1.04(0.52-2.07)	0.9
Hand (n=163)	27(64.3)	136(63.3)	1	
Disinfectant use				
Yes(n=76)	8(19.0)	66(30.3)	0.54(0.23-1.34)	0.14
No (n=186)	34(81.0)	152(69.7)	1	

HARCF1: Agricultural Research Center Farm (Holeta branch), HARCF2: Agricultural Research Center Farm(Adaberga branch), HCIF: Holeta Cattle Improvement Farm, BOCF: Bethel Orphan Center Farm, BxH= Borena cross with Holstein.

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 pencillin and ampicillin. However, it was found out that about 90% of *S. aureus* isolated from patients was found resistant in 1980s. In this study, a higher resistance to pencillin was determined in both dairy farm workers and dairy cows although penicillin resistance was found to be significantly higher in mastitic dairy cows than in dairy farm workers. Similarly, the result of the present investigation showed high percentage of resistance to oxacillin in dairy cows

Table 5. Number and percentages of dairy farm workers colonized with *S. aureus*. and bivariate predictors of isolation rate.

Risk factor	Colonized (n=9) Number (%)	Not colonized (n=59) Number (%)	Bivariate OR (%95)	P-value
Type of the farm				
HARCF1 (n=20)	3(33.3)	17(28.8)	2.2(0.3-14**)**	
HARCF2 (n=15)	2(22.2)	13(22.2)	5.4	0.418
HCIF (n=17)	0(0)	17(28.8)	1	
BOCF (n=16)	4(44.4)	12(20.3)	1.9(0.4-10)	
Type of occupation				
Milkier(n=29)	3(33.3)	26(44.1)	1.1(0.08-15.2)	
Farm cleaner(n=13)	1(11.1)	12(20.3)	2.4(0.124-46)	
Veterinarians(n=7)	2(22.2)	5(8.5)	1.7(0.15-20)	0.562
Animal feeder(n=13)	2(22.2)	11(18.6)	1.1(0.05-38)	
Others(n=6)	1(11.1)	6(8.8)	1	
Educational status				
Illiterate (n=17)	2(22.2)	2(3.4)	16(0.9-267	
Primary(n=30)	4(44.4)	26(44.1)	6.5(0.7-60)	0.107
High school(n=17)	2(22.2)	15(25.4)	7.5(0.65-87)	
Higher education(n=17)	1(11.1)	16(27.1)	1	
History of disease				
Yes(n=35)	8(88.9)	27(45.8)	9.48(1.07-215)	0.01
No(n=33)	1(11.1)	32(54.2)	1	
Antimicrobial use				
Yes(n=22)	5(22.5)	17(28.8)	3.09 (0.61-16.05)	0.11
No(n=46)	4(44.4)	42(71.2)	1	

HARCF1: Agricultural Research Center Farm (Holeta branch), HARCF2: Agricultural Research Center Farm (Adaberga branch), HCIF: Holeta Cattle Improvement farm, BOCF: Bethel Orphan Center Farm.

Table 6. The susceptibility pattern of *S. aureus* (n=51) isolated from milk samples and dairy farm workers.

Antimicrobial	Susceptible number (%)	Intermediate number (%)	Resistant number (%)
Amoxicillin+clavulanic	32(62.7)	-	19(37.3)
Cephalothin (KF)	28(54.9)	9(17.6)	14(27.5)
Chloroamphenicol	24(47.1)	15(29.4)	12(23.5)
Clindamycin (DA)	17(33.3)	25(49.)	9(17.6)
Oxacillin (OX)	33(64.7)	1(2.0)	17(33.3)
SXT	34(66.7)	6(11.8)	11(21.6)
Erythromycin (E)	11(21.6)	28(54.9)	12(23.5)
Gentamycin (CN)	21(41.2)	20(39.2)	10(19.6)
Pencillin (P)	3(5.9)	1(2.0)	47(92.2)
Rifampicin (RD)	49(96.1)	1(2.0)	1(2.0)
Tetracycline (TE)	12(23.5)	5(9.8)	34(66.7)
Vancomycin (VA)	45(88.2)	4(7.8)	2(3.9)

Sulphamethoxazole-timethoprim (SXZ).

and dairy farm workers. This may pose risk to consumers or individuals who have contact with animals especially for immune compromised ones. In contrast, Pereira et al. (2009) showed that 3.8% of *S. aureus* strains isolated

Table 7. Antimicrobial susceptibility pattern of *S. aureus* isolates (n=42) from dairy cows.

Antimicrobial	Susceptible number (%)	Intermediate number (%)	Resistant number (%)
Amoxicillin+clavulanic	27(64.3)	-	15(35.7)
Cephalothin (KF)	23(54.8)	7(16.7)	12(28.6)
Chloroamphenicol	19(45.2)	13(31.0)	10(23.8)
Clindamycin (DA)	13(31.0)	22(52.4)	7(16.7)
Oxacillin (OX)	28(66.7)	1(2.4)	13(31.0)
SXT	28(66.7)	5(11.9)	9(21.4)
Erythromycin (E)	8(19.0)	24(57.1)	10(23.8)
Gentamycin (CN)	16(38.1)	18(42.9)	8(19.0)
Pencillin (P)	1(2.4)	0(0.0)	41(97.6)
Rifampicin (RD)	41(97.6)	1(2.4)	0(0.0)
Tetracycline (TE)	6(14.3)	5(11.9)	31(73.8)
Vancomycin (VA)	37(88.1)	4(9.5)	1(2.4)

Sulphamethoxazole-trimethoprim (SXZ).

from various foods were resistant to methicillin but only 0.68% showed the presence of mec A gene. In the present study, the prevalence of oxacillin-resistant *S. aureus* was highly varible with type of farms. Especially, poorly managed farms showed high percentage of resistance to oxacillin. This is because of unhygienic status of farms that results in contamination of dairy cows and farm workers and leading to high chance of transfer of resistance strains (Rowe, 1999).

In this study, the resistance of *S. aureus* from human sources to some antimicrobials (amoxacillin–clavulinic acid, rifampicin and vancomycin) was generally greater than that from bovine sources. Moreover, study showed good managed farm had lower level colonization and lower percentage of resistance. 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 Witte (1998), Cruchaga et al. (2001) and Kidd et al. (2002). However, apparent difference were also noted by Erskine et al. (2002) and Iversen et al. (2004), who have shown that food-producing animals play an negli-gible role in the transmission of resistant strains.

In the present study, only 0 to 25% of the total isolates were found resistant to vancomycin, rifampicin, chloroamphenicol, gentamicin, erythromycin and sulphamethoxazole–trimethoprim. If the resistance rate is less than 55% antimicrobials are recommended for their application especially for treatment of infection in humans (Nihal et al., 2011). The reason why these antimicrobials were less resistant might be that they are not frequently used in the study area in veterinary services, and perhaps in human medicine. Similar suggestion was given by Jaims et al. (2002) that the development of antimicrobial resistance is nearly always as a result of repeated therapeutic and/or indiscriminate use of them. However, most of the isolates were resistant to penicillin and other β-lactams, and tetracycline in the present observation. This is due to the fact that tetracycline and penicillin are frequently and improperly used antimicrobials in animal and human health.

Staphylococci are frequently isolated from bovine mastitis which is one of the most common cause for the use of antimicrobial in lactating dairy cows. Similarly, the present investigation indicated that the resistance pattern of penicillin was found to be 96.7% (Table 7) which is similar to the finding made by Tariku et al. (2011) (87.2%) in Ethiopia, Landin (2006) (80%) in Sweden, Gooraninejad et al. (2007) (57%) in Iran and Myllys et al. (1998) (50%) in Finland. This is in contrast to findings observed by Adesiyun (1994) who reported 23% of resistance to pencillin G in West India.

Moreover, the present study showed the resistance of *S. aureus* to tetracycline (73.2%), amoxicillin-clavulinic acid (35.7%), oxacillin (31%), cephalothin (28.6%), chloramphenicol (23.8%), sulphamethoxazole-trimethoprim (21%), erythromycin (23.8%), gentamycin (19%), clindamycin (16.7%) observed in milk samples taken from dairy cows around Addis Ababa. This is in accordance with the findings of Tariku et al. (2011) who reported resistance of *S. aureus* to amoxicillin-clavulinic acid (46%), chloroamphenicol (16%), vancomycin (3%), but it disagree with the observation made by Tariku et al. (2011) in the case of tetracycline (0%), co-trimoxazole (0%) and clindamycin (4%) in dairy farms in Jimma town. The probable explanation could be that *S. aureus* strains have the capacity to change their resistance behavior to the exposed antimicrobials.

With a particular emphasis to tetracycline, the present observation agrees with preliminary finding conducted by Bayhun (2008) (55.3%). However, apparent difference was observed in the report of Tariku et al. (2011) (0%). This is due to the fact that tetracycline is the most commonly used antimicrobial in the treatment of infections in the livestock sector in Ethiopia. Moreover, tetracycline is widely used as growth factors in veterinary medicine for livestock rearing as well in the treatment of bacterial

Table 8. Antimicrobial susceptibility pattern of *S. aureus* isolates (n = 9) from dairy farm worker.

Antimicrobial	Susceptible number (%)	Intermediate number (%)	Resistant number (%)
Amoxicillin+clavulanic	5(55.6)	-	4(44.4)
Cephalothin (KF)	5(55.6)	2(22.2)	2(22.2)
Chloroampimicol	5(55.6)	2(22.2)	2(22.2)
Clindamycin (DA)	4(44.4)	3(33.3)	2(22.2)
Oxacillin (OX)	5(55.6)	0(0.0)	4(44.4)
SXT	6(66.7)	1(11.1)	2(22.2)
Erythromycin (E)	3(33.3)	4(44.4)	2(22.2)
Gentamycin (CN)	5(55.6)	2(22.2)	2(22.2)
Pencillin (P)	2(22.2)	1(11.1)	6(66.7)
Rifampicin (RD)	8(88.9)	0(0.0)	1(11.1)
Tetracycline (TE)	6(66.7)	0(0.0)	3(33.3)
Vancomycin (VA)	8(88.9)	0(0.0)	1(11.1)

Sulphamethoxazole-Trimethoprim (SXZ).

Table 9. Number and percentages of resistant *S. aureus* to antimicrobials in dairy farm workers and dairy cows.

Number of antimicrobials	Dairy cows' number (%)	Dairy farm workers number (%)	Overall number (%)
One	17(40.8)	4(44.4)	21(41.2)
Two	5(11.9)	2(22.2)	7(13.7)
MDR	20(47.6)	3(33.3)	23(45.1)
Total	42(82.4)	9(17.6)	51(100)

P-value = 0.681, MDR: multi-drug resistant.

infection occurring in human medicine (Ardic et al., 2005).

Furthermore, the resistance profile *S. aureus* to amoxicillin-clavulinic acid and oxacillin in dairy cows was found to be high. This is due to the fact that resistance of *S. aureus* to pencillin G, amoxicillin and oxacillin may be attributed to the production of β-lactamase, an enzyme that inactivates pencillin and closely related antimicrobial. It is believed that about 50% of mastitis causing *S. aureus* produces β-lactamase (Green and Bradely, 2004). Likewise, *S. aureus* showed resistance to vancomycin and clindamycin. This might indicate transfer of resistant strain among environment, livestock and human since this antimicrobials are not used in veterinary practice (Martel et al., 2002).

Regarding the antimicrobial resistance of individuals who have contact with dairy cow (Table 8), the result of the present study showed a comparable report with previously conducted study at Jimma Hospital by Barena and Fetene (2003) who stated resistance of *S. aureus* to oxacillin (51.1%), erythromycin (16.5%) and gentamicin (12.9%). In contrast, they reported resistance of *S. aureus to* chloroamphenicol (70.6%), sulphamethoxazole - trimethoprim (68.8%). This might be due to the methods they used in the identification of the strains, that is, coagulase as confirmatory element during isolation. Moreover, their study was conducted at hospital where more resistant strains were found than food source isolates (Nihal et al., 2011).

In addition, the present investigation disagree with Jakee et al. (2008) who reported resistance of *S. aureus* to amoxicillin-clavulinic acid (66.7%), gentamycin (66.7%), oxytetracycline (80%). This might be due to isolates obtained from clinical samples at different geographical location where practice of using these antimicrobials are common in Egypt (Nihal et al., 2011). Apart from this, the present observation is similar to the study result of Nwankwo and Nasiru (2011) who reported the resistance of *S. aureus* to gentamycin (7.6%), amoxicillin-clavulinic acid (37%), erythromycin (47.6%), chloroamphenicol (48.1%), pencillin G (92.9%) but his finding opposes the present finding in the case of resistance to tetracycline (68.9%), sulphamethoxazole-trimethoprim (84.5%) and vancomycin (0%). The present study also showed slightly higher level of resistance to rifampicin than the report of Chigbu et al. (2003) who detected 0% of resistance in Nigeria. This might be due to transfer of resistant gene from other bacteria for which rifampicin is commonly used for treatment or due the presence of intrinsic resistance gene.

S. aureus strains have developed multidrug resistance worldwide with broad diversity in prevalence rate in different regions (Normanno et al., 2007). In the present observation, 23 (45.1%) *S. aureus* strains (Table 9) showed multidrug resistance primarily to penicillin G, tetracycline, oxacillin and amoxicillin-clavulinic acid because of resistance to β-lactams and frequent use. This finding agrees

with the study result of Moon et al. (2007) who reported that even two out of 21 MRSA were resistant to more than three of non-β-lactam antimicrobials including gentamycin and ethromycin. This is lower than the findings of Normanno et al. (2007) who reported that 9.6 and 4% of *S. aureus* strains had resistance to three and four of the tested antimicrobials, respectively.

However, Chao et al. (2007) reported higher multidrug resistance rate (79%) among the isolates. Similarly, Barena and Fetene (2003) reported higher rate of multi-drug resistant *S. aureus* (80%) from Jimma Hospital than the present investigation. This is because their study was conducted at hospital level where more resistant strains were found than food source isolates (Nihal et al., 2011).

Moreover, the present investigation showed 20(47.6%) multidrug resistant *S. aureus* ssp. *aureus* isolated from milk of dairy cows. This is comparable with findings of Sharma et al. (2011) who reported slightly higher prevalence of multidrug resistant *S. aureus* from raw milk of dairy cattle in India, and found out that 70% of the isolates were resistant to amoxacillin-sulbactum, cloxacillin, erythromycin, kanamycin and vancomycin. The present study results demonstrated that a large proportion of resistant strains were isolated from raw milk. These indicate higher prevalence of multidrug *S. aureus* in dairy cows and its great risk for consumers and individuals who have contact with animals. Thus, special attention is needed to focus on multidrug resistant *S. aureus* isolates on farms in which the strains are closely related to human isolates as a possible source of human infections by consuming contaminated food products or having contact with infected animals (Lee, 2003).

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

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