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Study on the Prevalence and Distribution of *Staphylococcus aureus* in Raw Cow Milk Originated from Alage Atvet College Dairy Farm, Ethiopia

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Abstract

This investigation was carried out from October 2011-June 2012 to determine the prevalence of *Staphylococcus* in milk and rate of contamination at farm and milk distribution tank in Alage Agricultural Technical Vocational Educational Training College. Milk samples (row) were cultured on sheep blood agar and incubated. The plates were examined for gross colony morphology, pigmentation and haemolytic characteristics at 24-48 h then presumptive colonies of *Staphylococcus aureus* was selected and sub cultured on nutrient agar and incubated. Then, bacteria were identified according to its Gram reaction, morphology and the catalase test, tube coagulase test (4 h), haemolysis, pigment production (golden yellow), mannitol and maltose fermentation were used. A total of 170 milk sample of cow were examined for bacteriological status of *S. aureus*, an overall 28.2% prevalence of *Staphylococcus* was found. From this, 21.2% was directly from the farm and 35.3% were from milk distributing site to consumers. The total prevalence of *Staphylococcus* varied among the sample taking site. The prevalence of *S. aureus* from distributing raw milk site (35.3%) was significantly higher than the prevalence of *S. aureus* from samples taken directly from the farm (21.2%). From this the study it is considered that Staphylococcosis a potential hazard for the public and contamination rate is high in distribution site which needs improvement of the hygienic status of the personnel's at distribution site.

Keywords: Cow; Prevalence; Raw milk; *Staphylococcus aureus*

Introduction

Food must be visibly clean and free from noxious materials. It should be also nourishing and attractive as the aim of food hygiene should be the production and service of food which is both safe and suitable for consumption. However, most foods contain viable bacteria unless thoroughly heated or made sterile. Contamination of food products with pathogenic organisms may influence considerably their harmlessness, endanger the health of consumers and decrease shelf quality resulting in food-borne infections, intoxications and economic losses from food spoilage [1].

Staphylococcus aureus is an important food-borne pathogen. It is a versatile pathogen of both animals and humans and causes a variety of diseases with severity of slight skin infection to highly severe diseases like septicemia and pneumonia. *S. aureus* is present on the skin and mucosa of food-producing animal reservoirs, such as ruminants and it is mostly associated to subclinical mastitis leading to contamination of milk and dairy products [2].

The first description of food poisoning caused by staphylococci is thought to be that of Vaughan and Sternberg who investigated a large outbreak of illness in Michigan believed to have been caused by cheese contaminated with Staphylococci. Clear association of the organisms with food borne illness had to wait until Barber (in 1914) demonstrated that Staphylococci were able to cause poisoning by consuming milk from a cow with *Staphylococcal mastitis*. In 1930, Dack showed that staphylococcal food poisoning was caused by a filterable enterotoxin [3].

Staphylococcal Food Poisoning (SFP) is one of the most commonly occurring Food Borne Disease (FBD) worldwide with high occurrence second to salmonellosis [4]. In U.S. the annual number of SFP cases is 185,000 (with about 1750 hospitalizations) [5], and in Europe *S. aureus* caused 5.1% of the food-borne outbreaks between 1993 and 1998 [6].

In spite of the aforementioned prevailing situation and the presence of a number of public health problems due to food borne disease resulting from the consumption of different food items in Ethiopia there is paucity of well-documented information on the occurrence of *Staphylococcus* in raw bovine bulk milk [7].

Therefore, the objectives of this study were:

- To the prevalence and distribution of *Staphylococcus aureus* in raw bovine milk produced at Alage ATVET college dairy farm.
- To determine the risk of contamination at farm and distribution site.

Materials and Methods

Study area

The study was conducted at Alage Agricultural Technical and Vocational Education and Training (ATVET) College which is located about 217 kms south west of Addis Ababa and 32 km west of Bulbla town. The total area of the college covers 4200 hectares and it is situated at longitude of about 38°30' East and latitude of 07°30' North. It lies at an altitude of 1600 m above sea level in the agro-ecologically dry plateau of the south western part of the Ethiopian central rift valley. The area has three distinct seasons, namely main rainy (June to September), short rainy (March to May), and dry (October to

February) seasons. Based on ten years data (1996-2005), the mean annual rainfall of the area is 800 mm, with mean minimum and maximum temperatures of 11 and 29°C, respectively [8].

Study sample

The study was conducted on milk from apparently healthy exotic cows from Alage ATVET college.

Study design

Type of study

A longitudinal study was conducted from October 2011-June 2012. Sampling was carried out repeatedly from each critical point which is supposed to be the major risk areas for the consumers along the food value chain.

Sample collection and transportation

Samples collected from pooled udder milk of all lactating cows in the farm. Samples of milk were collected from milking bucket at the farm and storage tanks at sell (distribution) point were taken as well. During sampling of raw milk from the udder, the surface of the teat end was cleaned by wiping it with clean cotton dipped in 70% alcohol. Before sampling milk from milking bucket and storage container, the milk was thoroughly mixed after which 25 ml of milk was transferred into sterile screw capped sampling bottles aseptically. All, sample containing bottles then labeled and transported in an icebox to the Microbiology laboratory, Department of Animal Health at Alage ATVET College. Upon arrival, the samples were stored in a refrigerator at 4°C until analyzed.

Study methodology

Samples was kept in a refrigerator at 4°C until use and thawed for 3-5 h at room temperature when needed. Each raw milk sample was centrifuged separately and the pellets streaked directly onto 7% sheep blood agar and incubated aerobically at 37°C for 24-48 h [9].

The plates were examined for gross colony morphology, pigmentation and haemolytic characteristics at 24-48 h.

Presumptive colonies of *Staphylococcus aureus* was selected and sub cultured on nutrient agar and incubated aerobically at 37°C for 24-48 h to get a pure culture. Then, bacteria were identified according to its Gram reaction, morphology and the catalase test. *S. aureus* then identified by the tube coagulase test (4 h), haemolysis, pigment production (golden yellow), mannitol and maltose fermentation. Samples were considered positive for *S. aureus* when at least one colony is identified as *S. aureus*.

Gram's staining was used to determine gram's reaction, size, and shape and cell arrangements. Catalase test was performed by mixing bacterial culture with a drop of 3% H_2O_2 on a clean glass slide. The catalase positive cocci were considered as staphylococci. Yellowish discoloration of Mannitol Salt Agar (MSA) used as confirmative identification of the salt tolerant Staphylococci. For this, suspected colonies streaked on MSA plates and incubated at 37°C and examined after 24-48 h for growth and change in the colour of the medium.

The tube coagulase test was performed to differentiate pathogenic *Staphylococci* spp. from nonpathogenic spp. Sterile tubes containing 0.5 ml of selected isolates of *Staphylococcus*, grown on Tryptone Soya Broth (TSB) at 37°C for 24 h, and 0.5 ml of citrated rabbit plasma was 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 evaluated at 30 min intervals for the first 4 h of the test and then after 24 h 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) were visible within the tube and no degree of clotting taken as negative.

Staphylococcus aureus identified based on their maltose fermentation character on Purple Agar Base (PAB) media plate with 1% of maltose at 37°C for 24-48 h to differentiate pathogenic *staphylococci*, particularly coagulase positive isolates. *Staphylococcus aureus* rapidly ferment maltose and the acid metabolic products cause the pH indicator (bromocresol purple) to change the medium and colonies to yellow.

Sample size determination

The number of sample size was determined based on the desired absolute precision stated on Thrustfield [10]. 170 samples taken based on the expected prevalence of 29.1% found by Tesfaye [11].

Data collection, management and analysis

Data describing the presence of *Staphylococcus* in milk samples was classified filtered and coded using Microsoft Excel® 2007. The data was then be exported to SPSS windows version 18.0 (SPSS Inc., Chicago, IL) for appropriate statistical analysis. The prevalence of

Staphylococcus from all samples was determined by using descriptive statistics. Chi square (χ^2) was used and effects reported as statistically significant if p-value is less than 0.05 using 95% confidence intervals.

Results

Prevalence of *Staphylococcus aureus* in bovine milk

A total of 170 cow milk sample were examined for bacteriological status of *Staphylococcus aureus*, an overall 28.2% prevalence of *Staphylococcus* was found. From this, 21.2% (18 samples) was directly from the farm and 35.3% (30 samples) were from milk distributing site to consumers. The total prevalence of *Staphylococcus* varied among the sample taking site. The prevalence of *S. aureus* from distributing raw milk site (35.3%) was significantly higher than the prevalence of *S. aureus* from samples taken directly from the farm (21.2%) (Table 1).

Discussion

Milk contamination along the milk value chain could be high if there is no hygienic condition and cold chain facilities for transportation. Among the contaminants, *Staphlococcus aureus* is the one with high prevalence and potential public health hazard. So studying this contaminant is mandatory to see the hazard level of the milk distributed from Alage ATVET college dairy farm.

In the current study, *Staphylococcus aureus* was present in sample of raw milk both from the milk distributing tank and directly from the farm. The total prevalence in this study is in line with 29.1% [11] and 27% [12] in Debrezeit, Ethiopia. In the other side, the study done by Daka et al. [13], in Southern Ethiopia found very high prevalence than this study which is reported as 40.6%. The difference may come due to

the study site Southern Ethiopia includes small holders with a probability of high contamination due to poor hygienic standards.

Sampling site	No. of Sample	No. of positive	Prevalence (%)	p-value
Milk distributing site	85	30	35.3	0.041
Dairy farm	85	18	21.2	-
Total	170	48	28.2	-

Table 1: The prevalence of *Staphylococcus aureus* based on sample taking site.

There are so many results of prevalence by different researchers in different countries. For example in Morocco, it was found 40% prevalence of *S. aureus* [14], 36.9% in Palestine [15], 75% in Bangladesh [16] and 61% in India [17] which are higher than the result found in this study. The difference may be resulted from their sampling sites includes much number of local milk collection and distribution sites.

In the other hand there are researches done and found to have lower prevalence than this study like 18.18% prevalence of *S. aureus* found in Turkey by Ekici et al. [18]. The results in those studies are lower than this study might be due to environmental contamination at milk distribution site. In addition, those studies may have a better prevention and hygienic environment than study area of this research. Mastitis presence will also be the factor as Asperger and Zangeri said about 40% of the cases would be associated with the presence of mastitis. Environmental contamination during raw milk handling also results *S. aureus* to get a chance contaminating the milk [19-21].

There was a significantly high difference between the results found in Korea and this study as reported by Park et al. [22]. The lower prevalence (0.35%) in Korea than this study site could be the difference in farming system and indicate their high hygienic status.

A high rate of milk contamination found at collection centre in different studies. 75% of contamination found in Norway [20]. In this study the result showed a high rate of contamination at the milk collection centre than the farm. This may be due to poor hygienic handling and contamination from the environment and contamination of milk while mixing in a tank.

The high rate of milk contamination in distribution site than the farm was observed and this could be due to the contamination from human sources due to lack of hygiene, there could be also a transportation problem as there is no cold chain, the multiplication of the organism may also occur.

Conclusion and Recommendations

The presence of *Staphlococcus aureus* in raw milk both at farm level and bulk milk tank indicates that there is a potential hazard on the health of the public. The contamination was higher at collection centre than at the farm level that indicates proper handling of milk and awareness of hygiene is low among workers of the farm and distribution site. Therefore consumer should avoid consuming unpasteurized milk and milk quality control measures should be applied along the food value chain. In addition training should be given to personnel's of the farm, transportation method should be improved.

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