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Authors: Tesfaye, Kaleab, Gizaw, Zemichael, and Haile, Aklilu Feleke

Source: Environmental Health Insights, 15(1)

Published By: SAGE Publishing

URL: https://doi.org/10.1177/11786302211021297

Prevalence of Mastitis and Phenotypic Characterization of Methicillin-Resistant *Staphylococcus aureus* in Lactating Dairy Cows of Selected Dairy Farms in and Around Adama Town, Central Ethiopia

Environmental Health Insights
Volume 15: 1–8

DOI: 10.1177/11786302211021297

Kaleab Tesfaye[1], Zemichael Gizaw[2] and Aklilu Feleke Haile[3]

[1]College of Veterinary Medicine and Agriculture, Addis Ababa University, Addis Ababa, Ethiopia. [2]Department of Environmental and Occupational Health and Safety, Institute of Public Health, College of Medicine and Health Sciences, University of Gondar, Gondar, Ethiopia. [3]Aklilu Lemma Institute of Pathobiology, Addis Ababa University, Addis Ababa, Ethiopia.

ABSTRACT

BACKGROUND: The emergence of Methicillin resistant *Staphylococcus aureus* (MRSA) poses a serious public health threat. Strains of *Staphylococcus aureus* resistant to β-lactam antibiotics are known as MRSA. MRSA has gained attention as community pathogen. MRSA has been increasingly reported as emerging problem in veterinary medicine. However, little is known in Ethiopia. This study was, therefore, conducted to identify MRSA, to determine its drug susceptibility patterns, and mastitis infection in dairy cattle in and around Adama town, Central Ethiopia.

METHODS: A cross-sectional study was conducted to estimate the occurrence of MRSA in mastitic dairy cows in and around Adama town, central Ethiopia. A total of 384 lactating cows were included from the conveniently selected dairy farms in the study area. Approximately 10 ml of milk was aseptically collected from clinical and subclinical mastitic cows into sterile universal bottles after discarding the first 3 milking streams. Then, *Staphylococcus aureus* was isolated using the conventional bacteriological procedure. Resistance to methicillin was detected using the Kirby-Bauer disc diffusion antibiotic susceptibility method. Oxacillin disc was used to detect methicillin resistant *Staphylococcus aureus* strains. Antimicrobial susceptibility test was conducted against MRSA strains using streptomycin (S, 10 µg), amoxicillin (Am, 25 µg), kanamycin (k, 30 µg), nalidixic acid (NA, 30 µg), oxytetracycline (OT, 30 µg) sulphonamide (S, 300 µg) and ceftriazole (CRO, 30 µg).

RESULTS: The study found that the prevalence of mastitis was 121(31.5%). Among this 37(30.6%) were clinical mastitis and 84 (69.4%) of them were sub-clinical mastitis. Of 121 mastitis cases, *Staphylococcus aureus* was isolated in 37 (30.6%) of mastitic cow milk samples. The prevalence of mastitis was significantly affected by breed, age, floor type and hygienic status of the milkers ($P < .05$). Moreover, 32.4% of *Staphylococcus aureus* isolates were resistant to oxacillin. A total of 75% percent of MRSA isolates were resistant to amoxicillin, 66.7% were resistant to oxytetracycline, and 50% were resistant to sulphonamide. However, 75% of MRSA isolates were susceptible to kanamycin, 58.3% were susceptible to streptomycin, and 50% were susceptible to nalidixic acid.

CONCLUSION: The study revealed that relatively high number of strains are resistant to the antibiotics commonly used in the therapeutic protocol of many human and animal infections. Therefore, antimicrobial susceptibility test should be carried out at a regular basis and proper hygienic practices should be introduced at farm level. Creating public awareness about transmission, prevention and control of MRSA should also be considered.

KEYWORDS: Methicillin resistant, *Staphylococcus aureus*, mastitis infection, dairy cattle, Adama town

RECEIVED: March 20, 2021. **ACCEPTED:** May 11, 2021.

TYPE: Original Research

FUNDING: The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported by Addis Ababa University-Thematic research project "Pneumonia, diarrhea and mastitis in food animals: Host immunity, diversity of pathogens and antibiotics resistance, impact on food safety and trade, and intervention strategies".

DECLARATION OF CONFLICTING INTERESTS: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

CORRESPONDING AUTHOR: Zemichael Gizaw, Department of Environmental and Occupational Health and Safety, Institute of Public Health, College of Medicine and Health Sciences, University of Gondar, Gondar, Ethiopia. Email: zemichael12@gmail.com

Background

Milk produced from dairy cows is an important dietary source. Development of the dairy sector in Ethiopia can contribute significantly to poverty alleviation and nutrition in the country.[1] Nevertheless the quality and quantity of milk in the country deteriorates due to various reasons.[2] Mastitis occurs worldwide among dairy animals and it has been described to have an extreme zoonotic and economic impact.[3] Mastitis can be defined as clinical or subclinical and it is a complex and multi factorial disease. The occurrence of which depends on variables related to the animal, environment and pathogen.[4]

Mastitis can cause devastating effects to farmers because of a serious economic losses and a danger that the bacterial contamination of milk from affected cows may render it unsuitable for human consumption.[5] The economic impact of bovine mastitis is due to reduced milk production, cost of treatments and culling. However, the economic impact of mastitis varies and should be calculated at the farm or herd level and depends

on local, regional, epidemiological, managerial and economic conditions.[6-8] In developing countries, the economic impact of mastitis in small- and medium-sized farms varies according to the level of milk production per cow and the intensity of the production systems.[6] For instance, in Ethiopian crossbreed dairy systems, milk production was reduced by 1.2%, 6.3%, and 33%, respectively, in quarters with California mastitis test scores 1+, 2+, and 3+.[9] The estimates of the financial losses ranged from US$29.1 to US$66.6. A total loss of US$38 was estimated for each cow per lactation. Reducing mastitis could reduce the loss by US$35.[9]

Staphylococcus aureus is one of the major causes of bovine mastitis worldwide.[10] Many *Staphylococcus* strains are able to produce an extracellular exopolysacaride layer surrounding the cell wall. This capsular structure and production of slime have been associated with virulence against host defence mechanism. Various virulence factors operate together in the pathogenic process of *Staphylococcus aureus.* The broad range of infections caused by *Staphylococcus aureus* is related to a number of virulence factors that allow it to adhere to surface, invade or avoid the immune system, and cause harmful toxic effects to the host.[11,12] The virulence of *Staphylococcus aureus* is generally considered to be multifactorial and due to the combined action of several virulence determinants. One exception is the toxinoses, such as toxin shock syndrome and staphylococcal food poisoning, which are caused by toxic shock syndrome toxin, exfoliative toxins A and B, and different staphylococcal enterotoxins.[11]

The pathogenicity of *Staphylococcus aureus* is a complex process involving a diverse array of extracellular and cell wall components that are coordinately expressed during different stages of infection (ie, colonization, avoidance of host defence, growth and cell division and bacterial spread).[13,14] The coordinated expression of diverse virulence factors in response to environmental cues during infections (eg, expression of adhesins early during colonization versus production of toxins late in infection to facilitate tissue spread) hints at the existence of global regulators in which a single regulatory determinant controls the expression of many unlinked target genes.[15] These regulators help bacteria to adapt to a hostile environment by producing factors enabling the bacteria to survive and subsequently to cause infection at the appropriate time.

Antibiotics are used both for therapeutic and sub-therapeutic purpose to enhance feed efficiency and promote growth of animals in veterinary medicine. There is increasing public and scientific concern regarding extensive use of antimicrobials for therapeutic purpose or as growth promoters in food animals, due to the emergence and dissemination of multiple antibiotic resistant zoonotic bacterial pathogens.[16] The multidrug resistance has been increased globally that is considered a public health threat. Several previous investigations revealed the emergence of multidrug-resistant bacterial pathogens that could be transmitted to humans either by food chain or direct contact with the infected animal.[17-21] Antimicrobial resistance is a global public problem. The rate of mortality is high in people with antimicrobial resistant bacteria. For instance, people with *MRSA* infections are 64% more likely to die than people with drug-sensitive infections.[22] Each year, an estimated 23 000 deaths in the United States and 25 000 deaths in the European Union are extra deaths caused by bacteria resistant to antibiotics.[23]

Soon after the introduction of penicillin, around 1945, the majority of the *Staphylococcus aureus* population had become resistant to penicillin through the production of beta-lactamase, an enzyme that hydrolyzes penicillin. In the late 1950s, the beta-lactamase resistant Methicillin was introduced in human medicine. However, soon after its introduction, isolation of MRSA was reported.[24] Resistance of *Staphylococcus aureus* to antimicrobial agents can complicate treatment of its infections.[25] Methicillin resistance is caused by the acquisition of the *mec*A gene. This gene encodes an alternative penicillin-binding protein, called PBP2A, which has a low affinity for beta-lactam antibiotics.[26] The *mec*A gene is part of a large mobile genetic element called Staphylococcal Cassette Chromosome *mec* (SCC*mec*). MRSA are often multidrug resistant.[27]

The emergence of MRSA poses a serious public health threat. Strains of *Staphylococcus aureus* resistant to β-lactam antibiotics are known as MRSA.[28] First described as a cause of nosocomial infection in hospital settings, now MRSA has gained attention as community pathogen.[29] In recent years, MRSA has been increasingly reported as emerging problem in veterinary medicine. MRSA has been isolated from cattle, dogs, cats, pigs, horses and poultry worldwide.[30] However, little is known in Ethiopia. This study was, therefore, conducted to determine the occurrence of clinical and subclinical dairy cow mastitis in and around Adama town, central Ethiopia, to isolate and identify MRSA, and to determine its antimicrobial susceptibility of MRSA.

Methods

Study design and setting

A cross-sectional study involving microbiological analysis was employed from January to April 2015 to identify MRSA from mastitic dairy cows in and around Adama, central Ethiopia. Adama is one of the largest and most populated towns in Oromia National Regional State. It is about 100 km away from Addis Ababa in southeast direction at an altitude of 1650 m above sea level. Its annual temperature ranges from 13.9°C to −29°C. It is located at 8°33′35″N – 8°36′46″ N latitude and 39° 11′57″ E – 39°21′15″ E longitude.[31]

Study population

All holstein crossbreed and local zebu breed lactating dairy cows of large-scale and small-holder dairy farms found in and around Adama were considered as the study population.

Table 1. Interpretation of CMT results.

CMT SCORE	INTERPRETATION	VISIBLE REACTION
0	Negative	Milk fluid and normal
±	Trace	Slight precipitation
1	Weak positive	Distinct precipitation and no gel formation
2	Distinct positive	Mixture thickness with a gel formation
3	Strong	Viscosity greatly increased

Sample size determination sampling method

The sample size was calculated according to a method suggested by Thrusfield[32] from the expected prevalence of 50% because there was no similar studies in the area with defined precision of 5% and level of confidence of 95%.

$$n = \frac{(Z\alpha/2)^2 (P_{exp}(1 - P_{exp})}{d^2}$$

Where n = required sample size; P_{exp} = expected prevalence and a desired absolute precision (d) of 0.05, $Z = 1.96$. Therefore, a total of 384 dairy cows from the conveniently selected dairy farms were included in this study.

Sample collection

Milk samples were collected before milking and the teats were washed with tap water, dried and disinfected with cotton wool moistened with 70% ethyl alcohol. The first few streaks of milk were discarded and about 5 to 20 ml of milk collected in a sterile universal bottle. The samples were kept in ice box and transported to microbiology laboratory of the College of Veterinary Medicine and Agriculture, Addis Ababa University for examination. The samples were stored at +4°C until processed and examined next day.

California mastitis test (CMT)

California mastitis test was carried out using the methods described by Schalm et al.[33] and Quinn.[5] Briefly equal volume of commercial CMT reagent and milk were mixed in the cups of a plastic paddle by a horizontal swirling motion. Negative samples were identified as absence of gel formation; positive samples showed various degree of gel when the CMT reagent reacted with the nucleus of leukocytes which was a reflection of the number of inflammatory markers in the milk and thereby the degree of inflammation. The interpretation of the result was done as described by Quinn[5] (Table 1). The test has degrees like, negative (0) and trace (±) were considered as negative and different intensities of degree (1, 2 and 3) were considered as positive.

Isolation of Staphylococcus aureus

The milk sample were streaked aseptically onto sterile blood agar plates enriched with 7% heparinized sheep blood and incubated at 37°C for 24 to 48 hours under aerobic culture conditions. The plates were examined for the presence of *Staphylococcus* colonies. Isolates supposed to belong to *Staphylococcus* species on the basis of their morphological aspects (creamy, greyish, white or yellow colonies) and haemolytic pattern on the surface of blood agar plates were collected. Presumed staphylococcal colonies were then sub-cultured on nutrient agar plates and incubated at 37°C for 24 to 48 hours to get a pure culture.[5]

Identification of Staphylococcus aureus

Final identification of staphylococci organisms and species assignment were done based on Gram staining, catalase test, sugar fermentation and coagulase test.[34]

Gram's staining

All suspected cultures of *Staphylococcus* species were subjected to Gram's stain and observed under a light microscope for ram's reaction, size, and shape and cell arrangements. The Gram-stained smears from typical colonies that showed Gram-positive cocci occurring in bunched, grapelike irregular clusters were taken as presumptive *Staphylococcus* species.[35]

Catalase test

Pure culture of the isolates were picked using a sterile loop from the agar slant and mixed with a drop of 3% H2O2 on a clean glass slide. If the organism was positive, bubbles of oxygen were liberated within a few seconds and the catalase negative isolates did not produce bubbles. The catalase positive cocci were considered as staphylococci.[35]

Oxidation and fermentation test

The medium was green and if an acid is produced by the bacterium, as a result of glucose utilization, the medium become

yellow. Bacteria that can metabolize glucose under either aerobic or anaerobic condition are said to be facultative anaerobes and this is the feature of *Staphylococcus*.[5]

Mannitol salt agar

The colonies that were identified by Gram-staining reaction and catalase test and O-F test as *Staphylococcus* were streaked on MSA plates and incubated at 37°C and examined after 24 to 48 hours for growth and change in the colour of the medium. The presence of growth and change of pH in the media (red to yellow colour) was regarded as confirmative identification of staphylococci.[35]

Coagulase test

The tube coagulase test was performed in sterile tubes by adding 0.5 ml of selected isolates of *Staphylococcus* grown on Tryptone soya broth (TSB) at 37°C for 24 hours to 0.5 ml of citrated rabbit plasma. After mixing by gentle rotation, the tubes were incubated at 37°C along with a negative control tube containing a mixture of 0.5 ml of sterile TSB and 0.5 ml of rabbit plasma. Clotting was evaluated at 30 minutes intervals for the first 4 hours of the test and then after 24 hours incubation. The reaction was considered positive, if any degree of clotting from a loose clot to a solid clot that is immovable when the tube is inverted (tilted) and no degree of clotting would be taken as negative.[5]

Purple agar base

Purple agar base (PAB) with the addition of 1% maltose was used to differentiate the pathogenic staphylococci, particularly the coagulase-positive isolates. The suspected culture was inoculated on PAB media plate with1% of maltose and incubated at 37°C for 24 to 48 hours. The identification was based on the fact that *Staphylococcus aureus* rapidly ferment maltose and the acid metabolic products cause the pH indicator (bromocresol purple) to change the medium and colonies to yellow.[35]

Identification of Methicillin-resistant Staphylococcus aureus

Oxacillin disc was used to detect MRSA. Resistance to methicillin was detected using the Kirby-Bauer disc diffusion antibiotic susceptibility method. The test was performed using 1 µg oxacillin antibiotic discs on Mueller-Hinton agar plate. The results were interpreted by measuring the zone diameters with the aid of sliding calipers, including the disc diameter.[36]

Antimicrobial susceptibility test

Antimicrobial susceptibility test was conducted against MRSA strains using the following antimicrobial disks with their corresponding concentration: streptomycin (S, 10 µg), amoxicillin (Am, 25 µg), kanamycin (k, 30 µg), nalidixic acid (NA, 30 µg), oxytetracycline (OT, 30 µg) sulphonamide (S, 300 µg) and ceftriazole (CRO, 30 µg). It was also done using the Kirby-Bauer disc diffusion antibiotic susceptibility method according to the guidelines of the CLSI[37] to determine the antibiogram of the MRSA strains.

Data collection and analysis

The collected data about breed, age, lactation stage, hygienic status of the milkers and floor type of the dairy house were entered to excel spread sheet program and transferred to statistical package for social sciences (SPSS) version 20 for analysis. The age of the study animals was determined from birth records and categorized as <4 years, 4 to 7 years, and > 7 years. Lactation stage was classified as early (< 3 m), medium (3-6 m) and late (>6 m); hand washing before milking was recorded as yes or no; and the housing condition was categorized as good (house with concrete floor) and poor (house with muddy floor). Categorical variables were compared using the Chi-squared test and significant level was determined at 95% confidence interval and *P*-values <.05.

Results

From a total 384 animals included in this study, 121 (31.5%) cows had mastitis. Among this 37(30.6%) were clinical mastitis and 84(69.4%) of them were sub clinical mastitis. The prevalence of mastitis showed significant variation among different age groups (*P*= .00), breed (*P*= .00), and the prevalence of mastitis was significantly higher in late lactation stages (*P*= .03). Moreover, milkers hygiene (*P*= .00) and floor type (*P*= .00) were significantly influenced the prevalence of bovine mastitis. Generally, the prevalence of mastitis was significantly higher in holstein friesian cross breed cows (43.5%), old age (>7 years) cows (51.2%), late lactation stage (39.9%), muddy floor type (40.9%), and in those farms with poor milkers hygiene (49.2%) (Table 2).

From the 121 mastitic dairy cow milk samples, *Staphylococcus aureus* was identified in 37(30.6%) samples. From a total of thirty-seven *Staphylococcus aureus* isolates, 12 (32.4%) isolates were identified as MRSA strains. Regarding to drug susceptibility test, MRSA strains were highly resistant to amoxicillin (75%) and oxytetracycline (66.7%) (Table 3). Of the twelve MRSA isolates, 5 (41.7 %) were found to be resistant to 2 or more than 2 antimicrobials tested (Figure 1).

Discussion

The prevalence of mastitis in lactating cows in and around Adama town was found to be 31.5%. This finding was in agreement with a study in Hawassa town, 34.3%.[38] However, the present finding was lower than the prevalence around Adama, 46.7%[39] and around Sebeta, 52.8%.[40] This variability in prevalence of mastitis between different reports could be attributed to differences in farm management practices or differences in

Table 2. The association of potential intrinsic and extrinsic risk factors with mastitis (n = 384) in Adama town, central Ethiopia.

RISK FACTORS	NO OF EXAMINED	NO OF AFFECTED	PREVALENCE (%)	X^2 (*P* VALUE)
Breed				
Borena	170	28	16.5	31.97 (*P* = .00)
HF cross breed	214	93	43.5	
Age				
<4	137	27	19.7	17.69 (*P* = 0.00)
4-7	206	73	35.4	
>7	41	21	51.2	
Lactation stage				
<3	79	19	24.1	4.67 (*P* = 0.09)
3-6	222	69	31.1	
>6	83	33	39.8	
Floor type				
Muddy	220	90	40.9	21.085 (*P* = .00)
Concrete	164	31	18.9	
Hand washing				
No	185	91	49.2	51.698 (*P* = .00)
Yes	199	30	15.1	

Table 3. Antimicrobial susceptibility pattern of MRSA isolates (n = 12).

ANTIMICROBIAL USED	SENSITIVE (NO. (%))	INTERMEDIATE (NO. (%))	RESISTANT (NO. (%))
Amoxicillin	3 (25)	0 (0)	9 (75)
Ceftriazole	5 (41.67)	3 (25)	4 (33.33)
Kanamycin	9 (75)	3 (25)	0 (0)
Nalidixic acid	6 (50)	1 (8.33)	5 (41.6)
Oxytetracycline	2 (16.67)	2 (16.67)	8 (66.67)
Sulphonamide	4 (33.33)	2 (16.67)	6 (50)
Streptomycin (S)	7 (58.33)	1 (8.33)	4 (33.33)

study methods and instruments employed by the investigators. The prevalence of subclinical mastitis in this study was 69.4 % which is relatively closer with the findings in and around Addis Ababa, 55.1%[41] and in Gondar, 56%.[42] The present finding was higher than the prevalence reported in and around Sebeta, 36.67%,[40] in 2 major Ethiopian dairies, 38.2%[43] and in central Ethiopia, 22.3%.[44] In this study similar to previous studies,[38,43] the overall prevalence of clinical mastitis is lower than subclinical mastitis.

In the current study the rate of sub-clinical mastitis (34.5%) was higher than that of the clinical mastitis (17.2%). This is in agreement with a study in central Ethiopia.[40] This variation in prevalence between subclinical and clinical mastitis may be due to the fact that, the defence mechanism of the udder reduces the severity of the disease. In Ethiopia, the subclinical form of mastitis was neglected and efforts have been concentrated on the treatment of clinical cases.[45]

The increasing prevalence of mastitis with increasing age reported in this study is in agreement with the findings of a study in Adama town.[39] The finding of a high prevalence of mastitis in houses with muddy floors when compared with concrete floor types ($P < .05$) showed that the prevalence of mastitis is strongly associated with the bedding type of the farm. This can be justified as milker's hand and the

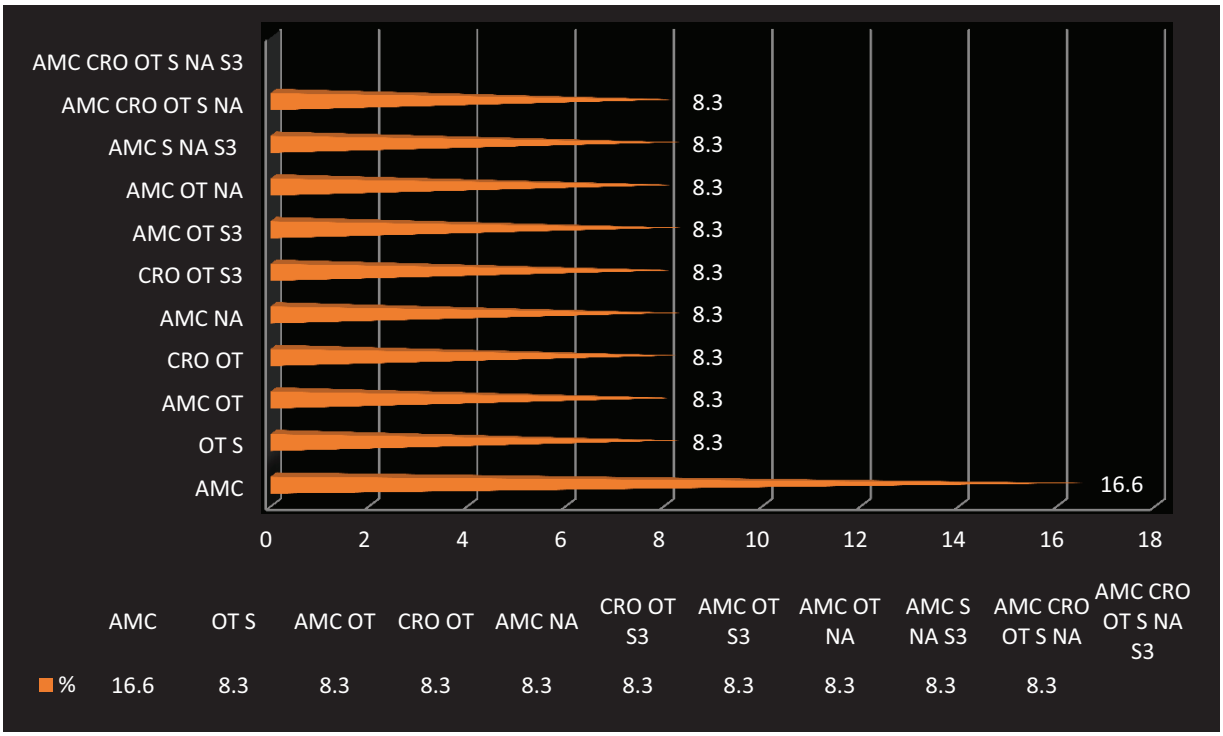

Figure 1. Antimicrobial resistance pattern of MRSA isolates.
The percentages of the phenotypes were calculated by dividing the number of the particular phenotype by the total number of antibiotic resistant isolates identified in a given area. Of the 12 MRSA isolates 5 of them shows resistant to more than 2 drugs (total MDR=41.65%).

environment (floor) in which the cows are kept are the main source of the infection.[46]

From 121 mastitic cow milk samples subjected to bacteriological examination, *Staphylococcus aureus* was isolated in 37 (30.6%) samples. This finding was comparable with a study in Adama, 32%[47] and in Jamaica, 27%.[48] The present finding was lower than the report of Hundera et al. (2005) (44.4%).[40] However, it was higher than the reports made by Hussein (1999) (10.6%).[49] The relative high prevalence of *Staphylococcus aureus* in this study could be associated with lack of effective udder and hand washing before milking, use of separate clothes for drying teats and disinfection of milking areas. The high prevalence of *Staphylococcus aureus* can be due to wide distribution of the organism inside mammary glands and has adapted to survive in the udder and establish chronic and subclinical infections.[46]

The results of this study showed that 32.4% of the isolates were MRSA. This result was lower than the report in Sothern Ethiopia, 60.3%.[50] However, this result was in agreement with a study in France, 34.7%.[51] This might be due to the fact that resistance to Methicillin in these isolates was not only due to the *mec*A gene since other modes of resistance to Methicillin such as the presence or over expression of ß-lactamase enzymes and chromosomal mutations have been documented.[52,53] The differences in the antimicrobial resistance rates to the various drugs observed might reflect a difference in regional antimicrobial usage and subsequent epidemiology due to inappropriate use of antibiotics in some regions. The choice of antibiotic for therapy of MRSA infections is usually complicated.

In this study high proportion of MRSA isolates were resistant to amoxicillin which was in agreement with findings of another study.[54] Consequently, penicillin and other ß-lactam antibiotics are not used in the therapy of MRSA infections. The most important resistance mechanism to ß-lactamase antibiotics is production of the ß-lactamase which inactivates ß-lactamase antibiotics (e.g. Amoxicillin) by hydrolyzing the ß-lactam ring. In our study, we employed the Amoxicillin disc diffusion zone edge test to detect ß-lactamase production and most of the MRSA isolates were positive for ß-lactamase production. Next to amoxicillin MRSA isolates were resistant to oxytetracycline (66.67%). This is due to indiscriminate use of antibiotics/anti-microbial agents for prophylactic as well as other therapeutic purpose. In our study, most of the MRSA strains were multi-drug resistant. This result was in agreement with global finding of MRSA strains are being multi-drug resistant.[55,56]

Conclusion

The study revealed that relatively high number of strains are resistant to the antibiotics commonly used in the therapeutic protocol of many human and animal infections. Therefore, antimicrobial susceptibility test should be carried out at a regular basis and proper hygienic practices should be introduced at farm level. Creating public awareness about transmission, prevention and control of MRSA should also be considered.

Acknowledgements

Authors acknowledged data collectors and supervisors for their participation during data collection. Authors also acknowledged Addis Ababa University for funding the research project.

Author Contributions

HT and AHF actively participated during conception of the research issue, development of a research proposal, data collection, analysis and interpretation, and writing various parts of the research report. ZG reviewed and controlled the quality issues of the research and prepared the manuscript. All the authors read and approved the final manuscript.

Availability of Data and Material

Data will be made available upon requesting the primary author.

Ethics Approval

The ethical and methodological aspects of this research was approved by the College of Veterinary Medicine and Agriculture, Addis Ababa University.

Consent Publication

This manuscript does not contain any individual person's data.

ORCID iD

Zemichael Gizaw https://orcid.org/0000-0002-6713-1975

REFERENCES

1. Ahmed MA, Ehui S, Assefa Y. *Dairy Development in Ethiopia*. International Food Policy Research Institute; 2004.
2. Bradley AJ. Bovine mastitis: an evolving disease. *Vet J*. 2002;164:116-128.
3. Al-Majali AM, Al-Qudah KM, Al-Tarazi YH, Al-Rawashdeh OF. Risk factors associated with camel brucellosis in Jordan. *Trop Anim Health Prod*. 2008;40:193-200.
4. Radostits O, Blood D, Gay C. *A Text Book of Diseases of Cattle Sheep, Pig, Goats and Horses*. 10th ed. Bailliere Tindall; 2007.
5. Quinn PJ. *Clinical Veterinary Microbiology*. Wolf/Mosby; 1994.
6. Romero J, Benavides E, Meza C. Assessing financial impacts of subclinical mastitis on Colombian dairy farms. *Front Vet Sci*. 2018;5:273.
7. Seegers H, Fourichon C, Beaudeau F. Production effects related to mastitis and mastitis economics in dairy cattle herds. *Vet Res*. 2003;34:475-491.
8. Petrovski K, Trajcev M, Buneski G. A review of the factors affecting the costs of bovine mastitis. *J S Afr Vet Assoc*. 2006;77:52-60.
9. Mungube E, Tenhagen B-A, Regassa F, et al. Reduced milk production in udder quarters with subclinical mastitis and associated economic losses in crossbred dairy cows in Ethiopia. *Trop Anim Health Prod*. 2005;37:503-512.
10. Piepers S, De Meulemeester L, de Kruif A, Opsomer G, Barkema HW, De Vliegher S. Prevalence and distribution of mastitis pathogens in subclinically infected dairy cows in Flanders, Belgium. *J Dairy Res*. 2007;74:478.
11. Lowy FD. *Staphylococcus aureus* infections. *N Engl J Med*. 1998;339:520-532.
12. Holmes A, Ganner M, McGuane S, Pitt T, Cookson B, Kearns A. *Staphylococcus aureus* isolates carrying Panton-Valentine leucocidin genes in England and Wales: frequency, characterization, and association with clinical disease. *J Clin Microbiol*. 2005;43:2384-2390.
13. Novick RP, Jiang D. The staphylococcal saeRS system coordinates environmental signals with agr quorum sensing. *Microbiology*. 2003;149:2709-2717.
14. Torres VJ, Attia AS, Mason WJ, et al. *Staphylococcus aureus* fur regulates the expression of virulence factors that contribute to the pathogenesis of pneumonia. *Infect Immun*. 2010;78:1618-1628.
15. Cheung AL, Bayer AS, Zhang G, Gresham H, Xiong Y-Q. Regulation of virulence determinants in vitro and in vivo in *Staphylococcus aureus*. *FEMS Immunol Med Microbiol*. 2004;40:1-9.
16. Normanno G, Corrente M, La Salandra G, et al. Methicillin-resistant *Staphylococcus aureus* (MRSA) in foods of animal origin product in Italy. *Int J Food Microbiol*. 2007;117:219-222.
17. Abolghait SK, Fathi AG, Youssef FM, Algammal AM. Methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from chicken meat and giblets often produces staphylococcal enterotoxin B (SEB) in non-refrigerated raw chicken livers. *Int J Food Microbiol*. 2020;328:108669.
18. Algammal AM, El-Kholy AW, Riad EM, et al. Genes encoding the virulence and the antimicrobial resistance in enterotoxigenic and shiga-toxigenic *E. coli* isolated from diarrheic calves. *Toxins*. 2020;12:383.
19. Enany ME, Algammal AM. The occurrence of the multidrug resistance (MDR) and the prevalence of virulence genes and QACs resistance genes in *E. coli* isolated from environmental and avian sources. *AMB Express*. 2019;9:192.
20. Algammal AM, El-Sayed ME, Youssef FM, et al. Prevalence, the antibiogram and the frequency of virulence genes of the most predominant bacterial pathogens incriminated in calf pneumonia. *AMB Express*. 2020;10:99.
21. El-Sayed M, Algammal A, Abouel-Atta M, Mabrok M, Emam A. Pathogenicity, genetic typing, and antibiotic sensitivity of *Vibrio alginolyticus* isolated from *Oreochromis niloticus* and *Tilapia zillii*. *Rev Med Vet*. 2019;170:80-86.
22. World Health Organization. Antimicrobial resistance. 2020. Accessed April 22, 2021. https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance.
23. Lim C, Takahashi E, Hongsuwan M, et al. Epidemiology and burden of multidrug-resistant bacterial infection in a developing country. *Elife*. 2016;5:e18082.
24. Robinson DA, Enright MC. Evolutionary models of the emergence of methicillin-resistant *Staphylococcus aureus*. *Antimicrob Agents Chemother*. 2003;47:3926-3934.
25. Lowy FD. Antimicrobial resistance: the example of *Staphylococcus aureus*. *J Clin Invest*. 2003;111:1265-1273.
26. Vanderhaeghen W, Hermans K, Haesebrouck F, Butaye P. Methicillin-resistant *Staphylococcus aureus* (MRSA) in food production animals. *Epidemiol Infect*. 2010;138:606-625.
27. Lee JH. Methicillin (oxacillin)-resistant *Staphylococcus aureus* strains isolated from major food animals and their potential transmission to humans. *Appl Environ Microbiol*. 2003;69:6489-6494.
28. Kumar R, Yadav B, Singh R. Antibiotic resistance and pathogenicity factors in *Staphylococcus aureus* isolated from mastitic Sahiwal cattle. *J Biosci*. 2011;36:175-188.
29. Saïd-Salim B, Mathema B, Kreiswirth BN. Community-acquired methicillin-resistant *Staphylococcus aureus*: an emerging pathogen. *Infect Control Hosp Epidemiol*. 2003;24:451-455.
30. Leonard F, Markey B. Meticillin-resistant *Staphylococcus aureus* in animals: a review. *Vet J*. 2008;175:27-36.
31. CSA. *The 2007 Ethiopian Agricultural Sample Enumeration (EASE), Executive Summary*, 2008; 2008.
32. Thrusfield M. *Veterinary Epidemiology*. John Wiley & Sons; 2018.
33. Schalm OW, Carroll EJ, Jain NC. *Bovine Mastitis*. Philadelphia Lea & Febiger; 1971.
34. Mekonnen A. *Isolation and Identification of Staphylococcus Species from Cottage Cheese (Ayib) and Raw Bovine Milk in Debre Zeit, Ethiopia*. MSc thesis. Addis Ababa University, Faculty of Veterinary Medicine Debre . . .; 2009.
35. Quinn P, Markey B, Carter M, Donnelly W, Leonard F. *Veterinary Microbiology and Microbial Disease*. 1st ed. Blackwell Publishing; 2002.
36. Clinical and Laboratory Standards Institute. *Performance Standards for Antimicrobial Disk Susceptibility Tests. Approved Standard M2-A10*. Clinical and Laboratory Standards Institute; 2009.
37. CLSI. Performance standards for antimicrobial susceptibility testing. Twenty forth information supplement (M 100-524). *Morb Mortal Wkly Rep*. 2014;54:1277-1280.
38. Bekele M, Admasu M, Mesele AAR, Fufa A. Mastitis in lactating cows at Hawassa Town: prevalence, risk factors, major bacterial causes and treatment response to routinely used antibiotics. *Am Eurasian J Sci Res*. 2012;7:86-91.
39. 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. *J Vet Med Anim Health*. 2010;2:29-34.
40. Hundera S, Ademe Z, Sintayehu A. Dairy cattle mastitis in and around Sebeta, Ethiopia. *Int J Appl Res Vet Med*. 2005;3:1525-1530.
41. 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*. 2013;23:50-55.
42. Moges N, Asfaw Y, Belihu K. 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 Adv*. 2011;3:455-459.
43. Workneh S, Bayleyegne M, Mekonen H, Potgreter L. Prevalence and aetiology of mastitis in cows from two major Ethiopian dairies. *Trop Anim Health Prod*. 2002;34:19-25.
44. Getahun K, Kelay B, Bekana M, Lobago F. Bovine mastitis and antibiotic resistance patterns in Selalle smallholder dairy farms, central Ethiopia. *Trop Anim Health Prod*. 2008;40:261-268.

45. Dego OK, Tareke F. Bovine mastitis in selected areas of southern Ethiopia. *Trop Anim Health Prod*. 2003;35:197-205.
46. Rodostits O, Blood D, Gay C. *Veterinary Medicine: A Text Book of the Disease of Cattle, Sheep, Pigs, Goats and Horses*. 8th ed. Boillière Tindall; 1994.
47. Rediet B, Kelay B, Alehegne W. Dairy cows mastitis survey in Adama Town, Ethiopia. *J Vet Med Anim Health*. 2013;5:281-287.
48. Zingeser J, Daye Y, Lopez V, et al. National survey of clinical and subclinical mastitis in Jamaican dairy herds, 1985–1986. *Trop Anim Health Prod*. 1991; 23:2-10.
49. Hussein N. *Cross-sectional and Longitudinal Study of Bovine Mastitis in Urban and Peri-Urban Dairy Systems in the Addis Ababa region, Ethiopia*. Faculty of Veterinary Medicine, Addis Ababa University School of Graduated Studies; 1999.
50. Daka D, Yihdego D. Antibiotic-resistance *Staphylococcus aureus* isolated from cow's milk in the Hawassa area, South Ethiopia. *Ann Clin Microbiol Antimicrob*. 2012;11:1-6.
51. Jones ME, Karlowsky JA, Draghi DC, Thornsberry C, Sahm DF, Nathwani D. Epidemiology and antibiotic susceptibility of bacteria causing skin and soft tissue infections in the USA and Europe: a guide to appropriate antimicrobial therapy. *Int J Antimicrob Agents*. 2003;22:406-419.
52. Gál Z, Kovács P, Hernádi F, et al. Investigation of oxacillin-hydrolyzing beta-lactamase in borderline methicillin-resistant clinical isolates of *Staphylococcus aureus*. *Chemotherapy*. 2001;47:233-238.
53. Banerjee R, Gretes M, Harlem C, Basuino L, Chambers HF. A mecA-negative strain of methicillin-resistant *Staphylococcus aureus* with high-level β-lactam resistance contains mutations in three genes. *Antimicrob Agents Chemother*. 2010; 54:4900-4902.
54. Moellering RC Jr. MRSA: the first half century. *J Antimicrob Chemother*. 2012;67:4-11.
55. Kesah C, Ben Redjeb S, Odugbemi T, et al. Prevalence of methicillin-resistant *Staphylococcus aureus* in eight African hospitals and Malta. *Clin Microbiol Infect*. 2003;9:153-156.
56. Peng Q, Hou B, Zhou S, et al. Staphylococcal cassette chromosome mec (SCC-mec) analysis and antimicrobial susceptibility profiles of methicillin-resistant *Staphylococcus aureus* (MRSA) isolates in a teaching hospital, Shantou, China. *Afr J Microbiol Res*. 2010;4:844-848.