

Prevalence and Drug Resistance Patterns of Staphylococcus Aureus in Lactating Dairy Cow's Milk in Wolayta Sodo, Ethiopia

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Abstract

A cross-sectional study was conducted on lactating dairy cows to determine the prevalence and drug resistance pattern of *S. aureus* in small holder dairy farms from November 2012 - April 2013 in Wolayta sodo, Ethiopia. The result of this study shown that the overall prevalence of *S. aureus* was found to be 32.14%. The prevalence of *S. aureus* showed significant variation among different breeds ($P= 0.001$), age ($P= 0.035$) and parity ($P= 0.001$). The prevalence of *S. aureus* was higher in cows that were cross breeds, were above five years and had parity of above two. There was no significant difference of *S. aureus* prevalence among different stages of lactation ($P > 0.05$). According to the results of antimicrobial susceptibility testing, *S. aureus* was highly susceptible to Ciprofloxacin (100%). *S. aureus* isolates were highly resistant to Penicillin G (93.3%), Streptomycin (53.3%), Tetracycline (40%) and Sulfamethoxazole-trimethoprim (26.7%). There is higher prevalence of *S. aureus* in lactating dairy cow's milk in the study area that needs further attention and investigation in line with its public health impact. Ciprofloxacin could be the drug of choice in the present study and indiscriminate use of antibiotics in the dairy farms should be avoided to minimize drug resistance.

Keywords: *Prevalence; Staphylococcus aureus; Antimicrobial resistance test; Wolayta sodo*

Introduction

Food-borne diseases are of a major concern, worldwide. Milk and milk products are the prime habitat to complex microbial ecosystems; these are responsible for the broad variations in taste, aroma and texture of milk and milk products [1].

Staphylococcus organisms are widely spread in many foods and low contamination levels that favor growth and multiplication could induce staphylococcal food poisoning [2]. In human and veterinary medicine, infections due to *Staphylococcus aureus* are of major importance. It can cause a wide range of infections in food producing animal and is recognized worldwide as a major pathogen causing subclinical intramammary infection in dairy cows causing significant losses in the dairy industry [3-6].

Staphylococcus aureus is a gram-positive, catalase-positive, usually oxidase-negative, facultative anaerobic coccus, which belongs to the family of *Micrococcaceae* and the group of *Staphylococci* [7]. Staphylococcal enterotoxins are a major cause of food poisoning, which typically occurs after ingestion of different foods, particularly processed meat and dairy products, contaminated with *S. aureus* by improper handling and subsequent storage at elevated temperatures [8]. Milk has been reported as a common food that may cause staphylococcal poisoning [9].

The rise of drug-resistant virulent strains of *Staphylococcus aureus* is a serious problem in the treatment and control of staphylococcal infections. *S. aureus* can cause hard-to-treat infections because they are becoming resistant to most of the antibiotics such as beta-lactams, aminoglycosides and macrolides and they have the potential to cause zoonotic infections in humans [10]. Thus the main objective of this study was to isolate *Staphylococcus aureus* from samples of lactating dairy cow's milk from Wolayta sodo and to determine their antibiotic resistance patterns.

Materials and Methods

Study Area

The study was conducted in Wolayta Sodo, Southern Ethiopia. Wolayta Sodo is located about 390 km south of Addis Ababa. It is located at latitude of 8°50'N and longitude of 37°45'E. The Altitude varies from 1100-2950 m.a.s.l. The area experiences mean annual temperature of about 20°C. The mean maximum temperature is 26.2°C and the average monthly minimum temperature is 11.4°C. The rainfall regimes over much of the area are typically bimodal with the big rainy season extending from June to September and a small rainy season occurring from February to April. The mean annual rain fall of the area ranges from 450-1446 mm with the lowest being in low land and highest in high land.

Study Design

A cross-sectional study was conducted to determine the prevalence of *S. aureus* from lactating dairy cows in Wolayta sodo from November 2012 - April 2013.

Sample Size Determination

Sample size was determined by using the formula for simple random sampling [11] at 95% confidence interval, 5% precision and with an expected prevalence of 15 % from previous study in similar study area [12].

Milk Sample Collection

Milk samples were collected by a standard milk sampling techniques [13] from cows that show CMT +ve reactions. The udder and teats were cleaned by tap water, dried and soaked in 70% alcohol to prevent contamination. The near teats were sampled first followed by the far once. 10 ml of milk was collected into a sterile test tube after discarding the first 3 milking stream, placed in racks for ease of handling and then finally transported to Sodo regional veterinary laboratory using an ice box and stored at 4°C until inoculation.

Bacteriological Examination

Bacteriological examination was done according to the NMC [13] and Quinn, *et al.* [14]. A loopful of milk sample was streaked on blood agar enriched with 7% defibrinated sheep blood (Oxoid, UK) using the quadrant streaking method for each quarter. Blood agar plates were incubated aerobically at 37°C for 24-48 hr. The plates were examined for gross colony morphology, pigmentation and hemolytic characteristics at 24-48 hr. Presumptive colonies of *S. aureus* were selected and sub cultured on nutrient agar (Oxoid, UK) and incubated aerobically at 37°C for 24-48 hr. Bacteria were identified according to their gram reaction, morphology, catalase test, tube coagulase test and mannitol fermentation. Samples were considered positive for *S. aureus* when at least one colony was identified as *S. aureus*.

Antimicrobial Resistance Test

After isolation and identification of *S. aureus*, then 30 isolates were evaluated using disk diffusion method for their antimicrobial resistance to 5 different antimicrobial drugs (Tetracycline, Streptomycin, Penicillin G, Ciprofloxacin and Sulfamethoxazole-Trimethoprim). Antimicrobial resistance testing was carried out in accordance with the guidelines published by the Clinical and Laboratory Standards Institute [15]. Selected isolates were first cultured in Tryptone soya broth overnight. A suspension of each test isolate was prepared in 0.9 % saline water to a turbidity equivalent to a 0.5 Mc Farland standard. Each suspension was streaked onto Mueller Hinton Agar (Oxoid, UK) by sterile swab following which antimicrobial discs (Oxoid, UK) were positioned onto the plates. Inoculated plates were incubated at 35-37°C for 24 hr (\pm 2 hr). The zones of inhibition were measured to the nearest millimeter and interpreted according to Clinical and Laboratory Standards Institute methods [15].

Data Analysis

The data was analyzed by using SPSS software version 20. The effect of risk factors such as breed, age, lactation stage and parity with possible association of the bacteria was analyzed using univariate logistic regression. Values were considered significant at $P < 0.05$ was considered significant in all analysis.

Result

Prevalence and Associated Risk Factors

From a total of 140 lactating cow's milk examined, the prevalence of *S. aureus* was 32.14% (45/140). The prevalence of *S. aureus* showed significant variation among different breeds ($P= 0.001$), age ($P= 0.035$) and Parity ($P= 0.001$). But there was no significant difference of *S. aureus* prevalence among different stage of lactation. The prevalence of *S. aureus* was higher in cows that were cross breeds, were above five years and had parity of above two.

Factors	Category	Total	Positive	Percent	P value	OR	LCL	UCL
Breed	Cross	80	35	43.8	0.001	3.889	1.730	8.742
	Local	60	10	16.7				
Age	>5	88	34	38.6	0.035	2.347	1.063	5.181
	<5	52	11	21.2				
Stage of Lactation	Mid	33	10	30.3	0.836	0.833	0.446	1.555
	Late	57	23	40.3				
	Early	50	12	24				
Parity	>2	85	37	43.5	0.001	4.529	1.910	10.740
	<2	55	8	14.5				

OR= Odds Ratio; LCL= Lower Confidence Level; UCL= Upper Confidence Level.

Table 1: Univariable Logistic Regression Analysis of the Association of Different Potential Risk Factors Associated with Prevalence of *S. Aureus* ($N =140$).

Antimicrobial Resistance Test

30 *S. aureus* isolates were subjected to antibiotic susceptibility tests and 5 antimicrobial agents, from different antibiotic classes were used. In this study *S. aureus* were found to be highly susceptible to chloramphenicol (100%). However *S. aureus* isolates were highly resistant to Penicillin G (93.3%), Streptomycin (53.3%), Tetracycline (40%) and Sulfamethoxazole-trimethoprim (26.7%).

Antimicrobials	Resistance	Intermediate	Susceptible
	No (%)	No (%)	No (%)
Tetracycline	12(40)	8(26.7)	10(33.3)
Ciprofloxacin	0(0)	0(0)	30(100)
Streptomycine	16(53.3)	5(16.7)	9(30)
Penicillin G	28(93.3)	-	2(6.7)
Sulfamethoxazole trimethoprim	8(26.7)	7(23.3)	15(50)

Table 2: Resistance of *S.aureus* Isolates to Different Antimicrobials ($n = 30$).

Discussion

The prevalence of *S. aureus* that was 32.14% in lactating dairy cows milk reported in this study is lower than the reports of 46.7% in cows by Abera., *et al.* [16] and 40.6% by Daka., *et al.* [17]. But it is higher than the study report of Binyam [12]. This study revealed the prevalence of *S. aureus* to be affected significantly with breed, age and parity. The prevalence of *S. aureus* was higher in cows that were cross breeds, were above five years and had parity of above two. The higher prevalence of *S. aureus* in cows greater than five years in this study is in agreement with the report of Abera., *et al.* [16]. The insignificant difference of *S. aureus* prevalence between different stages of

lactation in this study disagrees with the report of Abera, *et al.* [16] that revealed the significant difference among lactation stages. The differences in prevalence of *S. aureus* between different reports could be due to differences in farm management practices, study methodologies and time of sample collection and processing. The results of antimicrobial susceptibility testing, *S. aureus* was highly susceptible to Ciprofloxacin (100%). *S. aureus* isolates were highly resistant to Penicillin G (93.3%), Streptomycin (53.3%), Tetracycline (40%) and Sulfamethoxazole-trimethoprim (26.7%). The higher resistance of Penicillin G is comparable with the resistance report of Penicillin G (94.4%) by Abera, *et al.* [16]. The resistance of *S. aureus* to Penicillin may be due to the production of beta lactamase, an enzyme that inactivates Penicillin. There is higher prevalence of *S. aureus* in lactating dairy cow's milk in the study area that needs further attention and investigation in line with its public health impact. Antibiotics are used unconsciously to treat mastitis in different small holder dairy farms and it is observed that the level of antibiotic resistance has been rising more and more. Therefore restrictions on the irrational use of antibiotics should be applied and establishment of standardized monitoring systems in dairy farms are required.

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