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Research Article

Isolation and Identification of Staphylococcus Species from Cottage Cheese and Yoghurt in Selected Districts of East Wollega Zone, Ethiopia

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Abstract

A cross-sectional study was conducted to isolate and identify staphylococcus species from cottage cheese and yogurt in selected district of east Wollega zone, Ethiopia, from April 2017 to December 2017. A total of 188 milk product (62 cheeses and 126 yogurts) were collected from study areas. Of the total 188 milk product examined 69(36.7%) were positive for staphylococcus species. The result indicated that 25(13.3%) cottage cheese and 44(23.4%) yogurt samples were contaminated with staphylococcus species. The *S. aureus* was the most frequently isolated species among different samples accounting for 44(23.4%), followed by *S. intermedius* 11(5.9%), CNS 8(4.3%) and *S. hyicus* 6(3.2%). Prevalence of Staphylococcus were significantly higher ($p<0.05$) in plastics containers 76(40.4%) than others storage materials of milk product. This study revealed that, the prevalence of Staphylococcus species has statistically significant difference (P=0.002) among study districts of the area. The distribution of prevalent Staphylococci over different geographical area is indicators for lack of proper personal, environmental hygiene and sanitation; and absence of difference in animal husbandry practice in all study area. Hence, implementing strict hygienic control measures is important in order to guarantee the quality of cattle derivative food products.

Keywords: Staphylococcus Species; Cheese, Identification; Yogurt, East Wollega

Introduction

These days' food-borne diseases are becoming major public health concern worldwide particularly in the developing world. Among these food-borne concerns, consumption of contaminated raw milk either from infected cows or due to poor hygiene during production, handling, transportation and processing are prior issue in developing countries. The long tradition of consumption of fresh and fermented raw milk products was subject to an important change in the late 19th century, as the developed countries began wide-scale of pasteurization of milk to eliminate zoonotic bacterial pathogens [1].

Food borne pathogens causes illnesses and deaths in all populations, particularly in groups at risk such as infants, children, elderly and immunocompromised persons. The majority of the pathogens causing this significant disease burden are now considered to be zoonotic. The occurrence of some of these zoonotic pathogens seems to have increased significantly over recent years. The most important source of food borne disease is raw or improperly cooked food (meat and poultry, raw eggs, unpasteurized milk, shellfish and rice). Food handlers play a major role in ensuring food safety throughout the chain of food production [2]. Among the bacteria predominantly involved in these diseases, Staphylococcus aureus is leading cause of gastroenteritis resulting from the consumption of contaminated food. Staphylococcal food poisoning is due to the absorption of Staphylococcal enter toxins preformed in the food [3].

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. Contamination of milk and milk products with pathogenic bacteria is mainly due to processing, handling and unhygienic environment. The occurrence of these pathogenic bacteria in milk and milk products can cause severe health hazards to people as they are highly susceptible to variety of microorganism because

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of high nutritive value and complex chemical composition [4].

The importance of Staphylococcus aureus as a successful pathogen resides in its wide genetic diversity and host range and the different pathologies associated with infection. *S. aureus* is associated with hospital-acquired and community acquired infections and with human carriers [5] as well as livestock associated infections [6], for which Methicillin-Resistant S. Aureus (MRSA) and Methicillin-Sensitive S. Aureus (MSSA) isolates are important pathogens [7].

Culturally consumption of raw milk is common in Ethiopia and it is sometimes associated with bacterial diseases due to poor hygiene practice [8]. Illness caused by s*taphylococcus species* is acute food borne intoxication is highly spreading in developing countries like Ethiopia where food safety measurement has low consideration in general. To my knowledge, even though the occurrence of food borne intoxication is spreading in western oromia as the result of consumption of raw milk and milk product increasing, there is no scientific document on status of the microorganism identified in any food product in East Wollega zone.

✓ To identify species of staphylococcus from cheese and yogurt in Selected Districts of East Wollega Zone and

✓ To determine risk factors associated to occurrence of Staphylococcus in study area.

Materials and Methods

Study Area

The study was conducted in selected district of east Wollega zone, Ethiopia. The study was including different cafeterias, house storage and retailers located in (Sibu Sire, Jimma Arjo and, Nekemte town) of Eastern Wollega Zone, Oromiya regional state of Ethiopia. The area is found at 331 km of west of Addis Ababa, the capital city of Ethiopia. The area lies between 08°N 25 56 to 08°N 5805 and 034°E 33 41 to 035°E 28 48 and has average altitude of 1150 meters above sea level. The area has temperature of 33-35°C with more agricultural crops. The climatic condition alternates with long Summer May to August and short rainy seasons from March to April. The winter dry seasons (November to February) with mean annual rain fall of 1200mm [9]. Agriculture is the main livelihood in the area in which cattle and sheep kept as the major livestock which are highly important for the livelihood of the local population. The rearing system of cattle in study sites depends on natural grass and crop residues that kept in traditional management system [10].

Source of Study Population

All milk producing and processing center (cafeterias, house storage and retailers) located in each district and all of milk products available in and around indicated centers were included in this study.

Study Design and Period

A cross- sectional study design was used to undertake research work from May to December, 2017 in purposive selected districts of East Wollega zone depend on their potential milk productions. Cottage cheese and yogurt milk products were the samples used in this study. Cottage cheese and yogurt samples from cow milk were taken from different retailers, cafeterias and households storage container, which were supposed to be the major risk areas for the consumers as many people may share the pooled product.

Sampling Method and Sample size Determination

Simple random technique was employed to take cottage cheese and yogurt sample from selected different cafeterias and house storage and retailers. Using Thrusfield (2007) formula with 5% absolute precision at a 95% Confidence Interval (CI) the sample size for this study was calculated with expected prevalence of14.1% reported in Jimmazone [11], so that the required sample size for this study was estimated to be 188 samples.

Methods of Transportation and Submission of Samples

After collection of cottage cheese and yogurt samples, all samples were clearly labeled with appropriate identification number. After labeling, all samples were transported with ice box to the laboratory without delay. In the laboratory, samples were cultured immediately or stored at 4°C in any case of delay. Processing of specified samples was performed for isolation and identification of pathogenic bacteria based standard bacteriological tests in microbiology laboratory of school of Veterinary Medicine, Wollega University.

Isolation and Identification of Bacteria

Culture and isolation: All the samples was directly streaked onto 7% sheep blood agar and incubated aerobically at 37^0C for 24–48 hours. The plates were examined for the presence of Staphylococcus colonies. Isolates supposed to belong to Staphylococcus species on the basis of their morphological aspects (round, smooth and white or yellow colonies) and haemolytic pattern on the surface of blood agar plate. Presumed staphylococcal colonies were then sub-cultured on nutrient agar plates and incubated at 37^0C for 24-48 hours to get a pure culture (clone of cells derived from a single cell). After growth of presumptive colonies were identified by using conventional bacteriological techniques on the basis of colony characteristics, pigment production and hemolysis, final identification of the organisms and species was done based on Gram staining, catalase test, sugar fermentation and coagulase test. Pure cultures of a single colony type from nutrient agar plates were inoculated into nutrient slants and incubated at 37^0 C for 24-48 hours under aerobic culture conditions. The pure isolates in the nutrient slant were preserved and maintained at 4^0C for further need [12].

Biochemical tests: Then primary identification of suspected bacteria was performed based on Grams reaction, cellular morphology followed by other biochemical tests. All suspected cultures of Staphylococci species were subjected to Gram's stain and observed under a light microscope for Gram's reaction, size, shape and cell arrangements. The Gram-stained smears from typical colonies that showed Gram-positive cocci occurring in bunched, grapelike irregular clusters were taken as presumptive Staphylococcus species [12].

For Catalase test the center of an 18/24hour pure colony of the isolates were picked using a sterile loop form the nutrient agar plate and mixed with a drop of 3% H_2O_2 on a clean glass slide. If the organism positive, bubbles of oxygen liberated within a few seconds and the catalase negative isolates did not produce bubbles. The catalase positive cocci were considered as Staphylococci [12].

The colonies that were identified by Gram-staining reaction and catalase test as Staphylococcus were streaked on mannitol

salt agar plates and incubated at 37^{0}C for 24-48 hours to characterize the growth and change in the colour of the medium. The presence of growth and change of pH in the media (red to yellow colour) were regarded as confirmative identification of Staphylococci. Phenol red pH indicator detected the acidic metabolic product of mannitol. Fermentation of mannitol by S. *aureus* causes yellow discoloration of the medium. Colonies that develop weak or delayed yellow colour after 24hours of incubation were taken as *S. intermedius* and colonies that failed to produce any change on the medium were considered as *S. hyicus* and coagulase negative staphylococcus species [12].

The tube coagulase test was performed in sterile tubes by adding 0.5 ml of selected isolates of Staphylococcus grown on Tryptone Soya Broth (TSB) at 37^{0}C for 24 hours to 0.5 ml of fresh rabbit plasma. After mixing by gentle rotation, the tubes were incubated at 37^{0}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 was evaluated at 30 minutes intervals for the first 4 hours of the test and then after 24 hours 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) was visible within the tube and no degree of clotting would be taken as negative [12].

Purple Agar Base (PAB) with the addition of 1 % maltose was used to differentiate the pathogenic staphylococci, particularly the coagulase-positive isolates. The suspected culture was inoculated on PAB media plate with 1% of maltose and incubated at 37^{0}C for 24-48 hours. The identification was based on the fact that *S. aureus* rapidly ferment maltose and the acid metabolic products cause the pH indicator (bromocresol purple) to change the medium and colonies to yellow. *S. intermedius* gives a weak or delayed reaction and *S. hicus* did not ferment maltose but attacks the peptone in the medium producing an alkaline reaction (a deeper purple) around the colonies [12].

Data Management and Analysis

All collected date and laboratory results were entered into Excel databases and analyze using SPSS version 20 software programs. Descriptive statistics such as percentages and frequency distribution was used to describe the nature and the characteristics of the data to describe/present bacterial isolates. Moreover, comparison between each study districts, sample source, sample type, study subject against each test was done. Logistic regression was used to reveal the strength of the association of the potential risk factors with positivity of samples. In this line, the degree of association between risk factors and the prevalence of Staphylococcus was analyzed using test Odds Ratio (OR). In all analysis, the level of significance was set at 95% confidence interval with P<0.05 for significance value.

Results

Presence of Staphylococci was detected in 69 (36.7%) out of 188 analyzed samples. The contamination rate of the sample from yogurt 44(23.4%) was higher than that of the sample from cheese having prevalence 25(13.3%). The contamination of the sample was higher in house storage as compared with cafeterias and retailers sample source (Table 1). On the other hands, the prevalence of Staphylococci has statically significant difference (P=0.002) among geographic location of the area sampled. The prevalence of staphylococcus species has statically significant difference (P=0.00) among type of container used. Milk collected from pots was the most contaminated with Staphylococci with a prevalence of 34(18.1%) followed by kabes 24(12.8%) milk products (Table 1).

Table 1: Association between factors for occurrence of Staphylococci in cottage cheese and yogurt in selected areas of the East Wollega zone.

	Positive results n (%)	P –value	Odd ratio	95% CI	
Type of samples				**Lower**	**Upper**
Cottage cheese	25(13.3)	0.470	0.794	0.425	1.485
Yogurt	44(23.4)		1		
Districts					
Nekemte areas	15(8.0)	0.276	0.60	0.24	1.49
JimmaArjo	43(22.9)	0.002	0.29	0.13	0.64
Sibu Sire	11(5.9)		1		
Source of samples					
House storage	49(26.1)	0.380	0.62	0.21	1.81
Cafeteria	15(8.0)	0.053	0.28	0.08	1.01
Retailers	5(2.7)		1		
Containerof samples					
Plastics	3(1.6)	0.000	13.90	3.27	58.97
Pots	34(18.1)	0.011	0.25	0.08	0.72
Clay pots (Kabes)	24(12.8)	0.097	0.40	0.13	1.17
Churners	8(4.3)		1		

The prevalence of staphylococcus species is varying among different sample as indicated in table below. High prevalence of *S.aureus* was recorded in Yogurt (13.3%) as compared to cottage cheese (10.1%). *S.aureus* was the leading in predominance with a total of 16.5% isolates, of the total isolates of Staphylococci species followed by *S. intermidius* 3.7%, CNS 3.2% and *S. hycus* of 2.7% on sample collected from house storage. The prevalence staphylococcus *aureus i*n storage house, cafeteria and retailers were at 16.5%, 5.3% and 1.6% respectively. Milk product and by product contaminations with staphylococcus species was higher in clay pots containers as compared with plastics, kabe and churners used for samples storage (Table 2).

Table 2: Prevalence of staphylococcus species with their respective associated risk factors.

Variables	Species of staphylococcus (%)				
Types of samples	***S. aureus***	***S. intermedius***	***S. hycus***	**CNS**	**other bacteria**
Cottage Cheese	19(10.1)	2(1.1)	1(.5)	3(1.6)	37(19.7)
Yogurt	25(13.3)	9(4.8)	5(2.7)	5(2.7)	82(43.6)
Source of samples					
House storage	31(16.5)	7(3.7)	5(2.7)	6(3.2)	91(48.4)
Cafeteria	10(5.3)	3(1.6)	1(0.5)	1(0.5)	13(6.9)
Retailers	3(1.6)	1(0.5)	0(0.0)	1(0.5)	15(8.0)
Districts					
Nekemte town	10(5.3)	3(1.6)	1(0.5)	1(0.5)	33(17.6)
JimmaArjo	26(13.8)	8(4.3)	3(1.6)	6(3.2)	46(24.5)
Sibu Sire	8(4.3)	0(0.0)	2(1.1)	1(0.5)	40(21.3)
Containers					
Plastic	2(1.1)	1(0.5)	0(0.0)	0(0.0)	73(38.8)
Clay Pot	22(11.7)	7(3.7)	1(0.5)	4(2.1)	15(8.0)
Kabe	15(8.0)	2(1.1)	5(2.7)	2)1.1)	17(9.0)
Churner	5(2.7)	1(0.5)	0(0.0)	2(1.1)	14(7.4)

Discussion

Staphylococcus species is a major opportunistic pathogen in humans and one of the most important pathogenic bacteria in veterinary medicine. *S. aureus* is dangerous because of its deleterious effects on animal health and its potential for transmission from animals to humans and vice-versa [13]. It is prevalent food borne bacterial pathogens that cause food poising in human when it ingested in contaminated foods including dairy product such as cottage cheese and yoghurt. In the present study, the existence of Staphylococci was detected in 69 out of 188 examined samples. The analyzed data was conducted on Cottage cheese and Yogurt that were collected from housed hold, retailers and cafeteria.

The types of sample specific prevalence of Staphylococcus were found to be 13.3% and 23.4%, from cottage cheese and yogurt samples, respectively. 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. Contamination of milk and milk products with pathogenic bacteria is mainly due to processing, handling and unhygienic environment [4]. In contrary to this finding, the highest prevalence of *S. aureus* was found in traditional cheese (11.1%), followed by traditional ice-cream (5.9%), cream (5.6%), and butter (5.3%) [14]. The high prevalence staphylococcus species recorded in current finding in yogurt than in cottage cheese might be due to yogurt was the most examined samples than cottage cheese and difference in preparation.

From a total of 69 Staphylococci isolates originated from different sources, the highest 49(26.1%) prevalence was recorded in house storage followed by 15(8.0%) cafeteria and 5(2.7%) retailers' were contaminated. In modern milking systems, *S. aureus* is a common pathogen in cow's udders. The agent is transmitted by means of milking machines or the milker's hands, and enters through the milk duct or superficial lesions on the teat [14]. In addition to milking machine and milk handlers; and the common source of samples contamination might be due to poor hygienic condition of milk storage and transportation processes through food chain, as indicators in this result as high as 26.1% prevalence of staphylococci in milk stored in house storage.

The contamination rate of the sample from pots (18.1%) and plastics (1.6%) were statistically significantly (P=0.01) higher than that of the sample from churner (4.3%). A high prevalence of Staphylococcus was recorded in farm raw tank milk than in farm tanks swab and buckets swab. This finding comparable with finding of [16,17] who reported 33% and 29.5% Staphylococcus prevalence in tank milk, respectively, in Debrezeit, Ethiopia. Factors that could be hypothesized to be causes of contamination of milk in this study include absence of pre-milking udder cleaning, cleaning of milkers' hands, milking buckets and storage containers. Plastic containers have characteristics that make them unsuitable for milking and milk handling. According to [18] plastic containers scratch easily and provide hiding places for bacteria during cleaning and sanitization and plastic containers are poor conductor of heat and hence will hinder effective sanitization by heat. Additionally, the numbers of personnel working at milking environment and milk handlers were higher which might have contributed to milk contamination.

Coagulase-positive Staphylococci (32.5%) isolates was dominated in prevalence than the coagulase negative Staphylococci with prevalence rate of 4.3%. This is similar with the finding of [19] who reported that Coagulase Negative Staphylococci (CNS) is the second most prevalent pathogens next to coagulase positive Staphylococcus in Ambo, Ethiopia. Moreover, the results are agreement with the finding of [20] in central Ethiopia, who reported that Coagulase-positive staphylococci isolates dominated in prevalence than the coagulase negative Staphylococci. This finding is in contrary to the findings of [21] who reported coagulase-negative Staphylococcus (54%) is the predominantly prevailing isolates. The differences in prevalence reports of Staphylococcus species in the present study and other reports could be attributable to difference in sample type, differences in the origin of the samples or by geographical location.

In this study, the overall prevalence of Staphylococcus in the study areas was 36.7%. This finding is higher than that of [22] 28% in Mekele, [23] 12%, [24] 2.28% in Jimma and [25] 14.1% prevalence in Jimma zone. On the other hands the current 36.7% of overall prevalence of staphylococcus species in east Wollega zone is less than the finding of [20] who reported that 39.4% from central Ethiopia. The inconsistence of these results among different findings is might be due to the difference in handling practices and geographical location among studies sites.

High prevalence of Staphylococcus *aureus* was recorded in Yogurt (13.3%) as compared to cheese (10.1%). *S.aureus* was the leading in predominance with a total of 16.5% isolates, followed by *S. intermidius* 3.7%, CNS 3.2% and *S. hycus* of 2.7% on sample collected from house storage. This is agreement with the previous reported which indicated that, the highest isolation rate was observed in raw milk samples (56%) followed by yoghurt samples (22%), white soft cheese samples and pasteurized milk samples (4% each) then meat and (2%)meat products samples [26].

In this study, the overall prevalence of *S. aureus* analyzed from milk product and by product was significant higher in Jimmaarjoat 13.8% than Nekemte town5.3% and Sibu sire 4.3%. The contamination of milk from all sample's collected areas are indicated that the pathogen is circulating in study areas, however, there is no statistically significance differences was observed among the study areas.

Conclusion

This study indicated that the high prevalence of Staphylococci species isolated from cheese and Yogurts. The *S. aureus* was the highest frequently isolated species followed by *S. intermedius*, CNS and *S. hyicus* respectively. Majority of risk factors analysis revealed that prevalence of Staphylococcus is statistically significant difference, however the prevalence of the pathogen is not statistical significantly variation with type of sample (cheese and Yogurt). The finding indicated that pots, plastic, kabe and churner storing contraries are a source of contamination of milk with bacteria Staphylococcus and their contamination were indicators for poor hygienic practice. The contamination of milk product and by product recorded in all studies areas concluded that lack of proper personal, environmental hygiene and sanitation; and absence of difference in animal husbandry practice in the study area. Based on the current findings the community should take attention to clean milk containers and milking environment before milk processing. Awareness creation is required for dairy cow owners and milkers, regarding the importance of regular health checkups of dairy cattle, adequate udder preparation, hygienic milking technique, and practices on sanitary condition of milker's hands.

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