Staphylococcus aureus isolates from bovine subclinical mastitis in central Ethiopia revealed the presence of antimicrobial resistance and resistant genes

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Abstract

Background: *Staphylococcus aureus* is one of the predominant causative agents of mastitis disease in dairy herds. Mastitis disease has a negative impact in the economic losses in the dairy sector across the globe. The aims of this study were to determine the prevalence and detect antimicrobial resistance genes in the *Staphylococcus aureus* isolated from milk samples of subclinical bovine mastitis in Central Ethiopia.

Methods: A total of 265 lactating dairy cows from various dairy farms in four different geographical locations were screened by California mastitis test (CMT) for bovine subclinical mastitis. One-hundred thirty CMT positive milk samples were collected and transported to laboratory. Different biochemical tests and polymerase chain reaction (PCR) were used for the identification of *S. aureus* isolates. Finally, phenotypic and genotypic methods were performed for detection of some antimicrobial resistance patterns and genes (*mec*A, *erm*A, *erm*C, and *msr*A), respectively.

Results: From total of 265 lactating dairy cows screened, 49% (n=130) were positive for bovine subclinical mastitis. One-hundred thirty mastitic milk samples were subjected to bacterial culturing, one hundred (76%) *S. aureus* isolates were identified based on phenotypic characters. Sixty-eight confirmed *S. aureus* isolates were obtained using PCR. Of the sixty-eight isolates tested 12 samples were contained the methicillin resistance gene A (*mec*A). No amplification was observed for the erythromycin resistance genes (*erm*A, *erm*C, and *msr*A).

Conclusion: The high resistance of *Staphylococcus aureus* to commonly used antimicrobials contribute in dairy farms may cause health problems in the community consuming raw milk purchased from these farms.

Background

Mastitis is a common disease of dairy cows and a major concern for the dairy industry because of economic losses due to decreased animal health and increased antibiotics usage (Heikkilä et al., 2018; Gussmann et al., 2019). Due to multiple etiologies, it always remained a challenge to veterinarian worldwide. Approximately, 140 species of microorganisms have been identified as etiological agents of bovine mastitis (Radostits et al., 2007). Of these various etiological agents, *Staphylococcus aureus* (*S. aureus*) is one of the major agents of contagious mastitis, responsible for mainly bovine subclinical and clinical infections in cattle worldwide (Barkema et al., 2006). This pathogen, in combination with both the bovine host and environmental factors, is characterized by low cure rates compared with other mastitis pathogens because of its capability to acquire antibiotic resistance and produce a wide array of virulence factors (Gao et al., 2012).

Programs for mastitis prevention and treatment use wide range of antimicrobials (Wald et al., 2019). The indiscriminate use of antibiotics has led to the development of multiple antibiotic resistances thereby rendering the antibiotic treatment ineffective (Alian et al., 2012). The resistant bacteria present in

environments are in contact with human beings and animals (Hu et al., 2017). Antimicrobial resistance is a major public health concern in many countries due to the persistent circulation of resistant strains of bacteria in the environment and the possible contamination of water and food (Gelbrand et al., 2015). Therefore, investigation of a wide spectrum of antimicrobial resistance genes of *S. aureus* isolated from cows with mastitis is crucial not only for bovine mastitis control, but also for public health (Xavier et al., 2017; Kadlec et al., 2019).

Although *S. aureus* responds poorly to treatment with many different antimicrobial agents, antibiotic therapy still plays a significant role in the prevention and cure of bovine Staphylococcal mastitis (Wald et al., 2019). The infection of cows with increasingly antibiotic-resistant strains can cause several therapeutic problems and is one of the main reasons for monitoring drug resistance (Saini et al., 2013). The measurement of *S. aureus* antimicrobial resistance using phenotypic susceptibility tests, such as disk diffusion, is essential in order to select the most appropriate and efficient therapy (Walker, 2006). These methods can be combined with molecular analysis, as phenotypic *S. aureus* resistance to the most commonly used antimicrobials is related to the expression of antibiotic-resistance genes (Cockerill, 1999).

Rapid methods for accurate detection and susceptibility determination of *S. aureus* isolates are necessary to minimize patient suffering by identifying the antimicrobial agents to which the isolated strains may be sensitive to and hence provide treatment options (Duarte et al., 2015). Owing to the poor discriminatory power of the phenotypic techniques, deoxyribonucleic acid (DNA) based identification and genotyping techniques are now considered the ideal methods for the detection of antimicrobial resistance genes of *S. aureus* (Perez-Roth et al., 2001; Song et al., 2015). In previous study that was conducted in Ethiopia, *S. aureus* isolates were identified using only the phenotypic antimicrobial susceptibility test and identification of *S. aureus* (Duguma et al., 2014; Marama et al., 2016). Presently, there are limited published studies conducted in central highland of Ethiopia concerning molecular characterization of antimicrobial resistance of *S. aureus* in dairy farms. Therefore, the aim of this study was to determine antimicrobial resistance (AMR) patterns of *S. aureus* isolated from bovine subclinical mastitis in central highland of Ethiopia.

Methods

Study areas

This study was conducted in selected areas of central Ethiopia including Adaberga, Ambo, Bishoftu and Holeta as indicated in Fig. 1. The areas were purposively selected based on the abundance of dairy farms that constituting the known milk sheds to the Addis Ababa, and based on their agro-ecological differences.

Study Design And Study Population

A cross sectional study design was employed to determine the prevalence, antimicrobial resistance and resistant genes of *S. aureus* from bovine subclinical mastitis in central highland of Ethiopia from October

2018 to May 2019. The study population were lactating exotic Zebu cross-breed dairy cows which had not treated for mastitis either intra-mammary or systemic route during the study period. All dairy farms with herd size ranging from 20–110 cows, which were managed under semi intensive or intensive management system, were included as study population inclusion criteria.

Sample Collection And Transportation

The mastitis milk samples were taken from CMT positive dairy cows and collected according to earlier protocol (Quinn *et al.*, 2004). Briefly, quarters were washed with tap water and dried with clean towel. The teat ends were then cleaned with cotton soaked with 70% ethyl alcohol. Then, after discarding the first three streams of milk, 10 ml milk was collected aseptically into a sterile screw-cupped, pre-labeled test tube, by holding it in inclined position, so that, the pathogen that going to be recovered come from mammary gland. Finally, milk sample was held in an ice box for transportation to respective laboratory (National Agricultural Biotechnology Research Center, Holeta, Ethiopia) for isolation and identification of bacteria from milk samples. The samples were immediately cultured or stored at 4°C for a maximum of 24hr until cultured on standard bacteriological media.

Bacterial Isolation And Identification

Isolation and identification of *S. aureus* isolates were performed using the technique described beforehand (Quinn et al., 2015). Briefly, all samples were first inoculated onto freshly prepared nutrient agar (HiMedia, India) and incubated at 37°C for 24hours. Bacterial colonies were identified based on colonial morphology, cultural characteristics, Gram's staining and biochemical tests. *Staphylococcus aureus* produces golden colonies on nutrient agar; it is non-motile; coagulase positive, catalase positive, ferments mannitol and produces double pattern of haemolysis on sheep blood agar. The isolates that were suspected to be *Staphylococcus* species were again sub-cultured onto freshly prepared Mannitol salt agar (MSA) (HiMedia, India) and incubated at 37°C for 24 hours. Golden yellow colonies were presumptively identified as *S. aureus*. Furthermore, a single golden yellow colony from the culture plate was sub-cultured on freshly prepared nutrient agar slants at 37°C for 24hours, after which the slants were stored in the refrigerator for further analysis.

Bacterial DNA Extraction

Each isolate was inoculated into Brain Heart Infusion (BHI, Hampshire, UK) broth and incubated at 37°C for 24 hr. Aliquots of each culture were centrifuged and the supernatant was discarded. The pellet was used to extract DNA using Wizard® Genomic DNA extraction Kit (Promega Corporation, Madison, USA), according to manufacturer's instructions. Extracted DNA was quantified at 260/280 nm by Nanodrop (Thermo Fisher Scientific, Germany) and stored at -20 ∘C for further molecular work.

Molecular conformation of *Staphylococcus aureus*

Reference primers (Integrated DNA Technologies (IDT), San Diego, USA) *Sau*234 (F) (5′-CGATTCCCTTAGTAGCGGCG-3′) and *Sau*1501 (R) (5′-CCAATCGCACGCTTCGCCTA-3′) targeting the *23SrRNA* gene (Gene bank database *S. aureus*, GI no. 288516) were used to amplify the 23SrRNA gene, as previously described (Riffon et al., 2001). The primers (20 pmol) used in this study generated an amplicon of 1267 bp as visualized by gel electrophoresis (CBS Scientific, United Kingdom). The PCR reaction volume was 25µl and consisted of 12.5µl 2x PCR master mix (Promega Corporation, Madison, WI, USA), 1µl forward primer, 1µl reverse primer, 8.5µl nuclease-free water, and 2µl DNA template in each PCR tube. The PCR reaction was conducted using thermocycler (Gene Atlas, Astec, Kasuya, Japan) from a previously described method (Riffon et al., 2001). The PCR reaction conditions were as follows: initial denaturation at 94°C, 1 cycle for 2min; denaturation at 94°C for 45s; annealing at 58°C, 60.5°C, 60.9°C, 61.3°C (gradient) for 60s; extension at 72°C for 2min for 35 cycles; and a final extension at 72°C for 10min. The amplicons were visualized using 2% agarose gel (Promega Corporation, Madison, WI, USA). Marker 100 bp Plus DNA ladder was used in this study.

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing of the PCR confirmed *S. aureus* isolates were tested against nine commonly used antimicrobial agents (Abetek, Liverpool, UK) using the Kirby-Bauer disc diffusion method on Mueller Hinton agar (HiMedia, India) following the guidelines of Clinical Laboratory Standards Institute (CLSI) (CLSI, 2012). The isolates were classified in accordance with the guideline of CLSI (CLSI, 2012) as susceptible, intermediate or resistance for each antimicrobials tested according to the manufacturer's instructions by measuring the zone of inhibition around the antimicrobials disc (in millimeter). The details of the nine antimicrobial agents utilized used in this study was shown in Table 1 below.

Table 1

The list of a panel of antimicrobial agents utilized, their symbols, concentration and break points

Antimicrobial agent (disk code)	Content (µg/disk)	Inhibition zone diameter interpretive criteria (nearest whole mm)		
		Susceptible	Intermediate	Resistant
Ampicillin (AMP)	10µg	≥ 15	12−14	≤ 11
Chloramphenicol (CHL)	30µg	≥ 18	13−17	≤ 12
Ciprofloxacin (CPR)	5µg	≥ 21	16−20	≤ 15
Cefoxitin (CXT)	30µg	≥ 22	-	≤ 21
Erythromycin (ERY)	15µg	≥ 23	14−22	≤ 13
Gentamicin (GEN)	10µg	≥ 15	13−14	≤ 15
Penicillin (PEN)	10 units	≥ 29	21−28	≤ 20
Streptomycin (STR)	10 µg	≥ 15	12−14	≤ 11
Tetracycline (TET)	30µg	≥ 19	15−18	≤ 14

Source: - (CLSI, 2012)

Molecular Detection Of Antimicrobial Resistance Genes

Polymerase chain reaction amplifications of four antimicrobial resistant genes, which included methicillin resistant gene (*mec*A), various erythromycin resistant genes (*erm*A, *erm*C and *msr*A) were carried out with a pair of specific primers and using previously described protocol (Sawant et al., 2009; Melo et al., 2014) (Table 2). Details of primer sequences, their specific targets and expected amplicon sizes were summarized in Table 2. The reactions were performed in a final volume of 25µl each made by 12.5µl of 2X *Taq* PCR Master Mix, 1µl of 10 µM primer (each forward and reverse), 3µl of DNA template and 7.5µl sterile nuclease free water. PCR conditions were described by the original designers of primers (Table 2). Following completion of reactions, PCR products were run on a 1.5% agarose gel using electrophoresis, stained with gel red at 120 volts for 45min and visualized under UV light using a BioDoc-it™ imaging system (Cambridge, UK). We use 100bp plus DNA marker as molecular marker.

Table 2
Primers used in this study and the PCR conditions for amplifying some antimicrobial resistant genes

Target gene	Primer name and its sequence (5' 3')	Product size (in bp)	Amplification conditions	References
Methicillin resistance (*mec*A)	MECA_F: GGCTATCGTGTCACAATCGTT MECA_R: TCACCTTGTCCGTAACCTGA	689	95°C for 45 sec 55°C for 30 sec 72°C for 45 sec	(Melo et al., 2014)
Erythromycin resistance A (*erm*A)	ERMA_F: ATCGGATCAGGAAAAGGACA ERMA_R: CACGATATTCACGTTTTACCC	486	94°C for 1 min 49°C for 30 sec 72°C for 30 sec	(Sawant et al., 2009)
Erythromycin resistance (*ermC*)	ERMC_F: TGAAATCGGCTCAGGAAAAG ERMC_R: CAAACCCGTATTCCACGATT	272	94°C for 1 min 52°C for 30 sec 72°C for 30 sec	(Sawant et al., 2009)
Macrolide resistance A *msrA*	MSRA_F: TGGTACTGGCAAAACCACAT MSRA_R: AAACGTCACGCATGTCTTCA	1000	94°C for 30 sec 52°C for 30 sec 72°C for 30 sec	(Sawant et al., 2009)

Statistical analysis

Data generated from the study was arranged, coded and entered to excel spread sheet (Microsoft® office excels 2010) and subjected to descriptive statistics. The Chi-square test was applied to determine the existence of any association between sampling areas and prevalence of *S. aureus* using 26.0 *versions* of SPSS. The prevalence of mastitis was calculated by dividing the number of positive animals for CMT to the total number of animals examined times 100% (Thrusfield, 2005). The significance level was set at P-value (0.05) and 95% confidence level. In all cases, 95% confidence level and p-value less than 0.05 was consider as statistical significance.

Results

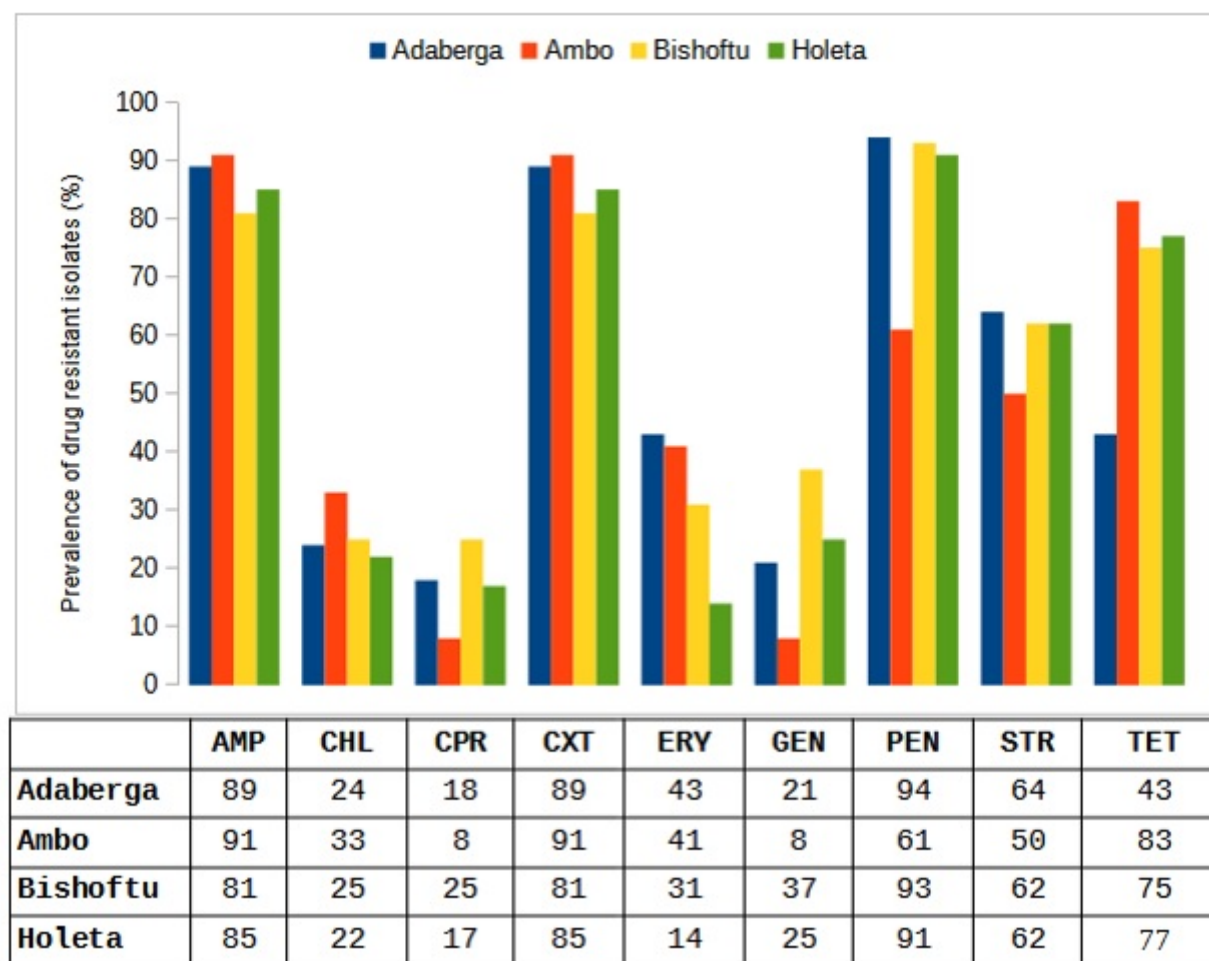## Prevalence of bovine subclinical mastitis and *S. aureus* isolates

A total of 265 lactating dairy cows from various dairy farms from central highland of Ethiopia were screened by using California mastitis test (CMT) for bovine subclinical mastitis. Out of 265 lactating dairy cows screened, 130 (49.06%, 95% CI: 43.08–55.06) of them were found positive by CMT for either of the four quarters. CMT positive milk samples were obtained from different sampling areas which included 54 (61.36%, 95%CI: 38.09–80.39) from the Adaberga, 16 (69.57%, 95% CI: 37.40-89.74) from Ambo and 16 (40%, 95%CI: 17.96-67.00) from Bishoftu and 44 (38.60%, 95% CI: 30.12–47.82) from Holeta. In the present study, the highest and lowest prevalence of bovine subclinical mastitis was recorded samples were collected from Ambo and Holeta with prevalence of 69.57% and 38.60%, respectively. The prevalence of bovine subclinical mastitis was statistically significant between different sampling areas (X^2 = 15.70; P = 0.0013).

On the other hand, 100 (76.92%, 95% CI: 68.79–83.44) milk samples from 130 CMT positive milk samples were tested positive for *S. aureus* based on different biochemical tests and PCR. Figure 2 depicts a gel image with 1267bp PCR gene amplicons demonstrating the presence of the *23SrRNA* (*S. aureus*-specific) gene that was amplified on representative *S. aureus* isolates. *S. aureus* isolates were obtained from different sampling areas, which included 37 (68.52%, 95% CI: 54.80-79.62) from Adaberga, 12 (75%, 95% CI: 47.97–90.71) from Ambo and 16 (100%) from Bishoftu and 35 (79.55%, 95% CI: 64.80-89.15) from Holeta. Regarding the sampling areas, the highest and lowest recovery rate of *S. aureus* isolates were observed in Bishoftu and Adaberga from CMT positive milk samples, respectively.

Antibiotic Susceptibility Test

One-hundred *S. aureus* isolates were tested to evaluate their susceptibility patterns a panel of nine antimicrobials agents. In the present study, *S. aureus* isolates were found variably resistant to the antimicrobials tested. Data depicting the susceptibilities of the isolates were presented as percentages are shown in Fig. 3. A large proportion (50–94.6%) of the *S. aureus* isolates obtained from Adaberga, Ambo, Bishoftu and Holeta were resistant to ampicillin, cefoxitin, penicillin and streptomycin. Despite the fact that a relatively large proportion (75–83.3%) of the isolates from Ambo, Bishoftu and Holeta were resistant to streptomycin, on the contrary, only a small proportion (43.24%) of the isolates from Adaberga sampled were resistant to this antimicrobial agent. Also intermediate sensitivity of *S. aureus* isolates was highest towards Erythromycin (50%), Ciprofloxacillin (35%) and followed by Gentamycin (24%) and Streptomycin (14%). Moreover, isolates obtained from Ambo were relatively highly susceptible to ciprofloxacillin and gentamycin. Similarly, low level resistance was observed against chloramphenicol as summarized in Table 3.

Table 3: Prevalence of drug resistant *S. aureus* isolates from various sampling sites

	AMP	CHL	CPR	CXT	ERY	GEN	PEN	STR	TET
Adaberga	89	24	18	89	43	21	94	64	43
Ambo	91	33	8	91	41	8	61	50	83
Bishoftu	81	25	25	81	31	37	93	62	75
Holeta	85	22	17	85	14	25	91	62	77

Similarly, multi-drug resistance (MDR) patterns were generated from 100 *S. aureus* isolates showing resistance to three or more antibiotics according to (Coyle, 2005). The MDR pattern PEN-AMP-CXT-TET-GEN was observed in 45% of the isolates from Adaberga and in 42% of Ambo isolates. The MDR pattern PEN-AMP-CXT was dominant among 27%, 22% and 26% of Adaberga and Bishoftu and Holeta samples, respectively. The predominant MDR pattern for isolates from Bishoftu and Holeta were PEN-AMP-CXT-STR-TET and PEN-AMP-CXT-STR was obtained at 33 and 24%, respectively. These results indicate that in the present study, MDR *S. aureus* was isolated from milk samples.

Table 4: Antimicrobial resistance patterns of *Staphylococcus aureus* isolates from various sampling sites

Sampling area	Number of isolates	Antibiotic resistance patterns	Multidrug resistance	
			Number	Percentage
Adaberga	11	PEN-AMP-CXT	3	27
		PEN-AMP-CXT-TET	1	9
		PEN-AMP-CXT-TET-GEN	5	45
Ambo	7	PEN-AMP-CXT-TET-GEN	3	42
		PEN-AMP-TET-STR	1	14
Bishoftu	9	PEN-AMP-CXT	2	22
		PEN-AMP-CXT-STR	1	11
		PEN-AMP-CXT-STR-TET	3	33
Holeta	15	PEN-AMP-CXT	4	26
		PEN-AMP-CXT-STR	6	24
		PEN-AMP-CXT-STR-TET	3	20
		PEN-AMP-CXT-TET	1	6

Discussion

The overall cow-level apparent prevalence of bovine subclinical mastitis in the central highland of Ethiopia was 49.05% (130/265). This result was almost in agreement with findings from similar studies by Ayana et al. (2017) from Bishoftu, Arga et al. (2012) from Ambo, Dego and Tareke (2003) from southern Ethiopia and Mungube et al. (2004) from Addis Ababa, which reported prevalence of 46.09%, 58.82%, 40.4% and 39.8%, respectively. However, the result of the present study was lower than the reports of Duguma et al. (2014) who reported a prevalence of bovine subclinical mastitis was 81% in Holeta, central highland of Ethiopia, Bishi (1998) who reported 69.8% in dairy farms of Addis Ababa and its vicinity and Mekibib et al. (2010) who reported 74.7% around Addis Ababa. Moreover, the present finding was higher than previous reports by Workineh et al. (2002) who reported a prevalence of 25.1% in Addis Ababa and Delesse (2010) who reported a 10.3% with prevalence of mastitis in dairy farms around Holeta town, Ethiopia. As mastitis is a complex disease, the difference of prevalence observed from the current study could be due to interactions of various factors such as dairy cow management system and husbandry practice, environmental conditions, animal risk factors and virulence factors of the circulating causative agents (Radositis et al., 2007) and also difference in study methods used, period of investigation and specific farm-level intervention might contribute in the variation of the prevalence of mastitis.

According to the biochemical tests and PCR finding in this study, 79.92% (100/130) of *S. aureus* isolates were recovered from 130 CMT positive milk samples. The finding of the current study was higher than the previous findings which were done around Sebeta (44.03%) by Sori et al. (2005), in Holeta agricultural research centre (43.3%) by Duguma et al. (2014), in and around Holeta town (47.1%) by Mekibib et al.

(2010) and in Bishoftu area (39.5%) by Asrat et al. (2013). This high rate of *S. aureus* in this study might suggest the high rate transmission of *S. aureus* infection which might occurred because of poor hygienic conditions during milking process in which contamination with *S. aureus* might occurred from contaminated milker's hands and milking equipment (Radositis et al., 2007). Apart from Ethiopia, *S. aureus* has also been reported as the chief etiological agent of mastitis in cattle by many studies from African and Asian countries (Abebe et al., 2016). Though direct comparisons among studies might be difficult, but in general, the variation in the prevalence between the present and previous studies might be due to differences in detection methods, geographical location of the study sites and differences in farm management practices in each studied farms. .

Previous studies have revealed that an increasing trend towards the occurrence of *S. aureus* isolates that portray multiple antimicrobials resistance phenotypes (Normanno et al., 2007; Pesavento et al., 2007) ; hence, *S. aureus* isolates that harbour multiple antimicrobials resistant traits have been reported to negatively impact on the treatment of staphylococcal infections. The present study showed a higher level of resistance of *S. aureus* to penicillin (93%), ampicillin (87%), tetracycline (75%) and cefoxitin (87%). The current finding was in line with the findings of Abebe et al. (2016) who reported resistance of *S. aureus* to penicillin (94%), tetracycline (73.8%) around Addis Ababa and (Abera et al., 2013) who recorded 94.4% to penicillin around Adama and Sori et al. (2005) who recorded 87.2% *S. aureus* isolates were found to be resistant to penicillin. In general, the current study able to show that susceptibility of *S. aureus* to commonly used antimicrobials, penicillin, ampicillin, cefoxtin and tetracycline in study area was very low. The possible justification for this could be the development of alarming level of resistance of *S. aureus* due to the regular use of these commonly used antibiotics for the treatment of cows that might resulted in the spread of resistant strain in study area. This result was in accordance with reports from earlier studies in other countries, suggesting a possible development of resistance from prolonged and indiscriminate usage of some antimicrobials (Enright et al., 2002). Previous report indicated that the prevalence of antimicrobials resistance in *S. aureus* isolates become a serious problem in a dairy herds (WANG et al., 2009).The over-use of antibiotics in dairy farms is one of the major factors responsible for the emergence of drug resistant bacteria. Furthermore, isolates that are resistant to ampicillin may cross select for resistance to other beta-lactams including penicillin, therefore, resistance to ampicillin is an indication of the resistance of the isolates to other beta-lactam antibiotics (WANG et al., 2009). Methicillin-resistant *Staphylococcus aureus* (MRSA) is currently a major burden in veterinary and human medicine (Tangka et al., 2002). This type of resistance is considered to be one of the most important and has been implicated in many animal and human illnesses that have resulted in high mortality. It is therefore, very important to implement a systemic application of an in vitro antibiotic susceptibility test prior to the use of antibiotics in both treatment and prevention of intra-mammary infections.

In the present study, multiple drug resistant (MDR) *S. aureus* strains defined as isolates that were resistant to three or more antibiotics were obtained in 54.26% of some of the milk samples analyzed. Development of multiple antibiotic resistances among most of these isolates may be attributed to transmission of resistance (R-factor) which is a plasmid-mediated genetic determinant. *S. aureus* often contain multiple plasmids that may contain various numbers of antibiotic resistant genes (Yamamoto et

al., 2013). Given the fact that antibiotic resistance traits in bacteria species including *S. aureus* may occur either spontaneously by mutation and selection or by acquisition of new genetic material from other resistant organisms through transformation, transduction and conjugation it is usually not surprising that the antibiotic resistance profiles of isolates from the same region may vary considerably.

In the present study, the isolates of *S. aureus* were tested for four antimicrobial resistance genes which included methicillin resistance gene (*mec*A), various erythromycin resistance genes (*erm*A, *erm*C) and macrolide resistance A gene (*msr*A). Among the four antimicrobial resistance genes screened, only *mec*A gene was amplified, 12% (12/100) of *S. aureus* isolates out of 100 *S. aureus* isolates and other antimicrobial resistance genes which included *erm*A, *erm*C *and msr*A were not detected in any of the isolates in the present study. In this study, *mec*A positive *S. aureus* was detected in milk samples collected from different sampling areas, including Ambo 16.67% (2/12), Bishoftu 25% (3/12) and Holeta 53.33% (7/12) based on the presence of the *mec*A gene amplicon. However, the present study was disagree with the report from the Central highlands of Ethiopia by Seyoum et al. (2016) and (Mekonnen et al., 2018) from North-West Ethiopia, who did not detect any *mec*A positive *S. aureus* in their study. Variation in the proportion of *mec*A positive *S. aureus* in comparison to other researcher might be due to the difference in sample size, antibiotic use in animal husbandry and hygiene practices among the dairy farms.

The *mec*A gene was detected in some isolates that resist cefoxitin. The existence of *mec*A positive cefoxitin resistance *S. aureus* in milk has been reported in many previous studies (UGWU et al., 2015). The presence of *mec*A-positive MRSA strains in bovine milk samples has been reported in many countries (Kreausukon et al., 2012). The presence of *mec*A negative MRSA strains in bovine milk samples has also been reported by (Kumar et al., 2011). However, *mec*A-negative cefoxitin resistance *S. aureus* has been also recovered from bovine milk; the resistance revealed by *mec*A-negative cefoxitin resistance *S. aureus* isolates might be attributed to the presences of other beta-lactam resistance mechanisms (Malik et al., 2007). This indicated the presence of incompatibility between the detection of methicillin resistance phenotypically using cefexitin discs and the absence of *mec*A gene in some MRSA isolates. This finding is in accordance with (García-Álvarez et al., 2011) who identified phenotypic MRSA isolates without *mec*A gene. This may be attributed to the presence of PCR inhibitors or other physical factors that may have compromised the sensitive of PCR in the detection of *mec*A gene.

Conclusion

The present study has revealed that bovine mastitis is a widely prevalent disease in the dairy farms of the central Ethiopia. The present investigation also explored that *S. aureus* is an important cause for bovine mastitis, which warns the higher public health risk due to consumption of raw milk and its products. The study also demonstrated that isolates are characterized by multiple drug resistance to commonly prescribed drugs in veterinary and human pharmacies. Dairy cows in the study area had high rates of infection by multi-drug resistant *S. aureus* isolates, especially methicillin resistance A (*mecA)* gene which may hold a serious threat to human and animal health. Though the development of antimicrobial

resistant determinants in *S. aureus* is associated with the uncontrolled usage of antimicrobial agents in human and veterinary medicine, the incidence of drug-resistant *S. aureus* in bovine milk samples warrants closer monitoring. Therefore, careful monitoring for the resistance status is an utmost need since the transmission of this pathogen is dynamic and involves human, animals, and likely the farm production environment.

List Of Abbreviations

AMP = Ampicillin; CHL = Chloramphenicol; CPR = Ciprofloxacillin; CXT = Cefoxitin; ERY = Erythromycin; GEN = Gentamycin; PEN = Penicillin; STR = Streptomycin; TET = Tetracycline; CMT = California mastitis test; MDR = Multi-drug resistance, DNA = Deoxyribonucleic acid; P−value = predictive value; PCR = polymerase chain reaction; χ2 = chi-square; MRSA = Methicillin-resistant *Staphylococcus aureus*

Declarations

Ethics approval and consent to participate

This study was conducted after gaining full approval by the ethical review board of the College of Veterinary Medicine and Agriculture, Addis Ababa University, Ethiopia. Informed written consent was taken from all participants prior to participation in this study. Also, permission from dairy farm owners/managers was obtained before collection of milk samples

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests

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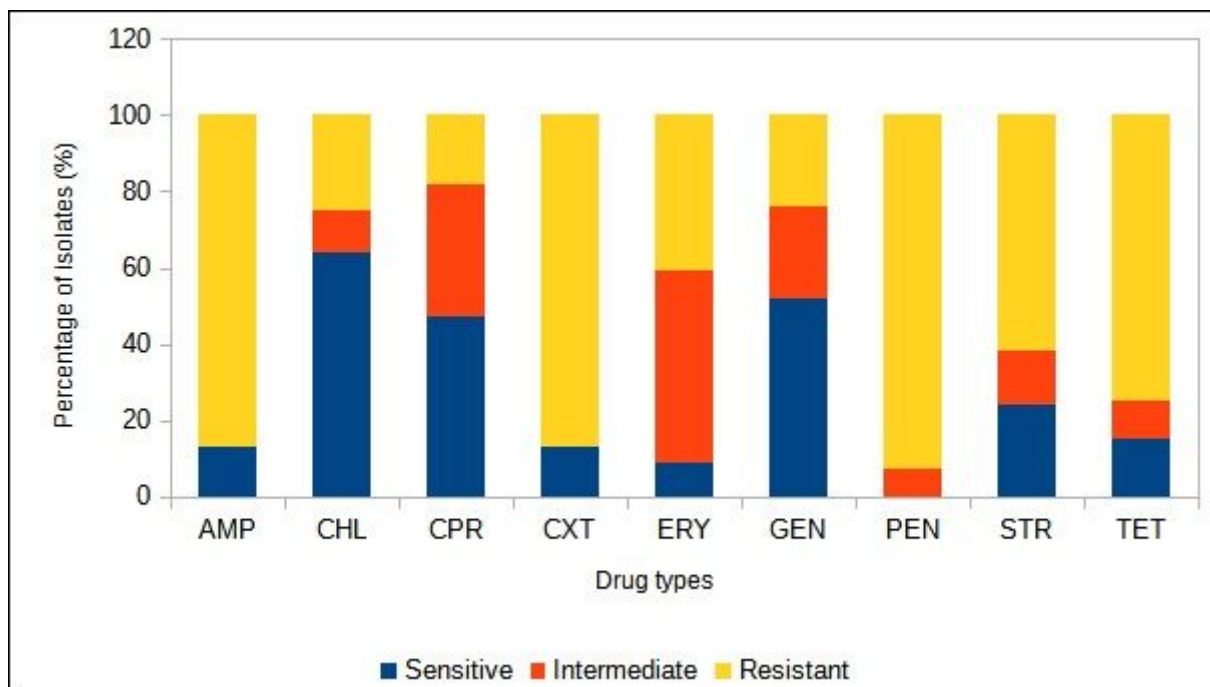
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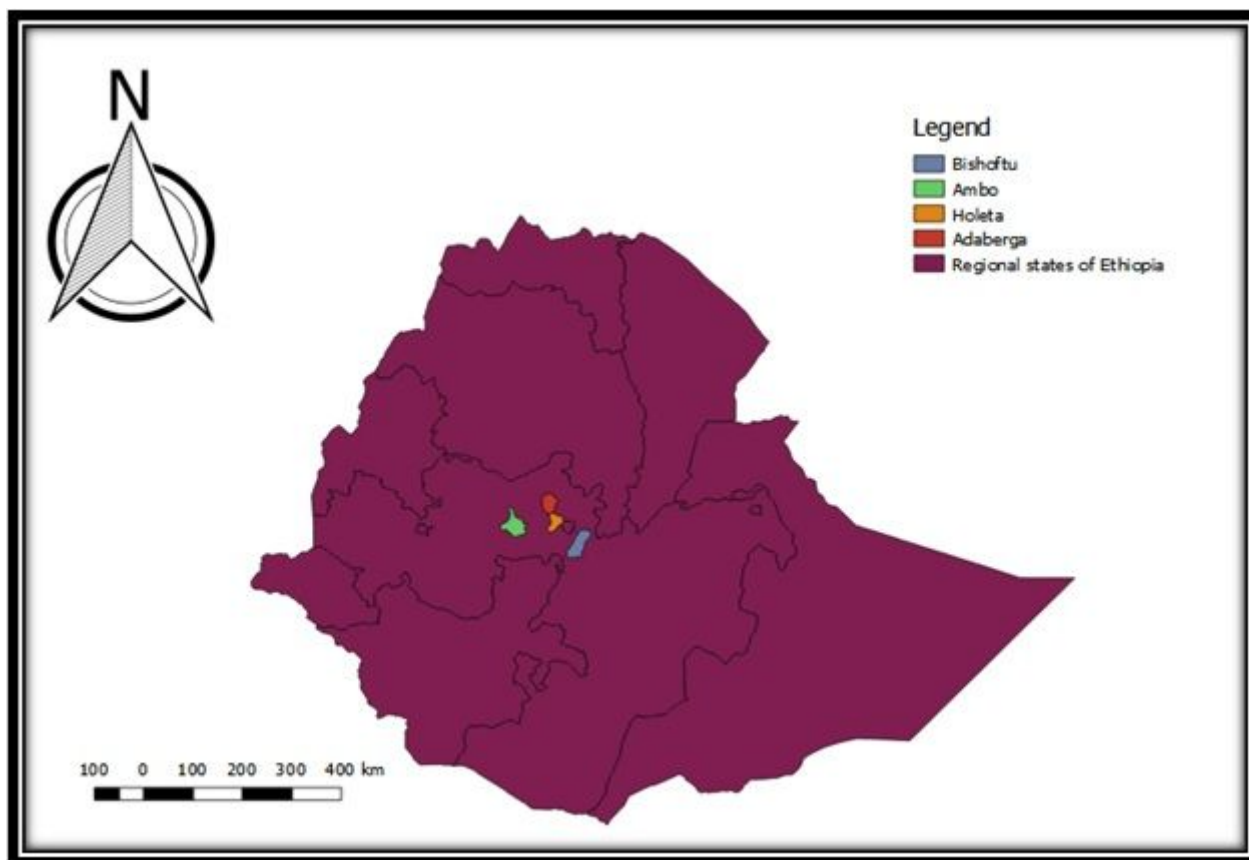
Figure 1

Map of Ethiopia indicating study areas of the research. Specific sampling areas are indicated in different colors that are explained in the legend.
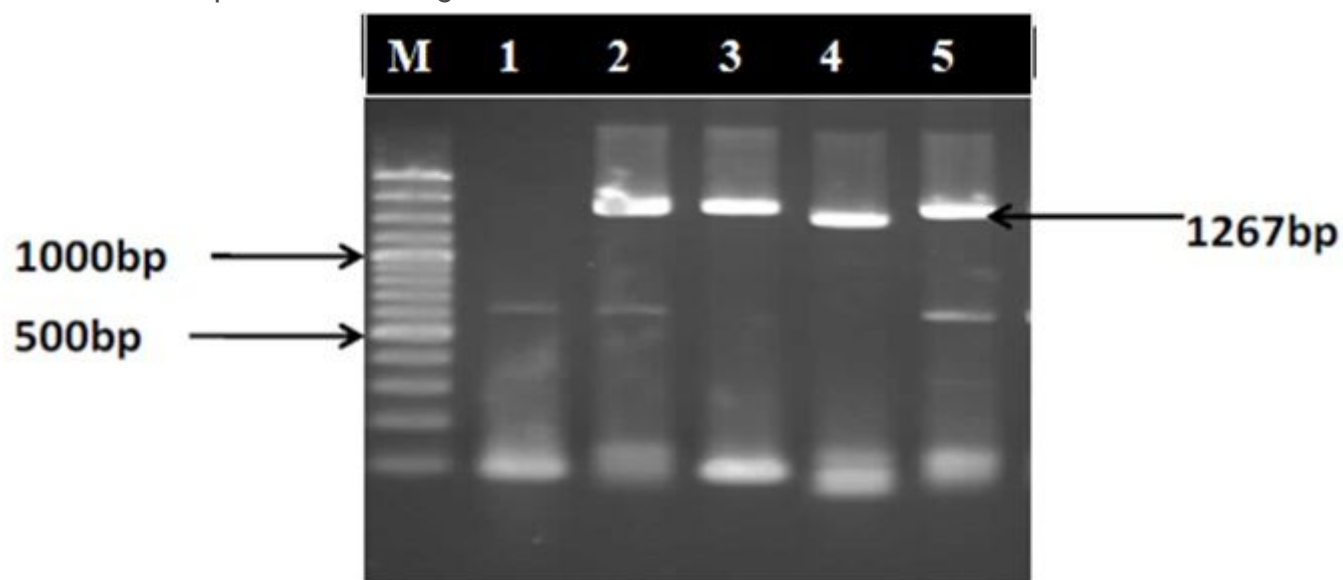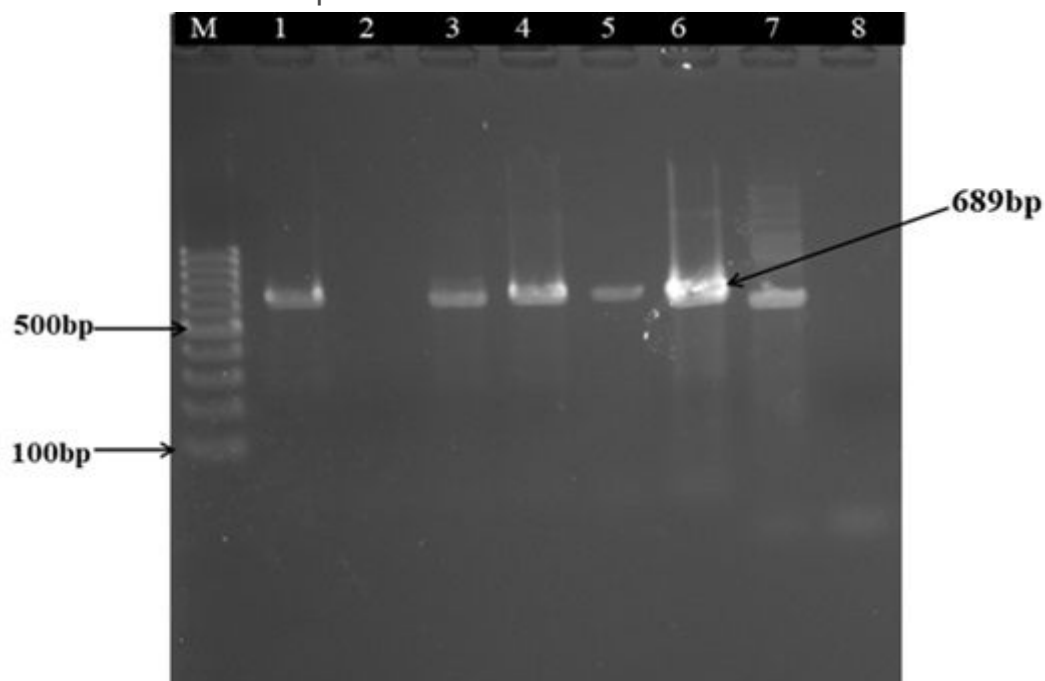

Figure 2

Amplicons of the 23SrRNA gene of representative S. aureus with a size of 1267bp. Lane M is a 100 bp plus DNA marker (DNA ladder, BioBasic); lanes 1 to 5 are test samples
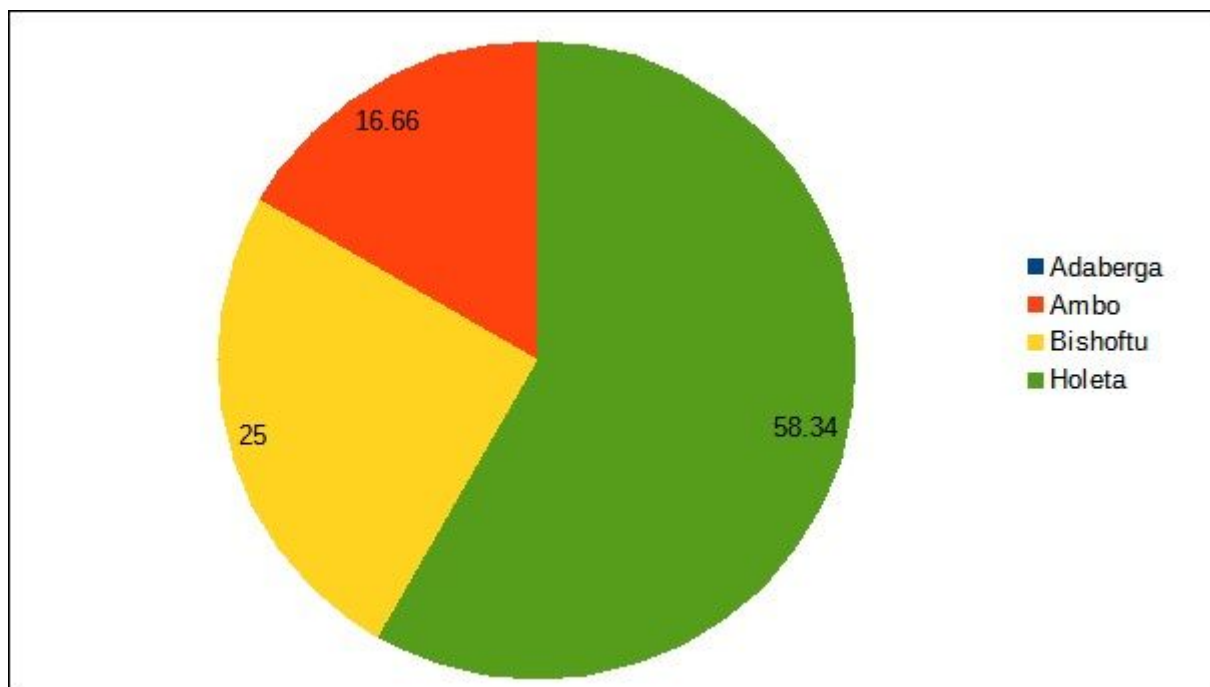
Figure 3

Antimicrobial resistance patters of S. aureus isolates

Figure 4

Agarose gel electrophoresis analysis for the mecA gene in S. aureus isolates, Lane M = l00bp DNA marker, Lanes 1-7 = test samples

Figure 5

Prevalence of S. aureus isolates that were positive for the targeted genes from sampling areas