Prevalence and antimicrobial susceptibility profiles of *Staphylococcus aureus* from cow's milk, nasal and environmental swabs in selected dairy farms in Morogoro, Tanzania

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SUMMARY

Staphylococcus aureus is a microorganism that is present as a commensal on the skin, the nose and mucous membranes of healthy humans and animals. However, it is also an opportunistic pathogen that can cause multiple infectious diseases of diverse severity. A cross-sectional study was carried out to determine the prevalence and antimicrobial resistance profiles of *S. aureus* from samples of cow's milk, farm environmental and cattle nasal swabs from three dairy farms in Morogoro.A total of 377 samples; raw milk (n = 100), nasal swabs (n=133) and environmental swabs (n = 144) were screened for the presence of *S. aureus*.California mastitis test (CMT) was used to establish the status of mastitis. Gram staining, oxidase, catalase, DNase, haemolysis and coagulase tests were employed for bacterial identification. Antimicrobial susceptibility testing was conducted using the Kirby- Bauer disk diffusion assay. Out of 200 cows (800 quarters milk) CMT screened for mastitis, (12.5%) quarters from 80 cows tested positive for subclinical mastitis, while twelve (1.5%) quarters from seven (3.5%) cows tested positive for clinical mastitis.Overall, 97 (25.7%) of 377 samples were positive for *S. aureus*, which were detected in 49.0%, 11.6%, and 40.0% of the milk, environment and nasal samples, respectively.Antimicrobial susceptibility testing revealed resistance to: Ampicillin (67.4%), Cefoxitin (14.8%), Erythromycin (21.1%), Gentamycin (3.2%), Oxacillin (37.9%), Tetracycline (55.8%), Trimethoprime-Sulfamethoxazole (29.5%) and Vancomycin(9.5%). 28.4% (n=27) of all isolates were resistant to Oxacillin and/or Cefoxitin, and therefore classified as Methicillin-Resistant *Staphylococcus aureus*(MRSA). 63%of the MRSA isolates originated from milk, 22.2% from nares and 14.8% from environmental samples. Over a half of all isolates were classified as multidrug resistant;of these 43.2% (n=41), 6.3% (n=6) and 1.1% (n=1) were simultaneously resistant to three, four and five antimicrobial agents, respectively. Taken together, this study revealed the prevalence of multidrug resistant *S. aureus* in cow's milk, nares and farm environment. Our findings also confirm the presence of livestock-associated MRSA, and thus underline the importance of applying biosecurity measures and good hygiene practices to prevent MRSA spread at the farm level and throughout the food production chain.
Key words: *Staphylococcus aureus*, Antimicrobial resistance, MRSA, Livestock environment

INTRODUCTION

Staphylococcus aureus, a non-spore forming and non-motile bacteria, is a microorganism that is present as a commensal on the skin, the nose and mucous membranes of healthy humans and animals. However, it is also an opportunistic pathogen that can cause multiple infectious diseases of diverse severity both in animals and humans (Luzzago*et al*., 2014; Petonand Le Loir, 2014). Bovine mastitis is an infection of the mammary gland andis the major illness for dairy ruminants that leads to reducemilk production, and is often

associated with cattle disorderssuch as fever and rumen motility. Other mastitis-related economiclosses include the reduction in milk yield, increased treatmentcosts, discarded milk, increase in culling, pharmacologiccosts, and increased labor costs (Cardozo *et al*., 2014).In cattle, *S. aureus* cause variety diseases ranging from simple abscesses and mastitis to the more severe toxic shock syndrome (Mdegela *et al*., 2005; Perssone*t al*., 2011; Sharma *et al*., 2015). *S. aureus*is responsible for approximately one-third of cases of clinical and subclinical mastitis in cattle (Bradley, 2002; Mdegela *et al*., 2005; Botrel*et al*., 2010).

Antibiotic use in the treatment of animal diseases including mastitis isfrequent; however, there has been an increase inthe bacterial resistance index.This fact reinforces the importance of adoptingappropriate treatment protocols with effectivemedicines, which must be selected from phenotypicand genotypic testing. Treatment of mastitis is mainly dependent on antimicrobial therapy such β-lactams, cephalosporin, aminoglycoside and macrolide (Kaliwal*et al*., 2011;Awandkar*et al*., 2013; Chandrasekaran *et al*., 2014). However, resistant *S. aureus* strains towards major classes of antibiotics have been reported, leaving veterinarians with limited options.The expression of the*mec*A gene in *S. aureus* confers resistance to most ofβ-lactams, including methicillin resistance (MRSA), agentsfrequently used for treatment of mastitis (Sawant *et al*., 2005). MRSA is an important human and animal pathogenthat can be implicated in a wide diversity of infections,including bovine mastitis (Stefani *et al*., 2012). Recently, a specific MRSA clone, CC398, has been found to be associated with pigs, veal calves, broiler chickens, companion animals and people in close contact with livestock (de Neeling *et al*., 2007; Denis *et al*., 2009; Graveland *et al*., 2011), and this strain shows resistance against tetracycline, macrolides, lincosamides, aminoglycosides and fluoroquinolones (Witte *et al*., 2007).

Methicillin resistant *Staphylococcus aureus* (MRSA) incriminatedin bovine mastitis has been evidenced in many countries including;Korea (Moon *et al*., 2007), Switzerland (Huber *et al*., 2010), Belgium (Vanderhaeghen *et al*., 2010), South Africa (Ateba *et al*., 2010), Turkey (Turkyilmaz *et al*., 2010), Serbia (Zutic *et al*., 2012), USA (Haran *et al*., 2012), China (Pu *et al*., 2014; Li *et al*., 2015),Tanzania (Mohamed, 2015) and Italy (Locateli *et al*., 2016). Similarly, *S. aureus*has been reported to colonize the nasal mucosa of different animal species,their owners, caretakers or veterinarians, and emphasized the importance of nasal MRSA carriage in terms of public and animal health (Faires*et al*., 2009; Gordoncillo*et al*.,2012; Loeffler *et al*., 2005, Van Duijkeren *et al*., 2010).

Due to the intensive use of antibiotics in public health and animal husbandry,antibiotic resistance in pathogens has been an increasing medical problem over the lastdecades. In addition to the spread of resistant zoonotic foodborne pathogens, there is alsoa possibility that food-related commensal bacteria or opportunistic pathogens are carriersof resistance genes, and therefore a potential hazard to consumers (Sharma *et al*., 2014).With regard to dairy production, the most relevant are resistant mastitis-causing bacteria,such as Staphylococci. Thepresence of resistant *S. aureus*in milk intendedfor human consumption or dairy products could be of public-health relevance. Hence, the aim of the present study was to determinethe presence and

antimicrobial resistance profiles of *S. aureus* in mastitis cases in dairy cattle, cattle nares and farm environment at three farms belonging to Sokoine University of Agriculture, Morogoro, Tanzania.

MATERIALS AND METHODS

Study area

The study was carried out in Morogoro municipality, Tanzania. Geographically, the municipality extends between longitude 35.6 to 39.5° E and latitude 5.7 to 10° S at an elevation of 500 to 600 m above sea level and is about 200 km west of Dar es Salaam. The municipality has a mixture of warm and cool temperatures ranging between 27 to 33.7°C in the dry/warm season and 14.2 to 21.7°C in cool/wet season. Morogoro Municipality experiences a sub-humid tropical climate with a bimodal rainfall pattern characterized by two rainfall seasons in a year with a dry season separating the short rains (October to December) and long rains (which fall from March to May/June). There are about 6 months of dryness, the peak being September. The mean annual rainfall is about 870 mm and the total annual evapotranspiration is about 1300 mm.

Study animals

The study was carried out in 3 dairy farms (A, B and C) that belonged to Sokoine University of Agriculture. All farms were located within the same climatic zone.Farm A included dairy herds and goat flocks in close proximity, whereas,farm B had dairy cattle, goats, sheep, pigs and camels also kept in close proximity, but with different attendants. Farm C had both dairy and pig herds kept in close proximity but with different management systems. Dairy herds were grazing on pasture for about 8 hours and fed with hay after returning to the housing pallor in the afternoon. Lactating cows were either hand-milked (farm B) or machinery-milked (Farm A and C) twice daily.

Study Design and sample collection

To investigate the prevalence of *S. aureus*, a cross-sectional study was conducted from January through April 2016. A total of 377 samples, including raw milk samples (n = 100), nasal swabs (n=133) and environmental swabs (n = 144), were collected from the three farms. The sample size was calculated according to Martin *et al.* (1987)to allow a detection level of 5% or with 95% certainty. From farm A, 140 samples (46 milk, 48 environmental swabs and 46 nasal swabs),93 from farm B (20 milk, 48 environment and 25 nasal swabs), and 144 samples from farm C (34 milk, 48 environmental and 62 nasal swabs) were collected. For milk samples, 300 milking cows were first screened using California mastitis test (CMT) for the presence of clinical or subclinical mastitis as previously described by Karimuribo *et al*. (2015). The CMT positive mammary quarters of 54 cows were sampled for isolation of *S. aureus*. All samples were analyzed at Microbiology laboratory in the College of Veterinary and Medical Sciences, Sokoine University of Agriculture.

Milk samples

A single aseptic milk sample was collected from each of CMT-positive quarter after udder preparation by the farm personnel. The milk samples collection was done according to the procedure previously described by Mdegela *et al*. (2009). Briefly, the teat ends were first disinfected with 70% alcohol, and approximately 10 mL of individual quarter milk samples

were collected into sterile receptacles. Samples were stored in cool box with ice packs before shipped to the laboratory for further processingwithin a maximum of 12 hours or frozen at −20°C until bacteriological analyses.

Environmental swabs

During the visit to each farm, twelve environmental samples consisting of floor, door and wall swabs (representative of all parts of animal houses/barns), at monthly interval, werecollected with sterilegauze.Each gauze pad was then placed in a sterile plastic bag (Ziploc®; SC Johnson), closed, labeled, and stored in a cool box with ice packs before transportation to the laboratory for further analyses within a maximum of 24 hours.

Nasal swabs

Nasal swab samples were taken from both nostrils (nares) of randomly selected healthy cows using cotton swabs. The cotton swabs were inserted into the anterior nares of both left and right nostrils and were gently rolled against the inner walls. Samples were then stored in a cool box with ice packs before transportation to the laboratory for further analyses within a maximum of 24 hours.

Isolation and identification of *S. aureus*

Nasal and environmental swabs were inoculated into tryptone soya broth (TSB, Oxoid Ltd., Basingstoke, Hampshire, UK), supplemented with 7% (w/v) sodium chloride, and incubated aerobically at 35°C for 24 hours. Then, 100 µL of the broth was inoculated onto Columbia blood agar (Oxoid Ltd., Basingstoke, Hampshire, UK), containing 7% sheep blood, and the plates were incubated under aerobic conditions incubated at 35–37°C for 24 hours. Similarly, 10 µL of each milk samplewas inoculated onto Columbia blood agar under the same condition as for broth. Presumptive colonies were provisionally identified based on Gram staining, morphology (presence of golden yellowish or creamy white colonies) and hemolytic pattern (with or without beta hemolysis). Identification *S. aureus*was achieved using standard conventional methods including, oxidase and catalase tests, slide and tube coagulase with rabbit plasma as well as DNase tests were used (Kateete *et al*., 2010). In case of discrepancy between the coagulase and the DNAse tests, a latex agglutination test (Slidex Staph Test, England) was carried out (Ridenour *et al*., 2007). MRSA ATCC 33591 and MSSA ATCC 25923 were used as quality control strains. *Staphylococcus aureus* isolates were stored in TSB containing 15% glycerol at -20°C until needed.

Antimicrobial susceptibility test

The Kirby-Bauer Disk Diffusion Susceptibility test was used to obtain the antimicrobial resistance profile of the isolates. This was done following the protocol by CLSI, (2014). Samples were cultured in nutrient broth at 37ºC for 18-24 hours. For phenotypic detection of MRSA antibiotic susceptibility testing, *S. aureus* isolates were tested against the antibiotics including oxacillin (1µg) and cefoxitin (30µg), whereas, Vancomycin (30 µg), Tetracycline (30 µg),Trimethoprim-sulfamethoxazole (25 µg), Ampicillin (10 µg), Erythromycin (15 µg) and Gentamycin (10 µg)for the routine drug susceptibility test.Methicillin was not included as a test antibiotic because it is no longer produced, and oxacillin remains a second option, though it was demonstrated that cefoxitin was more reliable than oxacillin for detecting MRSA (Zurita *et al*.,

2010). A suspension of the test organism were prepared in sterile saline equivalent to 0.5 McFarland using isolated colonies and were incubated at 37°C for 2 hour. Using a sterile cotton swab the organisms were inoculated on Mueller Hinton agar plate spread evenly over the entire surface. The antimicrobial-impregnated disks were placed on the surface of the agar plates and incubated at 37ºC except oxacillin which was at 35ºC for 24 hours. Following incubation, the diameter of the zone of inhibition was measured and the results interpreted according to the zone diameter interpretative standards (CLSI, 2014) for *Staphylococcus* spp.Determination of MRSA isolates was performed according to the results obtained in the evaluation of oxacillin and cefoxitin (Datta*et al*., 2011). Any isolates that was resistant to cefoxitin and/oroxacillin were considered as MRSA (CLSI, 2014).

Statistical analysis

The prevalence and antimicrobial resistance of *Staphylococcus aureus* from milk samples, nasal and environmental swabs in all three farms were compared using the Chi-squared (X^2) test. A value of $P< 0.05$ was considered statistically significant.

RESULTS

Prevalence of *Staphylococcus* spp

The CMT screening for clinical or subclinical mastitis in 200 cows (800 samples of milk) revealed that 100 quarters of 80out of 200 (40.0%) cows tested had subclinical mastitis in different number of quarters, while seven (3.5%) cows tested positive for clinical mastitis (Table 2).With regards to farm distribution, 46 out of 288(16.0%) mammaryquarters testedfrom farm A were CMT positive to mastitis. Twenty out of 140 mammary quarters (14.3%) and 8.2% (34 out of 416) mammary quarters tested positive to CMT on farm B and C, respectively.

Milk bacterial culture from the 100 mammary quarters yielded 49 *Staphylococcus aureus* intramammary infections (IMI) coming from the 80 cows. The overall prevalence of *S. aureus* was 25.7 % (97/377). Of these, 49 (49.0%) were from milk, 11 (7.9%) from environmental swabs, and 37 (27.8%) from nasal swabs (Table 1).

Table 1. The prevalence of *S. aureus* in cow milk, environment and nares from three dairy farms

Farm	Sources			
	Milk (No & %)	Environment (No & %)	Nares (No & %)	Total (%)
Farm A	22 (47.8)	8 (16.7)	10 (21.7)	40 (28.6)
Farm B	11 (55.0)	0	9 (36.0)	20 (2.5)
Farm C	16 (47.1)	3 (6.3)	18 (29.0)	37 (25.7)
Total	47 (49.0)	11 (7.6)	37 (27.8)	97 (25.7)

Table 2. Prevalence of *S. aureus* in cows with mastitis in three dairy farms

Source	No examined	No (%) of cows with subclinical mastitis	No (%) of cows with clinical mastitis	No (%) of cows with *S. aureus* mastitis	No (%) of mammary quarters affected with *S. aureus*
Farm A	72	25 (34.7)	2 (2.8)	17 (63.0)	22 (47.8)
Farm B	35	15 (42.9)	3 (8.6)	7 (38.8)	11 (55.0)
Farm C	104	40 (38.7)	2 (1.9)	17 (40.5)	16 (47.1)
Total	211	80 (37.9)	7 (3.3)	41 (51.3)	49 (49.0)

Antimicrobial susceptibility testing

To better assess the potential public health impact of *Staphylococcus* spp., a total of 95 of 97(2 isolates from milkcould not be retrieved fromglycerol stocks) were assayed for their potential to resist eight antimicrobials. Analysis of the antimicrobial resistance profiles showed that 90 of the 95 isolates (94.7%) were resistant to one or more antimicrobials, whereas five (5.3%) isolates were pan-susceptible to all antimicrobials tested. Nineteen isolates(20.0%; 10 milk samples, 6 nasal and 3 environmental swabs) were resistant to a single antimicrobial and 23 isolates (24.2%; 9 milk samples, 13 nasal and 1 environmental swabs) showed resistance to two antimicrobials. Forty-eight (50.5%)ofallisolates(26 milk, 16 nasal and 6 environmental swabs) were resistant to three or more antimicrobials tested. Two *S. aureus* isolates recovered from milk were resistant to Gen, where as 67.4%ofallisolates were resistant to Amp(Table 3).Approximately14.7%(6 from milk, 3 nasal and 3 from environmental swabs) and 37.9%(six 16 from milk, 6 nasal and 4 environmental swabs) of the isolates were resistant to Fox and Oxa, respectively (Tables 3).While resistance to Tet was significantly higher ($P< 0.05$) in isolates recovered from nares in comparison to those from milk, there were no significant differences ($P> 0.05$) in resistance associated with the remaining antimicrobials.

Table 3. Antimicrobial resistance profiles of *Staphylococcus aureus* isolated from dairy cow milk, environmental and nasal swabs

Source of isolates	Antimicrobial agent (%)[1]							
	Amp[1]	Fox	Ery	Gen	Oxa	Tet	CXT	Vac
Milk (n=47)	61.7	17.0	23.4	6.4	34.0	44.7	40.4	10.6
Nasal swabs (n=37)	75.7	8.1	16.2	0	16.2	73.0	21.6	10.8
Environmental swabs (n=11)	63.6	27.3	27.3	0	36.4	45.5	9.1	0
Total (n=95)	67.4	14.7	21.1	3.2	37.9	55.8	29.5	9.5

[1]Amp,ampicillin; Fox,cefoxitin; Gen, gentamycin; Ery, erythromycin; Oxa, oxacillin; Tet, tetracycline; CXT, Trimethoprim-Sulfamethoxazole; Vac, vancomycin.

Multiple antibiotic resistance (MAR) phenotypes of *S. aureus*

The predominantMAR phenotypes for *S. aureus* isolated from this studywere Amp-Tet-CXT, Amp-Ery-Tet and Amp-Fox-Oxa in20.0%, 9.5% and 8.4% of the isolates, respectively (Table 4). Furthermore,the most commonly observed resistance pattern was between Amp-Tet (46.3%) followed by Amp-CXT (27.4%),Amp-Oxa (13.7%), Fox-Oxa (11.6%), Amp-Fox (11.6%), Ery-Oxa (5.3%),and Oxa-Vac (4.2%).Forty-one (43.2%) among of the isolates in this study revealed resistance with three types of antimicrobial agents in ten different combinations. Six (6.3%) of all isolates were resistant to four antimicrobial agents in four different combinations, while resistance to five antimicrobial agents was observed once (Table 4).

Prevalence of Methicillin resistant *Staphylococcus aureus* (MRSA)

Twenty-seven (28.4%) of all isolates were resistant to oxacillin and/or cefoxitin, and therefore classified as MRSA. Of these, thirteen (56.5%) were resistant to both cefoxitin and oxacillin, one isolate showed resistant only to cefoxitin while 13 isolates were resistant only to oxacillin among of the two antibiotics. Majority of the MRSA isolates(63%) originated from milk with 22.2% and 14.8% isolated from nasal and environmental swabs, respectively.

Table 4. The predominant multiple antibiotic resistant phenotypes for *Staphylococcus aureus* isolated from cow's milk, environmental and nasal swabs

Phenotype[1]	Number observed	Percentage
Apm-Gen-Tet	1	1.1
Apm-Gen-CXT	1	1.1
Amp-Oxa-CXT	1	1.1
Amp-Fox-Gen	1	1.1
Fox-Oxa-Tet	1	1.1
Fox-Ery-Gen	1	1.1
Amp-Fox-Oxa	8	8.4
Amp-Ery-Tet	9	9.5
Amp-Tet-CXT	18	20.0
Amp-Tet-CXT-Vac	1	1.1
Amp-Fox-Oxa-Vac	1	1.1
Amp-Ery-Oxa-CXT	1	1.1
Amp-Fox-Oxa-CTX	1	1.1
Amp-Ery-Tet-CXT	2	1.1
Amp-Fox-Oxa-Tet-Vac	1	1.1

[1]Amp, ampicillin; Fox, cefoxitin; Gen, gentamycin; Ery, erythromycin; Oxa, oxacillin; Tet, tetracycline; CXT, Trimethoprim-Sulfamethoxazole; Vac, vancomycin.

DISCUSSION

Mastitis in both clinical and subclinical forms is the main disease that affects milk

production. The present study revealed higher prevalence of subclinical mastitis compared to clinical mastitis. Similar findings have been observed from other studies conducted elsewhere (Kivaria*et al*., 2004; Sori *et al*., 2011; Zeryehun *et al*., 2013). This observation could be attributed to the invisibility and silent nature of subclinical mastitis which is usually given little attention by farms when it comes to treatment unlike clinical mastitis towards which treatment and control efforts are concentrated (Karimuribo *et al*., 2006; Girma, 2010).

Bovine mastitis is generallyassociated with the intensive farming system. For implementationof therapeutic and preventive measures, appropriate knowledgeof the bacteriological population, antibiotic sensitivity pattern andepidemiological aspect holds key for the management of bovinemastitis in a dairy farm. The present study showed prevalence of 3.3% for clinical mastitis that is closer to the reports of 3.8% in smallholder dairy herds in the Dar es Salaam region (Kivaria*et al*., 2004), 3.4% in Ethiopia (Abebe*et al*., 2016), but lower than 11.5– 14.8% in smallholder dairy herds in the urban and peri-urban areas of the Dodoma municipality in Central Tanzania (Mdegela et al., 2005), and 10.0 – 22.4% reported in Ethiopia (Abera *et al*., 2013; Zeryehun*et al*., 2013). However, the present finding was much higher than the findings of 0.93% in Gondar, Ethiopia (Nibret *et al*., 2011). Risk factors which influence the occurrence of clinical mastitis include animal, pathogen, and environment, which could contribute in the discrepancies of mastitis prevalence (Radostits*et al*., 2007).

The present study also showed prevalence of 37.9% for subclinical mastitis that was closer to the reports of 33.3% in Kilosa district (Karimuribo*et al*., 2008), and 31.8% to 37.0% in Ethiopia (Sori*et al*., 2005; Girma, 2010; Nibret *et al*., 2011). However, the present results are lower than51.6 % (n = 91) reported in smallholder dairy farms in Tanzania (Mdegela *et al*., 2009), 75.9% in dairy cattle kept by smallholders in Iringa and Tanga regions of Tanzania, 87.9% in Uganda (Kasozi *et al*., 2014), and 61.2%in smallholder dairy of the Dodoma municipality (Mdegela *et al*., 2005).The CMT results were found to be higher than the 13.5% to 22.0% reported in Kosovo (Sylejmani *et al*., 2015), 10% in Ethiopia (Kerro and Tareke, 2003), and 8.33% from lactating cows in Peshawar district of Pakistan (Alam *et al*., 2016). This variability in prevalence of subclinical mastitis in present and earlier studies may be attributable to various factors including climate, season, study methodology and farm management practices such as general hygiene, care of teat injuries, care and working of milking machine, and adoption of various mastitis control measuresincluding teat dipping and dry cow therapy.

Staphylococcal mastitis accounts for single largestcause of economic losses in terms of depressed milk production, costof treatment and culling of high genetic vigor animals(Le Mare'chal*et al*., 2011). The present study indicates that 49.0%of milk samples from mastitic cattle were positive for *S.aureus*. Similar studies conducted in Morocco, Brazil, Ethiopia and Kenya reported prevalence of *S. aureus* in milk to be 40%, 68%, 48.75% and 30.6% respectively (Bendahou *et al*., 2008; de Oliveira *et al*., 2011; Daka*et al*., 2012; Shitandi and Sternesjö, 2004).The overall *S. aureus* nasal carriage (27.8%) observed in this study is relatively lower than the 43.7% reported in Saudi Arabia (Alzohairy, 2011), but higher than 5.06%

reported in Iran (Rahimi *et al*., 2015),16.0% in Norway (Mork *et al*., 2012), and 19.8% in Belgium (Nemeghaire *et al*., 2014).Environmental contamination with *S. aureus*was alsoobserved in this study. Environmental prevalence of *S. aureus*(7.6%) observed in the present study was consentient to the findings observed elsewhere (Khan *et al*., 2016; Locateli *et al*., 2016). High prevalence of *S. aureus*observed in the nasal swabs of cattle and environment may be a predisposing factor for subsequent Intra-Mammary Infection (IMI), which needs additional comparative studies (Vautor *et al*., 2005; Mork *et al*., 2012). However, *S. aureus*prevalence can vary depending on many factors including geographical locations, sampling methods, laboratory testing methods, and age of the animals tested.

Antibiotic-resistant *S. aureus* isolates pose a severe challenge to both veterinary and health professions and dairy cattle producers because they have a negative impact on therapy (Brouillette and Malouin, 2005). The antimicrobial resistance level in our study was relatively high. Forty-eight out of 95Staphylococcalisolates were resistant to 3 or more antimicrobial classes including most of the commonlyused antimicrobials in the region. Multiple antibiotic resistant *S. aureus* strains have been isolated from milk obtained from cattle, beef and human samples in many parts of the world (Waage *et al*., 2002; Shitandi and Sternesjö, 2004;Zhanel*et al*., 2005; Pesavento *et al*., 2007; Ateba*et al*., 2010). The prevalence of antibiotic resistance between *S. aureus* usually varies between isolates from the different sampled stations and even between isolates from different herds and/or flocks of the same farm (Waage *et al*., 2002). Furthermore, *S. aureus* has developed multidrug resistance in many regions of the world (WHO, 2000) and the usage of antibiotics correlates with the emergence and maintenance of antibiotic-resistant traits within pathogenic strains (Shitandi and Sternesjö, 2004).

The highest resistance to the antimicrobials used was to ampicillin (67.4%).Ampicillin and oxacillin are beta-lactam antibiotics, and resistance to the beta-lactam antibioticsis most often due to a plasmid-encoded penicillinase/beta-lactamase (Lowy, 2003; Petinakiand Spiliopoulou, 2012). Beta-lactam antibiotics such as Penicillin is commonly used on dairy cattle farms in the study area, and this could be the reason for high Ampicillin resistance profiles of *S. aureus* observed in this study.Tetracyclines have been used extensively to treat animal infections because of their relative safety and broad-spectrum activity (Kuang *et al*., 2009). Nevertheless, increasing tetracycline resistance rates in *S. aureus* strains from bovine mastitis have been reported (Kumar *et al*., 2010; Wang *et al*., 2012; Intorre *et al*., 2013). Accordingly, a relatively high (55.8%) level of Tetracycline resistance was found in our study.

The antimicrobial susceptibility test for *S. aureus* revealed a relatively low resistance to cefoxitin (14.7%), vancomycin (9.5%), gentamycin (3.2%), and moderate resistance to oxacillin (37.9%), trimethoprim-sulfamethoxazole (29.5%) and erythromycin (21.1%). Similar observations have been reported from Ethiopia (Mekuria *et al*., 2013) and China (Wang *et al*., 2012). However, Daka*et al*. (2012) reported higher resistance in isolates from cow's milk to oxacillin (60.3%) and vancomycin (38.5%), whereas, a study reported in South Africa showedthat 100% of isolates from commercial farms were susceptible to Vancomycin (Ateba*et al*., 2010). The

variations could be associated with the use of these antibiotics in dairy farms. Vancomycin is no longer used in veterinary medicine in many countries (Pace and Yang, 2006) including Tanzania, which may account for the results reported in this study.

The gold standard method for identifying MRSA is to detect the *mecA* gene or its product, PBP2a, by latex agglutination (Gosbell *et al*., 2001) However, these tests are relatively expensive. Cefoxitin and oxacillinhave been reported as surrogate markers for the detection of methicillin resistance (Fernandes *et al*., 2005; Anand *et al*., 2009; CLSI, 2014).In this study, 14.7%, 37.9% and 13.8% of isolates were phenotypically resistantto Cefoxin, Oxacillin and co-resistance to bothCefoxitin and Oxacillin, respectively. This is an indicator of MRSA. MRSA strains in livestock including bovine milk samples have also been reported in China (Wang *et al*., 2014), Pakistan (Alam *et al*., 2016), USA (Smith, 2015), European countries (Johnson, 2011;Kock et al., 2011), and African continent (Lozano *et al*., 2016).Interestingly, variations in prevalence of MRSA have been reported in different Sub-Saharan Africa including 81.2 – 93.2% milk samples from communal farms versus 5.7% – 7.0% from commercial farms in South Africa (Ateba *et al*., 2010), 60.3% in Ethiopia (Daka *et al*., 2012), 28.57% in Nigeria (Maisiyama *et al*., 2014), 15% in Morocco (Bendahou *et al*., 2008), 7.8% in Kenya (Shitandi and Mwangi, 2004), and 2.38% to 4.17% in raw milk samples in Tanzania (Mohammed, 2015).

Based on the findings of this study, it is concluded that milk production is extremely vital to million households around the globe. In most developing countries, including Tanzania, milk is produced by smallholders, and milk production contributes to household livelihoods, food security and nutrition. Milk provides relatively quick returns for small-scale producers and is an important source of cash income. Bovine mastitis is one of the most important diseases that have substantial consequences on the dairy industry and the public health as a whole. The detection of *S. aureus* in the milk samples indicates that the product is unwholesome for human consumption. A large proportion of the isolates obtained were resistant to three or more antibiotics, and a good number of these isolates were phenotypically confirmed as MRSA. Appropriate use of antimicrobials in animal husbandry could help to control the emergence of resistant strains and limit the acquisition of additional antimicrobial resistance genes in existing strains.A cost-effective policy in dairy cattlefarms would consist of preventing MRSA colonizationor intra-mammary infection by enforcing the same measures implementedfor the *S. aureus* control, particularly focusing on thehygienic milking procedures and on the application ofstrict biosecurity measures.

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REFERENCES

Abebe R, Hatiya H, Abera M, Megersa B, Asmare K. Bovine mastitis: prevalence, risk factors and isolation of *Staphylococcus aureus* in

dairy herds at Hawassa milk shed, South Ethiopia. *BMC Vet Res* 12:270, 2016.

Abera M, Demie B, Aragaw K, Regassa F,Regassa A. Isolation and identification of *Staphylococcus aureus* from bovine mastitic milk and their drug resistance patterns in Adama town, Ethiopia. *Afr J Dairy Farm Milk Prod* 1(2): 19 – 23, 2013.

Alam S, Abro SH, Khan MA, Abro R, Wagan H, Ali S. Emerging of Methicillin Resistant *Staphylococcus aureus* in Clinical and Sub-Clinical Bovine Mastitis in Peshawar. *SciInt (Lahore)*28(3):3055 – 3059, 2016.

Alzohairy M. Colonization and antibiotic susceptibility pattern of Methicillin resistance *Staphylococcus aureus* (MRSA) among farm animals in Saudi Arabia.*J Bacterio Res* 3(4):63– 68, 2011.

Anand KB, Agrawal P, Kumar S, Kapila K. Comparison of cefoxitin disc diffusion test, oxacillin screen agar, and PCR for *mec*A gene for detection of MRSA. *Indian J Med Microbiol* 27(1): 27-9, 2009.

Ateba CN, Mbewe M, Moneoang MS, Bezuidenhout CC. Antibiotic-Resistant *Staphylococcus aureus* Isolated from Milk in the Mafikeng Area, North West Province, South Africa.*S Afr J Sci* 106 (11/12): 1–6, 2010.

Awandkar SP, Bhikane AU, Kulkarni MB. Antibiotic Resistance trends in clinical bovine mastitis. *Biolife*1: 139–143, 2013.

Bendahou A,Lebbadi M,Ennanei L,Essadqui FZ,Abid M. Characterization of *Staphylococcus* species isolated from raw milk and milk products (lben and jben) in North Morocco. *J Infect DevCtries* 2: 218–225, 2008.

Botrel MA, Haenni M, Morignat E, Sulpice P, Madec JY, Calavas D. Distribution and antimicrobial resistance of clinical and subclinical mastitis pathogens in dairy cows in Rhone-Alpes, France. *Foodborne Pathog Dis* 7(5): 479 – 487, 2010.

Bradley AJ. Bovine mastitis: an evolving disease. *Vet J* 164(2): 116–128, 2002.

Brouillette E, Malouin F. The pathogenesis and control of *Staphylococcus aureus*-induced mastitis: Study models in the mouse. *Microb Infect* 7: 560 – 568, 2005.

Cardozo VF, Lancheros CAC, Narciso AM, Valereto ECS, Kobayashi RKT, Seabra AB, Nakazato G. Evaluation of antibacterial activity of nitric oxide-releasing polymeric particles against *Staphylococcus aureus* and Escherichia coli from bovine mastitis. *Int J Pharm* 473: 20-29, 2014.

Chandrasekaran D, Nambi AP, Thirunavukkarasu PS, Vairamuthu S, Venkatesan P, Tirumurugaan KG. A study on treatment of resistant mastitis in dairy cows.*J Appl Nat Sci* 6: 786–791, 2014.

Clinical and Laboratory Standards Institute. (CLSI) Performance Standards for Antimicrobial Susceptibility Testing: Twenty-Fourth Informational Supplement M 100-S24. Vol. 34. Wayne, PA, USA: Clinical and Laboratory Standards Institute, 2014.

Daka D, Solomon G,Yihdego D. Antibiotic-resistance *Staphylococcus aureus* isolated from cow's milk in the Hawassa area, South Ethiopia. *Annals Clin Microbiol Antimicrob* 11:26, 2012.

Datta P, Gulati N, Singla N, Rani Vasdeva H, Bala K, Chander J, Gupta V. Evaluation of various methods for the detection of meticillin-resistant *Staphylococcus aureus* strains and susceptibility patterns. *J Med Microbiol* 60(1): 1613–1616, 2011.

de Neeling AJ, van den Broek MJ, Spalburg EC, van Santen-Verheuvel MG, Dam-Deisz WD, Boshuizen HC, van de Giessen AW, van Duijkeren E, Huijsdens XW. High prevalence of Methicillin resistant *Staphylococcus aureus* in pigs.*Vet Microbiol* 122: 366–372, 2007.

de Oliveira LP, Soares e Barros LS, Silva VC, Cirqueira MG. Study of *Staphylococcus aureus* in raw and pasteurized milk consumed in the Reconcavo area of the State of Bahia, Brazil.*J Food Process Technol* 2:128, 2011.

Denis O, Suetens C, Hallin M, Catry B, Ramboer I, Dispas M, Willems G, Gordts B, Butaye P, Struelens MJ. Methicillin-resistant *Staphylococcus aureus* ST398 in swine farm personnel, Belgium. *Emerg Infect Dis* 15: 1098–1101, 2009.

Faires M, Tater K, Weese JS. An investigation of Methicillin-resistant *Staphylococcus aureus* colonization in people and pets in the same household with an colonization in people and pets in the same household with an infected person or infected pet. *J Am Vet Med Assoc* 235: 540 – 543, 2009.

Fernandes CJ, Fernandes LA, Collignon P.Cefoxitin resistance as a surrogate marker for the detection of Methicillin-resistant

Staphylococcus aureus.*J Antimicrob Chemother* 55(4): 506 – 510, 2005.

Gordoncillo MJ, Abdujamilova N, Perri M, Donabedian S, Zervos M, Bartlett P. Detection of Methicillin-resistant *Staphylococcus aureus* (MRSA) in backyard pigs and their owners, Michigan, USA. *Zoonoses Public Hlth* 59: 212 – 216, 2012.

Gosbell IB, Neville SA, Mercer JL, Fernandes LA, Fernandes CJ. Evaluation of the MRSA-Screen test in detecting oxacillin-resistance in community and hospital isolates of *Staphylococcus aureus*. *Pathol* 33(4): 493–495, 2001.

Graveland H, Duim B, van Duijkeren E, Heederik D, Wagenaar JA. Livestock-associated Methicillin-resistant *Staphylococcus aureus* in animals and humans.*Int J Med Microbiol* 301: 630–634, 2011.

Haran KP, Godden SM, Boxrud D, Jawahir S, Bender JB,Sreevatsana S. Prevalence and Characterization of *Staphylococcus aureus*, Including Methicillin-Resistant *Staphylococcus aureus*, Isolated from Bulk Tank Milk from Minnesota Dairy Farms. *J ClinMicrobiol* 50(3): 688–695, 2012.

Huber H, Koller S, Giezendanner N, Stephan R, Zweifel C. Prevalence and characteristics of methicillin resistant *Staphylococcus aureus* in humans in contact with farm animals, in livestock, and in food of animal origin, Switzerland, 2009. *Euro Surveill* 15: 19542, 2010.

Intorre L, Vanni M, Meucci V, Tognetti R, Cerri D, Turchi B, Cammi G, Arrigoni N,Garbarino C.Antimicrobial resistance of *Staphylococcus aureus* isolated from bovine milk in Italy from 2005 to 2011. *Large Anim Rev* 19: 287 – 291, 2013.

Johnson AP. Methicillin-resistant *Staphylococcus aureus*: the European landscape. *J AntimicrobChemother*66 (4): 43–48, 2011.

Kaliwal BB, Sadashiv SO, Kurjogi MM, Sanakal RD. Prevalence and Antimicrobial Susceptibility of Coagulase- Negative *Staphylococci* isolated from Bovine Mastitis. *Vet World* 4:158–161, 2011.

Karimuribo ED, Fitzpatrick JL, Swai ES, Bell C, Bryant MJ, Ogden NH, Kambarage DM, French NP. Prevalence of subclinical mastitis and associated risk factors in smallholder dairy cows in Tanzania.*Vet Record* 163: 16 – 21, 2008.

Karimuribo ED, Fitzpatrickb JL, Bellc CE, Swai ES, Kambarage DM, Ogdene NH, Bryantf MJ, Frenchg NP. Clinical and subclinical mastitis in smallholder dairy farms in Tanzania: risk, intervention and knowledge transfer. *Prev Vet Med* 74(1): 84–98, 2006.

Karimuribo ED, Gallet PL, Ng'umbi NH, Matiko MK, Massawe LB, Mpanduji DG,Batamuzi EK. Status and factors affecting milk quality along the milk value chain: a case of Kilosa district, Tanzania. *Liv Res Rural Dev* 27: 51, 2015.

Kasozi KI, Tingiira JB, Vudriko P (2014).High Prevalence of Subclinical Mastitis and Multidrug Resistant *Staphylococcus aureus* are a Threat to Dairy Cattle Production in Kiboga District (Uganda). *Open J Vet Med* 4:35-43.

Kateete DP, Kimani CN, Katabazi FA, Okeng A, Okee MS, Nanteza A, Joloba ML, Najjuka FC. Identification of *Staphylococcus aureus*: DNase and Mannitol salt agar improve the efficiency of the tube coagulase test. *Annals ClinMicrobiolAntimicrob* 9:23, 2010.

Kerro OD, Tareke F. Bovine Mastitis in selected areas of Southern Ethiopia.*Trop Anim Health Prod* 35: 197 – 205, 2003.

Khan SA, Abro SH, Khan MA, Abro R, Alam S, Buriro RA.Presence of methicillin resistant *Staphylococcus aureus* in the environment and working personal at dairy farms in Peshawar (A case study).*SciInt (Lahore)*28(3): 3067 – 3070, 2016.

Kivaria FM, Noordhuizen JP, Kapaga AM. Risk indicators associated with subclinical mastitis in smallholder dairy cows in Tanzania. *Trop Anim Health Prod* 36(6):581 – 592, 2004.

Kock R, Mellmann A, Schaumburg F, Friedrich AW, Kipp F.The epidemiology of Methicillin resistant *Staphylococcus aureus* (MRSA) in Germany.*DtschArzteblInt* 108: 761–767, 2011.

Kuang Y, Jia H, Miyanaga K, Tanji Y. Effect of milk on antibacterial activity of tetracycline against *Escherichia coli* and *Staphylococcus aureus* isolated from bovine mastitis. *ApplMicrobiolBiotechnol* 84: 135 – 142, 2009.

Kumar R, Yadav BR, Singh RS. Genetic determinants of antibiotic resistance in *Staphylococcus aureus* isolates from milk of Mastitic crossbred cattle. *CurrMicrobiol* 60: 379 – 386, 2010.

Le Mare'chal C, SeyffertN, Jardin J, Hernandez D, Jan G. Molecular Basis of Virulence in *Staphylococcus aureus* Mastitis. *Plos One*6(11): e27354, 2011.

Li L, Zhou L, Wang L, Xue H, Zhao X. Characterization of Methicillin-Resistant and -Susceptible Staphylococcal Isolates from Bovine Milk in Northwestern China. *Plos One*10(3): e0116699, 2015.

Locateli C, Cremonesi P, capriole A, carfora V, Lanzano A, Barberio A, Morandi S, Casula A, Castiglioni B, Bronzo V,Moronill P. Occurrence of Methicillin-resistant *Staphylococcus aureus* in dairy cattle herds, related swine farms, and humans in contact with herds. *J Dairy Sci* 100: 608–619, 2016.

Loeffler A, Boag AK, Sung J, Lindsay JA, Guardabassi L, Dalsgaard A, Smith H, Stevens KB, Lloyd DH. Prevalence of Methicillin resistant *Staphylococcus aureus* among staff and pets in a small animal referral hospital in the UK.*J AntimicrobChemother* 56: 692 – 697, 2005.

Lowy FD. Antimicrobial resistance: the example of *Staphylococcus aureus*. *J ClinInvestig* 111(9): 1265 – 1273, 2003.

Lozano C, Gharsa H, Slama KB, Zarazaga M, Torres C.*Staphylococcus aureus* in Animals and Food: Methicillin Resistance, Prevalence and Population Structure; A Review in the African Continent. *Microorganisms* 4: 12, 2016.

Luzzago C, Locatelli C, Franco A, Scaccabarozzi L, Gualdi V, Vigano R. Clonal diversity, virulence-associated genes and antimicrobial resistance profile of *Staphylococcus aureus* isolates from nasal cavities and soft tissue infections in wild ruminants in Italian Alps. *Vet Microbiol* 170(1-2):157–61, 2014

Maisiyama, IB, Okon KO, Adamu NB, Askira UM, Isyaka TM, Adamu SG, Mohammed A. Methicllin-resistant *Staphylococcus aureus* (MRSA) colonization rate among ruminant animals slaughtered for human consumption and contact persons in Maiduguri, Nigeria.*Afr J Microbiol Res* 8: 2643–2649, 2014.

Mdegela RH, Karimuribo E, Kusiluka LJM, Kabula B, Manjurano A, Kapaga AM, Kambarage DM. Mastitis in smallholder dairy and pastoral cattle herds in the urban and peri-urban areas of the Dodoma municipality in Central Tanzania. *Liv Res Rural Dev* 17: 123, 2005.

Mdegela RH, Ryoba R, Karimuribo ED, Phiri EJ, Løken T, Reksen O, Mtengeti E, Urio NA. Prevalence of clinical and subclinical mastitis and quality of milk in smallholder dairy farms in Tanzania. *J S Afr Vet Assoc* 80(3): 163–168, 2009.

Mekuria A, Asrat D, Woldeamanue Y, Tefera G. 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.*Afr J Microbiol Res* 7(27): 3501–3510, 2013.

Mohammed J. The Prevalence of Methicillin-Resistant *Staphylococcus aureus* (MRSA) Isolated from Raw Bovine Milk in Morogoro Municipality, Tanzania. A dissertation submitted in partial fulfillment of the requirement for the degree of Master of Science in Molecular Biology and Biotechnology of Sokoine University of Agriculture. Morogoro, Tanzania. page 69, 2015.

Moon JS, Lee AR, Kang HM, Lee ES, Kim MN, Paik YH. Phenotypic and genetic antibiogram of Methicillin-resistant *Staphylococci* isolated from bovine mastitis in Korea. *J Dairy Sci* 90: 1176–1185, 2007.

Mork T, Kvitle B, Jorgensen HJ. Reservoirs of *Staphylococcus aureus* in meat sheep and dairy cattle.*Vet Microbiol* 155(1): 81– 87, 2012.

Nemeghaire S, Argudin MA, Haesebrouck F, Butaye P. Epidemiology and molecular characterization of methicillin-resistant *Staphylococcus aureus* nasal carriage isolates from bovines. *BMC Vet Res* 10:153, 2014.

Nibret M, Yilikal A, Kelay B. A cross sectional study on the prevalence of sub clinical mastitis and associated risk factors in and around Gondar, Northern Ethiopia. *Int J Anim Vet Advances* 3(6): 455–459, 2011.

Pace JL, Yang G. Glycopeptides: Update on an old successful antibiotic class.*Biochem Pharm*71: 968 – 980, 2006.

Persson Y, Nyman AKJ, Andersson UG. Etiology and antimicrobial susceptibility of udder pathogens from cases of subclinical mastitis in dairy cows in Sweden.*Acta Vet Scand* 53. 36, 2011.

Pesavento G, Ducci B, Comodo N, Lo Nostro A. Antimicrobial resistance profile of *Staphylococcus aureus* isolated from raw

meat: A research for methicillin resistant Staphylococcus aureus (MRSA). *Food Cont* 18 (3): 196 – 200, 2007.

Petinaki E, Spiliopoulou I. Methicillin-resistant *Staphylococcus aureus* among companion and food-chain animals: impact of human contacts. *ClinMicrobiol Infect* 18: 626–634, 2012.

Peton V, Le Loir Y. *Staphylococcus aureus* in veterinary medicine. Infect Genet Evol 21:602–15, 2014

Pu WX, Su Y, Li JX, Li CH, Yang ZQ, Deng HP. High incidence of oxacillin-susceptible *mec*A-positive *Staphylococcus aureus* (OS-MRSA) associated with bovine mastitis in China. *Plos One*9: e88134, 2014.

Radostits OM, GayKW, HinchcliffCC, Constable PD. "Mastitis," In: Veterinary Medicine: A Text Book of Disease of Cattle, Sheep, Pigs, Goats, and Horses. 10thBailliereTindall edition, London, UK. pp. 674–762, 2007.

Rahimi H, Saei HD, Ahmadi M. Nasal Carriage of *Staphylococcus aureus*: Frequency and Antibiotic Resistance in Healthy Ruminants. *Jundishapur J Microbiol* 8(10): e22413, 2015.

Ridenour G, Lampen R, Federspiel J, Kritchevsky S, Wong E, Climo M. Selective use of intranasal mupirocin and chlorhexidine bathing and the incidence of Methicillin-resistant *Staphylococcus aureus* colonization and infection among intensive care unit patients. *Infect Control Hosp Epidemiol* 28(10):1155 – 1161, 2007.

Sawant AA, Sordillo LM, Jayarao BM. A survey on antibiotic usage in dairy herds in Pennsylvania. *J Dairy Sci* 88: 2991 – 2999, 2005.

Sharma L, Verma AK, Kumar A, Rahat A, Nigam R. Incidence and Pattern of Antibiotic Resistance of *Staphylococcus aureus* Isolated from Clinical and Subclinical Mastitis in Cattle and Buffaloes. *Asian J AnimSci* 9: 100 – 109, 2015.

Sharma P, Tomar SK, Goswami P, Sangwan V, Singh R. Antibiotic resistance among commercially available probiotics. *Food Res Int* 57: 176 – 195, 2014.

Shitandi A, Mwangi M. Occurrence of multiple antimicrobial resistance among *Staphylococcus aureus* isolates from Kenyan milk. *J Food TechnolAfr* 9: 23–25, 2004.

Shitandi A, Sternesjö Å. Prevalence of multidrug resistant *Staphylococcus aureus* in milk from large and small-scale producers in Kenya.*J Dairy Sci* 87:4145–4149, 2004.

Smith TC. Livestock-Associated *Staphylococcus aureus*: The United States Experience. *PLoSPathog* 11(2): e1004564, 2015.

Sori T, Hussien J, Bitew M. Prevalence and susceptibility assay of *Staphylococcus aureus* isolated from bovine mastitis in dairy farms of Jimma town, South West Ethiopia.*J Anim Vet Advances* 10(6): 745–749, 2011.

Stefani S, Chung DR, Lindsay JA, Friedrich AW, Kearns AM, Westh H, Mackenzie FM. Methicillin resistant *Staphylococcus aureus* (MRSA): global epidemiology and harmonization of typing methods. *Int J Antimicrob Agents* 39: 273–282, 2012.

Sylejmani D, Ramadani N, Robaj A, Hamidi A. Prevalence and Antimicrobial Susceptibility of Bacterial Isolates from Subclinical Mastitis In Dairy Farms In Kosovo. *Bulg J Vet Med* 16: 1311 – 1477, 2015.

Turkyilmaz S, Tekbiyik S, Oryasin E, Bozdogan B. Molecular epidemiology and antimicrobial resistance mechanisms of methicillin-resistant *Staphylococcus aureus* isolated from bovine milk. *Zoonoses Public Health* 57: 197–203, 2010.

Van Duijkeren E, Moleman M, Van Oldruitenborgh-Oosterbaan MMS, Multem J, Troelstra A, Fluit AC, Van Wamel WJB, Houwers DJ, de Neeling AJ, Wagenaar JA. Methicillin-resistant *Staphylococcus aureus* in horses and horse personnel: an investigation of several outbreaks. *Vet Microbiol* 141: 96 – 102, 2010.

Vanderhaeghen W, Cerpentier T, Adriaensen C, Vicca J, Hermans K, Butaye P. Methicillin-resistant *Staphylococcus aureus* (MRSA) ST398 associated with clinical and subclinical mastitis in Belgian cows. *Vet Microbiol* 144: 166–171, 2010.

Vautor E, Abadie G, Guibert JM, Chevalier N, Pepin M. Nasal carriage of *Staphylococcus aureus* in dairy sheep. *Vet Microbiol* 106(3-4):235–239, 2005.

Waage S, Bjorland J, Caugant DA. Spread of *Staphylococcus aureus* resistant to penicillin and tetracycline within and between dairy herds. *Epidemiol Infect* 129: 193 – 202, 2002.

Wang X, Meng J, Zhou T, Zhang Y, Yang B, Xi M, Sheng J, Zhi S, Xia X. Antimicrobial susceptibility testing and genotypic characterization of *Staphylococcus aureus*

from food and food animals. *Foodborne PathogDis*9(2):95 – 101, 2012.

Wang X, Wang X, Wang Y, Guo G, Usman T, Hao D. Antimicrobial resistance and toxin gene profiles of *Staphylococcus aureus* strains from Holstein milk. *LettApplMicrobiol* 58: 527–534, 2014.

WHO, World Health Organization (2000).Overcoming Antimicrobial Resistance. Geneva, Switzerland, pp 67. Available at URL: http://www.who.int/infectious-disease-report/index.html

Witte W, Strommenger B, Stanek C,Cuny C. Methicillin-resistant *Staphylococcus aureus* ST398 in Humans and Animals, Central Europe. *Emerg Infect Dis* 13(2): 255–258, 2007.

Zeryehun T, Aya T, Bayecha R. Study on prevalence, bacterial pathogens and associated risk factors of bovine mastitis in small holder dairy farms in and around addis Ababa, Ethiopia.*J Anim Plant Sci*23(1): 50–55, 2013.

Zhanel GG, Hisanaga TL, Laing NM. Antibiotic resistance in outpatient urinary isolates: Final results from the North American Urinary Tract Infection Collaborative Alliance (NAUTICA). *Int J Antimicrob Agent* 26: 380 – 388, 2005.

Zurita J, Mejía C, Guzmán-Blanco M. Diagnosis and susceptibility testing of methicillin-resistant *Staphylococcus aureus* in Latin America. *Braz J Infect Dis* 14 (2): S97 - S106, 2010.

Zutic M, CirkovicI, Pavlovic L, Zutic J, Asanin J, Radanovic O,Pavlovic N. Occurrence of methicillin-resistant *Staphylococcus aureus* in milk samples from Serbian cows with subclinical mastitis. *Afr J Microbiol Res* 6: 5887 – 5889, 2012.