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Full Length Research Paper

Contamination of informally marketed bovine milk with *Staphylococcus aureus* in urban and peri urban areas of Debre-Zeit, Ethiopia

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A cross-sectional study was conducted from October, 2009 to March, 2010 to assess the level of contamination of informally marketed milk with *Staphylococcus aureus* at farms and milk collection centers in Debre-Zeit, Ethiopia. One hundred and seventy raw farm bulk milk samples, 25 mixed bulk milk samples and 20 pasteurized and packaged milk samples were collected from 14 milk collection centers and isolation and identification of *S. aureus* were carried out following standard method. *S. aureus* was isolated from 44% of farm bulk milk and 72% of milk collection centers' bulk milk and it was not detected from pasteurized milk samples. Contamination rates of farm bulk milk with *S. aureus* were significantly different among the collection centers (χ^2 = 31.8, df = 13, *p* = 0.003). The milk produced and collected in peri-urban areas was significantly more contaminated with *S. aureus* (64%) than milk produced and collected in urban areas (38%) (χ^2 = 7.18, df = 1, *p* = 0.007). The frequency of isolation of *S. aureus* in milk collection centers bulk milk varied between 67 and 100% among collection centers. However, the contamination rates were not significantly different among these collection centers (χ^2 = 1.5, df = 4, p = 0.83). The overall contamination rate at collection centers (72%) was significantly higher than that at the farm level (33%, χ^2 = 10.6, df = 1, p = 0.001). Overall, the study revealed that milk produced and collected in and around Debre-Zeit was found to be contaminated with *S. aureus*, raising the issue of quality control and improving the safety of milk to safeguard the consumer from associated health problems and enabling the producers to earn much more from milk sale.

Key words: Bulk milk, collection centers, Debre-Zeit, Ethiopia, *Staphylococcus aureus.*

INTRODUCTION

A facultative anaerobic Gram-positive bacterium, *Staphylococcus aureus,* is a major cause of food borne intoxications and outbreaks throughout the world because of its ubiquity and its ability to persist and grow under various conditions. It is able to survive and multiply in a variety of food substrates, at a variety of temperatures (7 to 48°C) and pH values (4.5 to 9.3) and at water activities of (0.83 to 0.99) (Shah, 2003).

S. aureus can cause Staphylococcal food poisoning (SFP) by ingestion of preformed toxin produced under specific environmental conditions when the population density of the pathogen reaches 10^5 CFU/ml. This bacterial load allows the production of 20 ng to 1 µg of SE sufficient to determine symptoms of the poisoning in human beings (Shah, 2003; Lourdes et al., 2004; Todar, 2008).

Staphylococcal food poisoning is characterized by,

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nausea, vomiting, diarrhea, sweating, abdominal cramping, and prostration in human beings (Jay, 2000; Acco et al., 2003). The duration of illness typically is 1 to 2 days. However, it usually takes three days to recove completely and sometimes longer in severe cases (Jay, 2000; Aycicek et al., 2005).

Milk and dairy products are implicated as the sources of illness associated with milk collection and normal processing conditions that may allow the presence of bacteria in the dairy cows and the dairy environment to be introduced directly into the milk. Once introduced, the highly nutritive milk medium supports rapid microbial growth. Consequently, the potential for food borne illness and intoxication from consumption of milk and dairy products is of concern (Halpin-Dohnalek et al., 1989; Fujikawa and Morozumi, 2006).

Although, some outbreaks have been associated with pasteurized milk, pasteurization is still considered an extremely effective method for reducing bacterial pathogens in milk, and these outbreak events usually are rare (Cohen, 2000). However, not all milk and dairy products are pasteurized, and raw unpasteurized milk is widely consumed throughout the world. An increasing number of people are drinking raw unpasteurized milk and/or consuming products made from raw unpasteurized milk despite the well-documented hazards associated with this practice. Increased interest in raw milk consumption is likely associated with some people's desire to purchase locally and consume natural, unprocessed foods as well as the promotion of raw milk consumption by certain groups (Jayarao et al*.,* 2006; Oliver et al., 2009).

Raw milk has been reported to be a known vehicle for pathogens for more than 100 years. Outbreaks asso-ciated with the consumption of raw milk occur routinely. Consumption of raw milk is a high-risk behavior and will continue to cause morbidity and mortality until people stop it (Keene, 1999; Gillespie et al., 2003).

Majority of Ethiopian population consume raw milk and raw milk products including cheese, cream, butter and yoghurt (Ashenafi, 1990; Ashenafi and Beyene, 1994). Besides, informal marketing of raw milk and milk products in and around Debre-Zeit is very common (Abera, 2008). As a result, the possibility of occurrence of SFP due to consumption of dairy products is common in our country (Ashenafi and Beyene, 1994; Yilma et al., 2007). How-ever there is a paucity of information on the occurrence and magnitude of *S. aureus* in bovine milk at different points of milk value chain in the country. Therefore, the main objective of the study was to assess the contamination of informally marketed bovine milk with *S. aureus* in urban and peri urban areas of Debre-Zeit.

MATERIALS AND METHODS

Study area

The study was conducted in and around Debre-Zeit town, from October, 2009 to March, 2010. Debre-Zeit is located at 9°N and 40°E, in Oromia National Regional State about 47 km southeast of the capital city of Ethiopia, Addis Ababa. It has a human population of about 95,000. The altitude is about 1850 m above sea level. It experiences a bimodal pattern of rainfall with the main rainy season extending from June to September (of which 84% of rain is expected) and a short rainy season from March to May with an average annual rainfall of 800 mm. The mean annual minimum and maximum temperatures are 12.3 and 27.7^{o}C, respectively, with an overall average of 18.7°C. The highest temperatures re corded in May and the mean relative humidity is 61.3%. Debre-Zeit is the center of Ada'a-Liben Woreda. The Woreda has a total land area of about 1610.56 km^{2} and divided in to three agro-ecological zones namely midland (94%), highland (3%) and lowland (3%) (CSA, 2006).

Type and origin of samples

The study was conducted on raw bovine bulk milk at farm and at collection centers and on pasteurized milk of Ada'a-Liben district dairy and dairy product producer and marketing co-operative society. The cooperative has around three hundred and sixty eight members having a dairy farm that were operational during the study period and supply milk to fourteen collection centers.

Study design

A cross sectional study was conducted to determine the contamination of *S. aureus* in informally marketed milk at farm and milk collection centers and pasteurized in Debre-zeit.

Sampling and sampling size

Stratified random sampling technique was employed to take farm milk samples (170) from the fourteen milk collection centers. To calculate the total sample size, the following parameters were pre-determined: 95% level of confidence (CL), 5% desired level of precision, size of study population 368 (farms currently supplying milk to collection centers of the cooperatives) and with the assumption of 29.1% (Tesfaye, 2008) expected contamination of *S. aureus* in bulk milk at farm level. Then, the sample size was determined using the formula for sampling from finite population recommended by Thrusfield (2005). Purposive sampling technique was employed in sampling raw bovine bulk milk (25) and pasteurized milk (20) from five collection centers (milk shop), where milk sold to consumers, to assess the contamination rate of *S. aureus* resulting from cross contamination and post pasteurization contamination, respectively.

Sample collection and transportation

Each bulk milk samples was collected in a sterile snap-cap milk collection vial from dairy producers and collection centers. Briefly, milk samples in the bulk containers were agitated before collection, and samples taken from the top of the bulk tank using a sanitized dipper. Identification of samples was made by date of collection and sources (farm name and Milk collection centers (MCC)) of the milk. All samples were kept in an icebox containing ice packs and taken immediately to the microbiology laboratory of Addis Ababa University, school of Veterinary Medicine, Debre-Zeit for microbiological analysis. Upon arrival, the samples were stored overnight in a refrigerator at 4°C until examined the n ext day. Pasteurized milk samples were purchased from the milk shop and

Table 1. Contamination of *S. aureus* in farm bulk milk among milk collection centers.

Collection center	Number of samples	Positive	Frequency	*p*-value
Center 1	19	6	31.58	0.1172
Center 2	3	1	33.33	0.9517
Center 3	28	8	28.57	0.8250
Center 4	17	6	35.29	0.8135
Center 5	8	6	75.0	0.0498
Center 6	6	5	83.33	0.0474
Center 7	18	4	22.22	0.5235
Center 8	6	4	66.67	0.1413
Center 9	16	6	37.5	0.7134
Center 10	10	3	30.0	0.9304
Center 11	9	4	44.44	0.5090
Center 12	16	8	50.0	0.2711
Center 13	7	6	85.71	**0.0308**
Center 14	7	7	100	-
Total	170	74		

immediately transported to laboratory and cultured for bacteriological analysis.

Isolation and identification of *Staphylococcus aureus*

The bacteriological investigation was performed following the standard microbiological technique recommended by Quinn et al. (1999). A loopful of milk was streaked on sterile 5% sheep blood agar and the plates were incubated aerobically at 37°C and examined after 24 to 48 h of incubation for growth. The colonies were provisionally identified based on staining reaction with Gram's stain, morphology and hemolytic pattern. The representative colonies were sub cultured on blood agar plate and nutrient agar plates and incubated at 37°C for 24 h. Pure colonies were preserved and maintained for characterizing the isolates on nutrient slants. Catalase, oxidase, coagulase, mannitol fermentation and maltose fermentation tests were done to characterize the bacterium.

Data management and analysis

Laboratory analysis results were entered into MS-EXCEL and analyzed using SPSS (2002), Statistical package of version 15. Contamination rate was computed as the number positive samples for *S. aureus* by divided the total number of samples examined in each type of samples items. The GLM with binomial errors and Chi-square tests were applied to see the difference in the contamination rate of *S. aureus* among the collection centers, between farm bulk milk and collection centers bulk milk and between urban and peri urban farming. Statistical significance was accepted at $P < 0.05$.

RESULTS

Isolation and identification of *Staphylococcus aureus* from farm bulk milk

Out of 170 raw milk samples tested, 44% (74/170) were contaminated with *S. aureus*. Comparison of the proportion of milk contaminated with *S. aureus* among different collection centers showed a significant differences (χ^2 = 34.9, df = 13, p = 0.001). Among all the collection centers, the percentage of contaminated milk with *S. aureus* was significantly higher than average at center 5 (75.0%, p = 0.0498), center 6 (83.3%, p = 0.0474), center 13 (85.7%, p = 0.0308). A hundred percent of contamination rate was observed at center 14 milk collection centers (100%), Table 1).

The milk produced and collected in peri-urban areas was significantly more contaminated with *S. aureus* (25/39, 61.5%) than milk produced and collected in urban areas (50/131, 38.2%, χ^2 = 7.18, df = 1, p = 0.007). Farming at Urban area was a preventive factor for milk contamination with *S. aureus* (odds ratio 0.346, 95% CI (0.332, 0.360)) whereas farming in peri-urban areas was a risk factor for contamination of milk with *S. aureus* (odds ratio = 2.89, 95% CI: 2.78 to 3.01). (Table 2)

Isolation and identification *Staphylococcus aureus* from collection centers bulk milk

Out of 10 urban collection centers, only five centers sold raw milk to consumers. Out of 25 raw milk samples tested, 72% (18/25) were contaminated with *S. aureus*. The frequency of isolation of *S. aureus* varied between 66.7% and 100% among collection centers. However, the difference was not statistically significant(χ^2 = 1.497, df = 4, p = 0.827).

The farm results from these five collection centers (Table 1) were added up and compared with the results of milk collection centers. Accordingly, the contamination of *S. aureus* at collection centers (72.0%, 18/25, Table 3)

Table 2. Contamination of *Staphylococcus aureus* in urban and peri-urban dairy farms.

Dairy farm	Positive	Contamination (%)	Total
Peri-urban	25	64.1	39
Urban	50	38.2	131
Total	75	44.1	170

Table 3. Contamination rate of *S. aureus* in bulk tank raw milk at milk collection centers selling raw milk directly to consumers.

Collection center	Number of samples	Positive	Frequency (%)
Center 5	6	4	66.7
Center 6	4	3	75.0
Center 7	6	4	66.7
Center 8	2	2	100
Center 9	7	5	71.4
Total	25	18	72.0

was significantly higher than that at the farm level (32.9%, 28/85, Table 1, $\chi^2 = 10.56$, df = 1, $p = 0.001$).

Isolation and identification of *Staphylococcus aureus* from pasteurized milk

None of the twenty pasteurized milk samples tested to assess the survival of *S. aureus* at pasteurization temperature and possibility of post processing contamination yielded *S. aureus*.

DISCUSSION

In all cases of staphylococcal food poisoning, the foodstuff or one of the ingredients was contaminated with an SE-producing *S. aureus* strain. Many different foods can be a good growth medium for *S. aureus*, and have been implicated in staphylococcal food poisoning, including milk and cream, cream-filled pastries, butter, ham, cheeses, sausages, canned meat, salads, cooked meals and sandwich fillings (Loir et.al., 2003).

In this study, *S. aureus* was isolated in 44% (74/170) of the farm bulk milk samples and 72% (18/25) of the milk collection centers bulk milk samples. It was isolated from none of the pasteurized milk samples. The result showed a high contamination rate at milk collection centers, which might attributed to cross contamination of milk while bulking and poor handling across the dairy value chain. The contamination of *S. aureus* at collection centers was nearly in agreement with the pervious work (Wubete, 2004) where *S. aureus* was isolated at recovery rate of 75%. The result of the present study showed a slight lower contamination rate (43.5%) than a recent report from Norway where *S. aureus* was recovered in 75% of 220 bovine farm bulk milk samples (Jorgensen et al., 2005). However, the result was relatively higher than the previous works, 29.1% (Tesfaye, 2008) and 27% (Wubete, 2004) done in the same study area. Pasteuri-zation of commercially distributed milk has greatly reduced the risk of infection resulting from the consumption of contaminated milk (Jayarao et al., 2006). The present study found absence *S. aureus* from pasteurized milk samples. This shows that *S. aureus* were inactivated during the pasteurization process and absence of post pasteurization contamination. This might be from effective maintenance of pasteurization temperature and proper packaging of pasteurized milk.

Some of the *S. aureus* bacteria have become resistant to several antibiotics, mainly penicillins. A previous study on antimicrobial resistance revealed that more than 27% of the *S. aureus* isolates were found to be resistant to penicillin (Tesfaye, 2008). Most of the resistance to penicillin was a result of penicillinases, produced by the bacteria. Therefore, methicillin which is a β-lactam anti-biotic and insensitive to penicillinases, was widely used to cure *S. aureus* cases in humans. However, methicillin-resistant isolates were reported (Jevons, 1961). These Methicillin-resistant *S. aureus* can spread from human to human, from animal to human and most likely from human to animal too having significant role in public health issues (Loo et al., 2007). In this study we did not consider the antibiogram pattern of the isolates.

Conclusion

The study undertaken has shown that milk informally marketed in Debre-Zeit is contaminated with *S. aureus* with a higher contamination at collection centers than at farms. Hence, proper handling of milk along the milk

value chain ought to be exercised to increase the shelf life of milk. Furthermore, it should be subjected to pasteurization or heat treatment at least equivalent to pasteurization temperature so as to make it safe for human consumption. Finally, most importantly, a well approved quality control measures should be implemented along the milk value chain at least at milk collection centers to exclude contaminated and unhygienic milk from bulking other milk. Future study should focus on determination of sensitivity *S. aureus* to available drugs to select an appropriate drug effective in treating associated clinical problems.

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