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Identification and Antimicrobial Resistance Pattern of *Staphylococcus aureus* Isolated from Bovine Raw Milk in Addis Ababa, Ethiopia

Mekiya Sani[a], Getachew Gugsa*[b], Abebe Mekuria[c], and Meselu Ahmed[b]
[a] Halal Food Industries Plc, Mojo, Ethiopia,
[b] School of Veterinary Medicine, Wollo University, Dessie, Ethiopia
[c] College of Health Sciences, Arsi University, Assela, Ethiopia

ABSTRACT

Milk and its products are highly susceptible to microbial attack because of their rich composition. Therefore, the current cross-sectional study was conducted from November 2013 to April 2014 to isolate *Staphylococcus aureus* (*S. aureus*) and evaluate its *in vitro* antimicrobial resistance pattern from bovine raw milk of lactating dairy cows of five private dairy farms of Addis Ababa, Ethiopia. A total of 267 milk samples were randomly and aseptically collected and tested using bacteriological and antibiotic susceptibility tests. The findings were analyzed using WHONET 5.6 and SPSS Version 16 Softwares. The phenotypic results showed that out of the total 267 cultured raw milk samples 14(5.24%) were found to be coagulase positive *S. aureus*. All the positive isolates were subjected to antibiotic susceptibility tests against twelve antimicrobial agents of different antibiotic classes. The antibiogram profile of the tested *S. aureus* results revealed as large proportions of the isolates were found to be highly susceptible to Streptomycin (71.4%), Trimethoprim/Sulfamethoxazole (71.4%), Kanamycin (64.3%), Chloramphenicol (57.1%), Gentamycin (57.1%), and Methicillin (57.1%). However these isolates were highly resistant to Amoxicillin (78.6%), Tetracycline (78.6%), Ampicillin (71.4%), and Cloxacillin (57.1%). But the isolates were showed intermediate response to Neomycin (57.1%) and Polymyxin B (28.6%). Of the tested isolates 92.9% were developed a multidrug resistant. Hence, there should be a practice of proper use of antibiotics and hygienic measures in the farms, and genotypic based studies should be undertaken.

Keywords: Addis Ababa, Antibiogram, Dairy Farm, Milk, Resistance, *S. aureus*.

INTRODUCTION

Milk is a very nutritional food that is rich in carbohydrate, proteins, fats, vitamins and minerals. However, milk and milk-based food products are highly susceptible to microbial attack because of their rich composition, which provides a favorable medium for growth of a host of spoilage agents (Bradely, 2002; De Buyser, 2001). The health risk to consumers can be due to the presence of zoonotic pathogens and antimicrobial drug residues (Bradely, 2002). The quality of milk may be lowered by a numbers of factors such as adulteration, contamination during and after milking and the presence of udder infections (Esron et al., 2005).

Bovine mastitis produces a wide variety of problems in the dairy farm (Getahun et al., 2008). Staphylococcal mastitis is the commonest and economically the greatest concern to the dairy industry, worldwide (Atasever, 2012; Hussain et al., 2012a; Momtaz et al., 2010). *Staphylococcus aureus* (*S. aureus*) is the most predominant contagious pathogen responsible for clinical and subclinical infections in lactating cows. The organism is well adapted to survive in the udder and shed into milk from infected quarters (Le Marechal et al., 2011).

Naturally, *S. aureus* isolates are inhabitants of mucous epithelia and skin of human, dairy cattle and other mammalians (Chu et al., 2012). Transmission occurs mainly at milking time through contaminated milking machines, clothes and hands of milkers or machine operators (Radostitis et al., 1994; Seki et al., 1998; Zadoks et al., 2000). *S. aureus* is an important and versatile food-borne pathogen of humans and animals and causes a wide variety of diseases ranging in severity from mild skin infections to more severe diseases such as pneumonia and septicaemia (Leonard & Markey, 2008; Lowy, 1998).

The pathogenic potential of *S. aureus* depends on numerous cell surface virulence factors and it has

*Corresponding author**:** gugsag@yahoo.com

capability of producing a variety of exotoxins and cell surface-associated proteins that enhances the cellular attachment, organism invasion to host immune system and stimulation of toxic tissue reactions (Hussain et al., 2012b; Kalorey et al., 2007).

In general, the treatment of bovine mastitis is based on the use of antibiotics which are not always effective. These drugs are also responsible for the presence of residues in the milk and the increase of antibiotic-resistant strains (Getahun et al., 2008). *S. aureus* has been reported to frequently show multiple antimicrobial resistance patterns (Enright, 2003). Improper use of antimicrobials has resulted in augmenting the bacterial resistance mechanism including the β-lactamase production and low-affinity penicillin binding protein 2a (PBP2a) (David & Daum, 2010). In addition, S. *aureus* strains are capable of mutation, clonal evolution and horizontal gene transfer that boost up the virulence and drug resistance (Brody et al., 2008).

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). Consequently, enumeration and identification of staphylococci in dairy products is a priority in developing public health measures to reduce food borne disease outbreaks (Ercolini, 2004; Palomares, 2003). Furthermore, surveillance of antimicrobial resistance is important to ensure optimal results of antimicrobial use and minimize the risk for selection and spread of antimicrobial resistance (Ochoa-Zarzosa et al., 2008; Piepers et al., 2007). 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 (Hundera et al., 2005; Mekonnen et al., 2005).

Hence, identification of pathogenic and resistant *S. aureus* from bovine raw milk is of vital importance for successful treatment. 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. Therefore, in view of this, the current study was intended to isolate *S. aureus* from bovine raw milk of lactating dairy cows and to evaluate the *in vitro* antimicrobial resistance pattern of *S. aureus* from dairy farms in and around Addis Ababa, Ethiopia.

MATERIALS AND METHODS

Study area:

The study was conducted from November 2013 to April 2014 in Addis Ababa. Addis Ababa is a seat both for Federal Democratic Republic of Ethiopia (FDRE) and Oromiya National Regional State Government. Addis Ababa is located between 8°55'and 9° 0 5'N Latitude and 38° 40' and 38°50'E Longitude. The city is located at the center of Ethiopia with an area of 540Km^2 of which 18.174m^2 is rural and its altitude ranges from 2000m-2800 m.a.s.l. It is bordered with Oromiya National Regional State in all directions (AACA, 1998). It has a humid sub tropical climate with mild dry winter, hot summer and moderate autumn and spring seasonality classification. The average temperature is 15.9°C and total annual precipitation averages 1089mm.

Study animal:

The study animals consisted of cross breed lactating dairy cows that were managed under the intensive production system. The dairy cows were comprised of cross and exotic breeds (HF) and adult in age group exclusively the lactating cows.

Study design:

A cross-sectional study was conducted from November 2013 to April 2014 in selected private dairy farms located in and around Addis Ababa city. The study farms were selected purposively based on the presence of potential lactating dairy cows. For sampling of individual animal's random sampling was utilized in the selected sites of the dairy farms.

Sample size and collection:

A total of 267 samples of bovine raw milk samples were collected using a purposive sampling technique from Addis Ababa. About 10ml of milk sample was aseptically collected from each of clinically and sub clinically (california mastitis test (CMT) positive) mastitis non-blind quarters of the selected cows using sterile universal bottle for bacterial isolation according to Quinn et al. (2002). Then, the collected samples were transported with an ice box to Shola Veterinary Laboratory, Urban Bureau of Agriculture, Addis Ababa City Administration, for microbiological examination. If immediate inoculation was not convenient, samples were kept at 4°C for a maximum of 24h until cultured on standard bacteriological media.

Bacteriological examination of milk samples:

A loopful of milk sample was streaked on 5% sheep blood agar and the plates were incubated aerobically at 37°C and examined after 24h for growth, gross colony morphology characteristics after 24-48h. The colonies were provisionally identified on the basis of staining reaction with Gram's stain, cellular morphology and hemolytic pattern on blood agar. The representative colonies

were sub cultured on mannitol salt agar then incubated at 37°C for 24-48h. The presumed colonies that grew on mannitol salt agar were then sub-cultured on nutrient broth and nutrient agar plates and incubated at 37°C for 24-48h to get a pure culture. The pure isolates were preserved, maintained and stored at 4°C until the different differential biochemical tests were performed for identification and characterization of the isolates (Quinn et al., 2002). The purified *S. aureus* isolates were identified through different biochemical tests: catalase test, oxidase test, oxidation-fermentation test, and tube coagulase. Cultures which showed hemolysis positive, Gram-positive, catalase positive, oxidase negative, oxidation-fermentation positive, and coagulase positive were considered as *S. aureus* (Bannerman, 2003; Quinn et al., 2002). Plates were incubated at 37°C for 24-48h. All media were prepared and used according to the manufacturer's specification.

Antibiotic susceptibility test:

The isolates of *S. aureus* were screened for *in vitro* antimicrobial susceptibility using the agar disk diffusion method by Kirby et al. (1966) on Mueller-Hinton agar to determine their antibiotic-resistance profiles. The following twelve different antibiotic discs, with their concentrations given in parentheses, were used in the antibiograms: Ampicillin (AP)(10µg), Amoxicillin (AMX) (25µg), Cloxacillin (COX) (5µg), Methicillin (MET) (5µg), Gentamycin (GEN) (10µg), Kanamycin (K) (30µg), Neomycin (N) (30µg), Streptomycin (S) (10µg), Trimethoprim/Sulfamethoxazole (SXT) (24µg), Polymyxin B (PB) (300µg), Chloramphenicol (C) (30µg), and Tetracycline(TE) (30µg). After 18-24h of incubation, the clear zones (inhibition zones of bacterial growth around the antibiotic discs (including the discs) diameter for individual antimicrobial agents were measured and then translated into Sensitive (S), Intermediate (I), and Resistant (R), categories according to the interpretation table of the National Committee for Clinical Laboratory Standards (NCCLS, 2012).

Data analysis:

All collected data were entered into Microsoft excel spread sheet and coded appropriately. The coded data was transferred and analyzed using WHONET 5.6 to test the antimicrobial resistance of the bacteria. In addition, descriptive statistics were used to determine the prevalence using Statistical Package for Social Sciences (SPSS, 2002).

Ethical consideration:

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

RESULTS

Antimicrobial resistance of *S. aureus*:

The phenotypic examination results showed that out of the total 267 raw milk samples collected from the different lactating cows of the five private dairy farms 14 (5.24%) were found to be coagulase positive *S. aureus*.

The antibiogram profile results of the tested *S. aureus* isolates indicated as large proportions of the isolates of this study were found to be highly susceptible to Streptomycin (71.4%), Trimethoprim/ Sulfamethoxazole (71.4%), Kanamycin (64.3%), Chloramphenicol (57.1%), Gentamycin (57.1%), and Methicillin (57.1%). However these isolates were highly resistant to Amoxicillin (78.6%), Tetracycline (78.6%), Ampicillin (71.4%), and Cloxacillin (57.1%). But the isolates were showed intermediate response to Neomycin (57.1%) and Polymyxin B (28.6%) as it is depicted below in Table 1 and Fig. 1.

Multiple drug resistance phenotypes of *S. aureus:*

In the current study, multiple drug resistance (MDR) phenotypes were determined for *S. aureus*. The results were showed that 92.9% of the tested

Table 1: *In vitro* antimicrobial resistance of pattern of *S. aureus* isolated from milk of bovine.

Antimicrobial agents	Interpretations		
	Sensitive (%)	Intermediate (%)	Resistant (%)
Ampicillin	21.4	7.1	71.4
Amoxicillin	14.3	7.1	78.6
Cloxacillin	14.3	28.6	57.1
Methicillin	57.1	14.3	28.6
Gentamycin	57.1	14.3	28.6
Kanamycin	64.3	14.3	21.4
Neomycin	28.6	57.1	14.3
Streptomycin	71.4	21.4	7.1
Trimethoprim/Sulfamethoxazole	71.4	14.3	14.3
Polymixin B	35.7	28.6	35.7
Chloramphenicol	57.1	28.6	14.3
Tetracycline	14.3	7.1	78.6

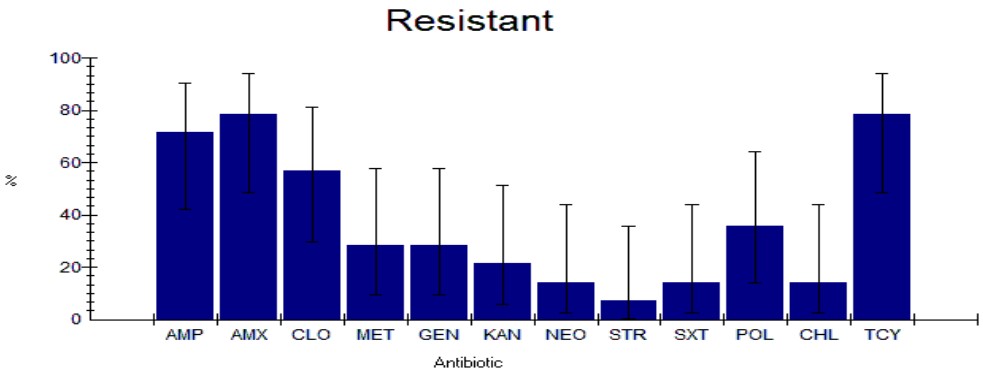

Fig. 1: *In vitro* antimicrobial resistance pattern of *S. aureus* isolated from milk of bovine.

AMP=Ampicillin, AMX=Amoxicillin, CLO=Cloxacillin, MET=Methicillin, GEN=Gentamycin, KAN=Kanamycin, NEO=Neomycin, STR=Streptomycin, SXT=Trimethoprim-sulfamethoxazole, POL=Polymyxin B, CHL=Chloramphenicol, TCY= Tetracycline

isolates were developed a multidrug resistant to more than two antimicrobial agents. The MDR was principally observed to Amoxicillin, Ampicillin, Tetracycline, and Polymixin B. The predominant MDR phenotypes of *S. aureus* (14.3% of the isolates) isolated from this study area were Tetracycline, Polymixin B, Amoxicillin, and Ampicillin (Table 2).

DISCUSSION

The present phenotypic results of *S. aureus* from the five different private dairy farms indicated a prevalence of 5.2%. More or less this finding is closely comparable with the reports of Hussein et al. (1997) who reported 10% prevalence in Addis Ababa, Ethiopia. But it is by far lower than the findings of Bitaw et al. (2010) in Bahir Dar town and its environs, Ethiopia (20.3%), Abebe et al. (2013) around Addis Ababa, Ethiopia (16.2%), Zeryehun et al. (2013) in and around Addis Ababa, Ethiopia (28.8%), Birhanu et al. (2013) in Asella, South Eastern Ethiopia (35.71%), Firaol et al. (2013) in and around Gondar town, North Western Ethiopia (18.44%), Fanta et al. (2013) in urban and peri urban areas of Debre-Zeit, Ethiopia (44%), Sant et al. (2013) in Faizabad, India (31.78%), Kapllan et al. (2013) in Fieri Region in Albania (18%), Purba et al. (2014) in Karnal, North India (73.6%), Forough et al. (2012) in Fars, Chahar Mahalva Bakhtiari and Ghom, provinces, Iran (17.9%), Deresse et al. (2012) in Hawassa area, Ethiopia (48.75%), Pant et al. (2013) from different regions of Dehradun (60%), Alekish et al. (2013) in Northern Jordan (53.4%), Gali et al.

Table 2: In vitro multi drug resistance (MDR) pattern of *S. aureus* isolated from milk of bovine

***S. aureus* Isolates**	**Resistance profile**	**No. of isolates**	**Isolates %**
*S. aureus*1	AMX	1	7.1
*S. aureus*2	TCY AMX AMP	1	7.
*S. aureus*3	TCY POL AMX	1	7.1
*S. aureus*4	KAN POL AMP	1	7.1
*S. aureus*5,6	TCY POL AMX AMP	2	14.3
*S. aureus*7	KAN TCY POL AMX	1	7.1
*S. aureus*8	TCY POL AMX AMP GEN	1	7.1
*S. aureus*9	TCY STR AMX AMP GEN	1	7.1
*S. aureus*10	TCY SXT STR AMP GEN	1	7.1
*S. aureus*11	TCY SXT STR AMX AMP GEN	1	7.1
*S. aureus*12	KAN TCY SXT POL AMX AMP	1	7.1
*S. aureus*13	KAN TCY STR POL AMX AMP GEN	1	7.1
*S. aureus*14	KAN TCY SXT POL AMX AMP GEN	1	7.1

(2014) in Nigeria (12.63%), and Gali et al. (2013) in Zaria and Kaduna, Nigeria (52.42%). These differences in the prevalence of *S. aureus* from the different studies might be due to differences in husbandry practices, awareness and skill of the farm owners, and animal health delivery systems.

The antibiogram profile results of the current study for the tested *S. aureus* indicated as large proportions of the isolates of the study were found to be highly susceptible to Streptomycin (71.4%), Trimethoprim/Sulfamethoxazole (71.4%), Kanamycin (64.3%), Chloramphenicol (57.1%), Gentamycin (57.1%), and Methicillin (57.1%). The reason why these antimicrobials were less resistant might be that they are not frequently used in the study area in veterinary services. However these isolates were highly resistant to Amoxicillin (78.6%), Tetracycline (78.6%), Ampicillin (71.4%), and Cloxacillin (57.1%). But the isolates were showed intermediate response to Neomycin (57.1%) and Polymyxin B (28.6%). In addition, the present *in vitro* antibiotic sensitivity test results indicated that as 92.9% of the tested isolates were developed a multidrug resistant to more than two antimicrobial agents. This finding is almost in agreement with the findings of Deresse et al. (2012) who reported that isolated strains were showed variable degree of resistant to Ampicillin (70.9%), Amoxicillin-Clavulanic acid (30.9%), and Trimethoprim/Sulfamethoxazole (7.7%), Abebe et al. (2013) whose finding revealed the development of resistance to Tetracycline (66.7%), Ampicillin-Clavulanic acid (37.3%), and low level of resistance to Chloramphenicol (23.5%), Trimethoprim/Sulfamethoxazole (21.6%), and Gentamycin (19.6%) and multidrug resistance was also observed in 47.6% of the total isolates, Abera et al. (2013) who reported that *S. aureus* were found to be highly susceptible to Chloramphenicol (100%) followed by Gentamycin (91.7%), Kanamycin (88.9%), and Streptomycin (86.1%), but were highly resistant to Amoxicillin (36.1%), Gali et al. (2014) who reported high frequency of resistance to Tetracycline (85%), Amoxicillin (65%), but susceptible to Chloramphenicol (100%), Trimethoprim/Sulfamethoxazole (90%) and Methicillin (40%), Alekish et al. (2013) who reported high rate of sensitivity to Gentamycin (47.1%) and high rate of resistance among the isolates was against Tetracycline (77.7%), Forough et al. (2012) who reported that most of the isolates (82.6%) were resistant to one or more antimicrobial agent and 13.0% were resistant to single antibiotic and 34.8% showed resistance to 2 antimicrobial agents and the development of resistance to Ampicillin (54.3%), Tetracycline (26.1%), and Trimethoprim/Sulfamethoxazole (17.4%) but all isolates tested for antibiotic sensitivity were susceptible to Methicillin and Chloramphenicol, and Indu & Brinty (2014) who revealed that *S. aureus* isolates were found to be highly susceptible towards Chloramphenicol (71.5%) and Gentamycin (78.58%) and resistance to Ampicillin (50%).

In the current study the pattern of susceptibility and resistance exhibited variations, which might be due to different geographical and environmental conditions. Moreover, this study has demonstrated the existence of alarming levels of resistance of *S. aureus* to commonly used antimicrobial agents in the study farms. These might be due to the fact that prolonged and indiscriminate usage, and frequent prescriptions of particular drugs often leads to possible resistance development in the animals, which were also suggested by other researchers from abroad (Edward et al., 2002; Gentilini, 2000).

The resistance of *S. aureus* to Amoxicillin and Ampicillin may be attributed to the production of beta-lactamase, an enzyme that inactivates penicillin and closely related antibiotics. It is believed that around 50% of mastitis causing *S. aureus* strains produce beta-lactamase (Green & Bradely, 2004). Moreover, Penicillin G and Tetracycline are the most commonly used antimicrobials for the treatment of infection or mastitis in the livestock sector in Ethiopia and Tetracycline is also widely used as growth factors in veterinary medicine for livestock rearing as well in the treatment of bacterial infection occurring in human medicine (Ardic et al., 2005).

In conclusion, the present phenotypic study demonstrated a low level of prevalence (5.2%) of coagulase positive *S. aureus* from the five different private dairy farms which indicated a progressive reduction as compare to the previous reports in the study area. However, the findings obtained from this study unequivocally proved the existence of alarming level of resistance of *S. aureus* to the commonly used antimicrobials like Ampicillin, Amoxicillin, and Tetracycline. This suggests uncontrolled, prolonged and indiscriminate usage and frequent prescriptions of some antimicrobials. The study also indicated the existence of alarming levels of multidrug resistant *S. aureus* in dairy cows. Thus, there is a great risk of transmission of these strains to the consumers and individuals who have contact with animals. Accordingly, there should be a proper pasteurization and fermentation of milk before human consumption, practice of proper hygienic measures in the farms and during milking, intensive public education on proper usage of antimicrobials, shifting in using of the relatively susceptible antimicrobials, controlling guidelines for their legal application and regular surveillance on effectiveness of the commonly prescribed drugs, special attention to the occurrence of multidrug resistance *S. aureus* and eventually genotypic epidemiological studies

should be undertaken in the study area at large in the country.

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REFERENCES

Abebe, M., Daniel, A., Yimtubezinash, Wo., & Genene, T. (2013). 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. *African Journal of Microbiology Research, 7(27),* 3501-3510.

Abera, M., Demie, B., Aragaw, K., Regassa, F., & Regassa A. (2013). Isolation and identification of *Staphylococcus aureus* from bovine mastitic milk and their drug resistance patterns in Adama town, Ethiopia. *African Journal of Dairy Farming and Milk Production, 1(2),* 019-023.

Addis Ababa City Administration (AACA) (1998). Improving urban environmental conditions in Addis Ababa.

Alekish, M.O., Al-Qudah, K. M., & Al-Saleh, A. (2013). Prevalence of antimicrobial resistance among bacterial pathogens isolated from bovine mastitis in Northern Jordan. *Revue de Médecine Vétérinaire, 164(6),* 319-326.

Ardic, N., Ozyurt, M., & Sareyyupoglu, B. (2005). Investigation of erythromycin and tetracycline resistance genes in methicillin-resistant Staphylococci. *International Journal of Antimicrobial Agents, 26,* 213-218.

Atasever, S. (2012). Estimation of correlation between somatic cell count and coagulation score of bovine milk. *International Journal of Agricultural and Biological Engineering, 14,* 315-317.

Ateba, C., Mbewe, M., Moneoang, M.S., & Bezuidenhout, C.C. (2010). Antibiotic-resistant *Staphylococcus aureus* isolated from milk in the Mafikeng Area, North West province, South Africa. *South African Journal of Science, 106,* 12-13.

Bannerman, T. L. (2003). Staphylococcus, micrococcus, and other catalase positive cocci that grow aerobically: In Murray, P. R., Baron, E. J., Jorgensen, J. H., Pfaller, M. A., and Yolken, R.H. (ed.), Manual of Clinical Microbiology, 8th edition', (ASM Press).

Birhanu, A., Diriba, L., & Iyob, I. (2013). Study of bovine mastitis in Asella government dairy farm of Oromia regional state, South Eastern Ethiopia. *International Journal of Current Research and Academic Review, 1(2),* 134-145.

Bitaw, M., Tefera, A., & Tolesa, T. (2010). Study on bovine mastitis in dairy farms of Bahir Dar town and its environs. *Journal of Animal and Veterinary Advances, 9,* 2912-2917.

Bradely, A.J. (2002). Bovine mastitis an evolving disease. *The Veterinary Journal,* 164, 116-128.

Brody, T., Yavatkar, A.S., Lin, Y., Ross, J., Kuzin, A., Kundu, M., Fann, Y., & Odenwald, W.F. (2008). Horizontal gene transfers link a human MRSA pathogen to contagious bovine mastitis bacteria. *PLOS ONE, 3,* 3074.

Chu, C., Yu, C., Lee, Y., & Su, Y. (2012). Genetically divergent methicillin-resistant *Staphylococcus aureus* and sec-dependent mastitis of dairy goats in Taiwan. *BMC Veterinary Research, 8,* 39.

David, M.Z., & Daum, R.S. (2010). Community associated methicillin-resistant *Staphylococcus aureus*: epidemiology and clinical consequences of an emerging epidemic. *Clinical Microbiology Reviews, 23,* 616-687.

De Buyser, M.L. (2001). Implication of milk and milk products in food-borne diseases in France and in different industrialized countries. *International Journal of Food Microbiology, 67,* 1-17.

De Silva-ciombra, M., Silva-carvalho, M.C., Wisplinghoff, H., Hall, G.O., Tallent, S., Wallace, S., Edmond, M.B., Figueredo, A.M., & Wenzel, R.P. (2003). Clonal spread of methicillin-resistant *Staphylococcus aureus* in a large geographic area of the United States. *Journal of Hospital Infection, 53,* 103-110.

Deresse, D., Solomon, G., & Dawit, Y. (2012). Antibiotic-resistance *Staphylococcus aureus* isolated from cow's milk in the Hawassa area, South Ethiopia. *Annals of Clinical Microbiology and Antimicrobials, 11(26),* 1-6.

Edward, M., Anna, K., Michal, K., Henryka, L., & Krystyna, K. (2002). Antimicrobial susceptibility of staphylococci isolated from mastitic cows.

Bulletin of the Veterinary Institute in Pulawy, 46, 289-294.

Enright, M. C. (2003). The evolution of resistant pathogen the case of MRSA. *Current Opinion in Pharmacology, 3,* 474-479.

Ercolini, D. (2004). PCR-based detection of enterotoxigenic *Staphylococcus aureus* in the early stages of raw milk cheese making. *Journal of Applied Microbiology, 96,* 1090-1096.

Esron, D., Karimuebo, E., Lughano, T., Kusiluka, R., Melegela, H., Aglowisye, M., Kapaa, M., & Kalvin, S. (2005). Study on mastitis, milk quality and health risk associated with consumption of milk from pastoral herds in Dodoma and Morgora region, Tanzania. *Journal of Veterinary Science, 6,* 213-221.

Fanta, D., Kohei, M., Akafte, T., & Delia, G. (2013). Contamination of informally marketed bovine milk with *Staphylococcus aureus* in urban and peri urban areas of Debre-Zeit, Ethiopia. *African Journal of Dairy Farming and Milk Production, 1(1),* 008-011.

Firaol, T., Sefinew, A., & Awoke, T. (2013). Aerobic microorganisms isolated from mastitic bovine milk and their antimicrobial susceptibility profiles, Ethiopia. *American-Eurasian Journal of Agricultural & Environmental Sciences, 13(7),* 920-925.

Forough, A., Ebrahim, R., Amir, S., Hassan, M., Majid, R., & Manochehr, M. (2012). Antimicrobial resistance of *Staphylococcus aureus* isolated from bovine, sheep and goat raw milk. *Global Veterinaria, 8(2),* 111-114.

Gali, A. U., Junaid, K., Veronica, J.U., Mohammed, B., & Jacob, K. P. K. (2013). Methicillin-resistant *Staphylococcus aureus* (MRSA) in fresh and fermented milk in Zaria and Kaduna, Nigeria. *International Journal of Drug Research and Technology, 3(3),* 67-75.

Gali, A. U., Junaidu, K., Veronica, J. U., Mohammed, B., & Jacob, K. P. K. (2014). Occurrence of vancomycin-resistant *Staphylococcus aureus* (VRSA) in fresh and fermented milk in Nigeria: A Preliminary Report. *International Journal of Public Health and Epidemiology, 3(8),* 054-058.

Gentilini, E., Denamiel, G., Llarente, P., Godaly, S., Rebuelto, M., & Degregorio, O. (2000). Antimicrobial susceptibility of *Staphylococcus aureus* isolated from bovine mastitis in Argentina. *Journal of Dairy Science, 83,* 1224-1227.

Getahun, K., Kelay, K., Bekana, M., & Lobago, F. (2008). Bovine mastitis and antibiotic resistance patterns in Selalle smallholder dairy farms, Central Ethiopia. *Tropical Animal Health and Production, 40,* 261-286.

Green, M., & Bradely, A. (2004). Clinical forum: *Staphylococcus aureus* mastitis in cattle. *UK VET, 9,* 1-9.

Hundera, S., Ademe, Z., & Sintayehu, A. (2005). Dairy cattle mastitis in and around Sebeta, Ethiopia. *The International Journal of Applied Research in Veterinary Medicine, 3(4),* 1525-1530.

Hussain, R., Javed, M.T., & Khan, A. (2012a). Changes in some biochemical parameters and somatic cell counts in the milk of buffalo and cattle suffering from mastitis. *Pakistan Veterinary Journal, 32,* 418-421.

Hussain, R., Javed, M.T., Khan, A., Mahmood, F., & Kausar, R. (2012b). Mastitis and associated histo-pathological consequences in the context of udder morphology. *International Journal of Agricultural and Biological Engineering, 14,* 947-952.

Hussein, N., Yehualashet, T., & Tilahuan, G. (1997). Prevalence of mastitis in different local and exotic breeds of milking cows, Ethiopia. *Journal of Agricultural Science, 16,* 53-60.

Indu, S., & Brinty, A. (2014). Isolation and identification of *Staphylococcus aureus* from bovine mastitis milk and their drug resistance patterns in Silchar town dairy farms, N.E India. *Online International Interdisciplinary Research Journal, 4,* 256-260.

Kalorey, D.R, Shanmugam, Y., Kurkure, N.V., Chousalkar, K.K., & Barbuddhe, S.B. (2007). PCR-based detection of genes encoding virulence determinants in *Staphylococcus aureus* from bovine subclinical mastitis cases. *Journal of Veterinary Science, 8,* 151-154.

Kapllan, S., Jorida, T., Renata, K., Kastriot, K., Sokol, D., Fejzo, S., Ilirian, K., & Bejo, B. (2013). Incidence of coagulase positive *Staphylococcus aureus* in raw cow milk produced by cattle farms in Fieri Region in Albania. *Journal of Life Sciences, 7(4),* 390-394.

Kirby, W. M., Bauer, A. W., Sherris, J. C., & Turck, M. (1966). Antibiotic susceptibility testing by a standard single disc method. *American Journal of Clinical Pathology, 45,* 493-496.

Le Marechal, C., Thiery, R., & Leloir, Y. (2011). Mastitis impact on technologie proprerties of milk and quality of milk products- review. *Dairy* Science *& Technology, 91,* 247-282.

Leonard, F.C., & Markey, B.K. (2008). Meticillin-resistant *Staphylococcus aureus* in animals: A review. *The Veterinary Journal, 175,* 27-36.

Lowy, F.D. (1998). *Staphylococcus aureus* infection. *The New England Journal of Medicine, 339,* 520-532.

Mekonnen, H., Workineh, S., Bayleyegne, M., Moges, A., & Tadele, K. (2005). Antimicrobial susceptibility profile of mastitis isolates from cows in three major Ethiopian dairies. *Revue de Médecine Vétérinaire, 176(7),* 391-394.

Momtaz, H., Rahimi, E., & Tajbakhsh, E. (2010). Detection of some virulence factors in *Staphylococcus aureus* isolated from clinical and subclinical bovine mastitis in Iran. *African Journal of Biotechnology, 9,* 3753-3758.

National Committee for Clinical Laboratory Standards (NCCLS) (2012). Performance standards for antimicrobial susceptibility testing, 7th informational supplement, Approved standard M100-S21.

Ochoa-Zarzosa, A., Loeza-Lara, P., Torres-Rodrı´guez, F., Loeza-Angeles, H., Mascot-Chiquito, N., Sanchez-Baca, S., & Lopez-Meza, J.E. (2008). Antimicrobial susceptibility and invasive ability of *Staphylococcus aureus* isolates from mastitis from dairy backyard systems. *Antonie van Leeuwenhoek, 94,* 199-206.

Palomares, C. (2003). Rapid detection and identification of *Staphylococcus aureus* from blood culture specimens using real time fluorescence PCR. *Diagnostic Microbiology and Infectious Disease, 45,* 183-189.

Pant, R., Nirwal, S., & Rai, N. (2013). Prevalence of antibiotic resistant bacteria and analysis of microbial quality of raw milk samples collected from different regions of Dehradun. *International Journal of PharmTech Research, 5 (2),* 804-810.

Piepers, P., De Meulemeester, L., De Kruif, A., Opsomer, G., Barkema, H.W., & Vliegher, S. (2007). Prevalence and distribution of mastitis pathogens sub-clinically infected dairy cows in Flanders, Belgium. *Journal of Dairy Research, 74,* 478-483.

Purba, S., Debasish, M., Sachinandan, D., & Chanchal, D. (2014). *Staphylococcus aureus* in dairy animals and farm workers in a closed herd in Karnal, North India: Assessment of prevalence rate and *CoA* variations. *International Journal of Innovative Research in Science, Engineering and Technology, 3(4),* 10962-10972.

Quinn, P. J., Markey, B. K., Carter, M. E., Donnelly, W. J. C., Leonard, F. C., & Maguire, D. (2002). Veterinary Microbiology and Microbial Disease, 1stedition', (Blackwell Science Ltd., UK).

Radostitis, O.M., Blood, D.C., & Gay, C.C. (1994). Veterinary Medicine: A Text Book of the Diseases of Cattle, Sheep, Pigs, Goats and Horses, 8th edition', (Bailliere Tindall, London).

Sant, P.T., Rajesh, K.J., & Namita, J. (2013). Characterization and antimicrobial sensitivity of *Staphylococcus aureus* isolates from subclinical bovine mastitis. *Journal of Animal Health and Production, 1(2),* 20-23.

Seki, K., Sakurada, J., Seong, H.K., Murai, M., Tachi, H., Ishii, H., & Masuda, S. (1998). Occurrence of coagulase serotype among *Staphylococcus aureus* strains isolated from healthy individuals-special reference to correlation with size of protein-A gene. *Microbiology and Immunology, 42,* 407-409.

Statistical Package for Social Science, Inc. (SPSS) (2002). SPSS for window (Version 11.5) SPSS, Chicago, IL, USA.

Thirapatsakun, T. (1999). Mastitis management. In: Small Holder Dairying in the Tropics'. In: Hunt and Chantalakhana (eds). ILRI, Kenya, Nairobi, 299-339.

Zadoks, R.W., Van Leeuwen, H., Barkema, O., Sampimon, H., Verbrugh,Y., Schukken, H., & Van Belkum, A. (2000). Application of pulsed-field gel electrophoresis and binary typing as tools in veterinary clinical microbiology and molecular epidemiologic analysis of bovine and human *Staphylococcus aureus* isolates. *Journal of Clinical Microbiology, 38,* 1931-1939.

Zeryehun, T., Aya, T., & Bayecha, R. (2013). Study on prevalence, bacterial pathogens and associated risk factors of bovine mastitis in small holder dairy farms in and around Addis Ababa, Ethiopia. *The Journal of Animal & Plant Sciences, 23(1),* 50-55.