Acta Tropica 177 (2018) 32–36

Contents lists available at ScienceDirect

Acta Tropica

journal homepage: www.elsevier.com/locate/actatropica

Prevalence, risk factors and antimicrobial susceptibility test of *Staphylococcus aureus* in Bovine cross breed mastitic milk in and around Asella town, Oromia regional state, southern Ethiopia

Befikadu Seyoum[a], Hailemariam Kefyalew[a], Birhanu Abera[b], Nejash Abdela[c,*]

[a] College of Veterinary Medicine, Haramaya University, Ethiopia
[b] Asella Regional Veterinary Laboratory, Asella, Ethiopia
[c] School of Veterinary Medicine, College of Agriculture and Veterinary Medicine, Jimma University, Jimma, Ethiopia P.O. Box. 307 Jimma, Ethiopia

ARTICLE INFO

Keywords:
Ethiopia
Mastitis
Milk
Prevalence
Staphylococcus aureus

ABSTRACT

A cross sectional study was undertaken from November 2016 to March 2017 in and around Asella town, Oromia regional state, southern Ethiopia, to determine the prevalence, associated risk factors and antimicrobial susceptibility of *Staphylococcus aureus,* in Bovine cross breed mastitis milk. A total of 384 lactating dairy cows were screened for mastitis based on clinical examinations and California mastitis test (CMT). Out of 230 lactating crossbred cows with either clinical or subclinical mastitis examined for the involvement of *Staphylococcus aureus. Staphylococcus aureus* was isolated at a rate of 47.2% (N = 92) and 42.9% (N = 15) of the sub- clinical and clinical cases, respectively. The overall prevalence of *Staphylococcus aureus* scored in this study was 46.5% (N = 107). Descriptive statistics and chi-square were used in order to assess the magnitude of the difference of comparable variables, as a result, among risk factors considered, Age, parity, and lactation stage were found significantly associated with the occurrence of *S. aureus in* mastitis milk ($p < 0.05$). The current study revealed that *S. aureus* has 0% susceptibility to penicillinG, followed by tetracycline (14.2%). However, these randomly selected isolates were found to be totally (100%) susceptible to the Kanamycin. The possible justification for, low antimicrobial susceptibility to these commonly used antimicrobials might be repeated and uncontrolled use of these drugs without veterinarian's prescription. Proper provention and regular antimicrobial sensitivity testing helps to select effective antibiotics and ultimately reduce the development of resistance towards commonly used antibiotics. To conclude, the study was able to show that, mastitis caused by *S. aureus* is one of the major problems of dairy cows in milk production and imposing public health hazard in study area. Hence, every possible control and prevention strategies should be implemented.

1. Introduction

Ethiopia is a resourceful country bestowed with the largest livestock resource in the African continent and the cattle population is estimated to be 57.8 million with the potential to export substantial numbers of live animals and their products (CSA, 2016). Cows represent the largest population of cattle of the country. However, the annual consumption of milk in Ethiopian is low as compared to the average milk consumption of developed and developing countries. Local milk production does not satisfy the country's milk requirement due to low input husbandry practice and wide spread livestock diseases (Mohammed et al., 2004).

In present day, there is a national drive to alleviate the existing food deficit by devising different agricultural strategies including improvements of the productivity of livestock sector by controlling some of the major infectious disease, has received little attention in the country, especially, mastitis, the common problem of dairies that is known by an inflammation of the mammary gland is the leading one, that can contribute to reduce, milk production (Mekonnen et al., 2005). It is primarily resulting from an invasion of mammary tissues by pathogenic microorganisms through the teat canal resulting in physical, chemical, pathological changes in glandular tissues and milk. Evidence to date shows that affected dairy cows may loss 15% of their production and the affected quarter a 30% reduction in productivity (Heeschen, 1997).

Mastitis is one of the most common diseases of dairy cattle throughout the world causing huge economic losses. (Kumar et al., 2011). It may be caused by either infectious or non-infectious agents.

* Corresponding author.
E-mail address: nejash.abdela@gmail.com (N. Abdela).

http://dx.doi.org/10.1016/j.actatropica.2017.09.012
Received 11 August 2017; Accepted 16 September 2017
Available online 19 September 2017
0001-706X/

Infectious mastitis results from bacterial, mycotic or algal pathogens. Non-infectious mastitis is the result of injury, chilling, bruising or rough or improper milking. But it is almost due to the effect of infection by bacteria or mycotic pathogens. A total of about 140 microbial species, subspecies and serovars have been isolated from the bovine mammary gland. Pathogens causing mastitis in cattle are divided into major pathogens (those that cause clinical mastitis) and minor pathogens those that normally cause subclinical mastitis and less frequently clinical mastitis (Firaol et al., 2013). Clinical mastitis refers to the condition where the cow's immune system responds with enough intensity to indicate signs of inflammation that is physically observable such as swelling, discoloration and pain; whereas, subclinical mastitis refers to inflammation of the mammary gland in the absence of visible gross lesion in the udder or it is secretion with the presence of pathogenic microorganisms and usually high number of somatic cells in the milk (Harmon, 2004).

Among several etiological agents, *Staphylococcus aureus* is a major mastitis-causing pathogen that also poses food safety and antimicrobial resistance threats (Kumar et al., 2011). It is a versatile pathogen of humans and animals that causes a wide variety of the disease (Abebe et al., 2013) and is an important cause of clinical mastitis in dairy cows causing a huge economic loss worldwide (Lundberg et al., 2014). Reports from Ethiopia also indicate that, *S. aureus* is the most predominant cause of mastitis in dairy cows (Getahun et al., 2007). It can express a wide array of potential virulence factors, including surface proteins that promote adherence to damaged tissue and/or exotoxins and enzymes that can cause variety of infections in skin and soft tissues, including intramammary mastitis (Iwasuki et al., 2006). Some evidence suggests that biofilm formation can be a virulence factor associated with *S. aureus* mastitis (Vasudevan et al., 2003).

The cure rate after antimicrobial treatment of clinical *S. aureus* mastitis is very variable due to both cow and bacterial factors such as parity of the cow, chronicity of the infection and bacterial genotype (Lundberg et al., 2014).To approach appropriate treatment and control measure, it is important to perform antibiotic susceptibility test on relevant and most frequently used antimicrobials. Currently, in Ethiopia, including the study area, the information on prevalence and distribution of S. *aureus* and the sensitivity to commonly used antimicrobials for treatment of *S. aureus* mastitis is scarce. Therefore, the study was aimed at providing information on the prevalence, of an important pathogen, *S. aureus* in Bovine cross breed mastitis milk; antibiotic susceptibility and its associated risk factors in the study area.

2. Material and methods

2.1. Study area

The study was conducted in and around Asella town, located in Oromia regional state, South Eastern Ethiopia. Asella town, the capital of Arsi zone, is located at about 175 km Southeast of Addis Ababa at 6° 59′ to 8° 49′N latitudes and 38° 41′ to 40° 44′E longitudes with an altitude of the area ranges from 2500 to 3000 m above sea level and the temperature, between, 10–25 °C. The mean rain fall is 1147 mm and agricultural production system of the area is of mixed crop and livestock production. Dairy farming using improved cross breeds is a common practice and farming system exists in the study area were intensive, semi-intensive and extensive (CSA, 2007).

2.2. Study population

The study population were lactating dairy cows of crossbreeds, introduced by the artificial insemination program that were found in study area and kept under intensive and semi-intensive husbandry practices.

2.3. Study design

A cross sectional study was undertaken from November 2016 to March 2017 in and around Asella town to determine the prevalence, associated risk factors and antimicrobial susceptibility of *Staphylococcus aureus* in Bovine cross breed mastitis milk.

2.4. Sampling methods and determination of sample size

Simple random sampling was carried out and the sample size of the study was determined based on sample size determination method as described by (Thrusfield, 2005) with a 95% confidence interval and 5% desired precision. Since there was no similar research conducted in the area, expected prevalence of 50% was assumed. The required sample size of the study animal was determined by the formula given in (Thrusfield, 2005) with 95% of confidence interval and 5% desired precision. The value of 50% was assumed and this was correspond to a required minimum sample size of 384 of lactating cows by the field formula given as follow.

$$N = \frac{(1.96)^2 \times Pexp(1 - Pexp)}{d^2}$$

Where: N = Number of sample size
Pexp = Expected prevalence = 50%
d2 = Absolute precision = 5%
CI = Confidence interval (95%)

Therefore the calculated sample size was 384 of lactating cross breed cows.

2.5. Study methodology

2.5.1. California mastitis test (CMT)

Each selected lactating cow was screened for mastitis based on clinical examinations and California mastitis test. Clinical examination of the udder was based on the method previously indicated (Radostitis et al., 2007). The clinical findings considered include abnormalities of the secretion, abnormalities of the udder and teat, and systemic reaction. The California mastitis test was performed according to previously established method (Quinn et al., 2004). About 2 ml of milk from each quarter of the udder was placed in each of four shallow cups in the CMT paddle and an equal amount of the reagent was added. A gentle circular motion was applied in a horizontal plane for 15 s. The result was scored based on the gel formation and categorized as negative if there was no gel formation, or positive if there was gel formation at least in one quarter ranging from Trace (T) to +3, based on gel formation. Hence, a cow was considered mastitis positive, if one or more quarters were CMT positive with or without isolation of the microorganisms.

2.5.2. Sample collection and transportation

The milk sample was taken from mastitis positive cows not treated early with either intra mammary or systematic antimicrobials agents and collected according to earlier protocol (Quinn et al., 2004).To explain: quarters were washed with tap water and dried. The teat ends were then cleaned with cotton soaked with 75% ethyl alcohol. Then, after discarding the first three streams of milk, 8–10 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 Asella regional laboratory for bacteriological examination to isolate *Staphylococcus aureus*. The samples were immediately cultured or stored at 4 °C for a maximum of 24 h, until cultured on standard bacteriological media. Bacterium isolation and identification was carried out based on standard bacteriological techniques previously established (Quinn et al., 2004). In addition, data on potential risk factors including age, parity, lactation stage, housing and husbandry system were collected from interview of owners and