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Isolation and Identification of *Staphylococcus* Species from Ethiopian Cottage Cheese (Ayib) in Debre Zeit, Ethiopia

[1]Mekonnen Addis, [2]Mahindra Pal and [2]Moses N. Kyule
[1]College of Agriculture and Veterinary Medicine, Jimma University, Jimma, Ethiopia
[2]Faculty of Veterinary Medicine, Addis Ababa University, Debre Zeit, Ethiopia

Abstract: In this study, investigation of the presence of Staphylococcus and determination of its prevalence and distribution, identification of *Staphylococcus* species and determination of their prevalence and distribution and characterization of the isolates in order to determine their ability in synthesizing coagulase from Ethiopian cottage cheese (Ayib) were conducted from October 2008-April 2009 in Debre Zeit, Ethiopia. About 200 cottage cheese (Ayib) samples were analyzed. The identification results showed 24%, Staphylococcus prevalence. The 48 isolates proved to be Staphylococcus were tested for species assignment. They were grouped into *S. aureus* with 10 (5%) isolates, *S. intermedius* with 11 (5.5%) isolates, *S. hycius* with 8 (4%) isolates and coagulase negative staphylococci with 19 (9.5%) isolates. All the isolates were tested for the production of coagulase to determine their pathogenicity. The prevalence of coagulase positive staphylococci in the study were found to be 14.5%. The high level of Staphylococcus isolate found in the Ethiopian cottage cheese (Ayib) in the present study represent a poor keeping quality and public health risk to the consumer. This suggests the need to implement strict hygienic control measures along the food chain to improve the hygienic conditions during manufacturing, handling, storage and commercialization of cheese in order to guarantee the quality of this highly popular product in Debre Zeit, Ethiopia in order to decrease the risk of staphylococcal food poisoning.

Key words: Ethiopian cottage cheese (Ayib), Debre Zeit, prevalence, staphylococcus, poisoning, Ethopia

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 (Le Loir *et al*., 2003; Ash, 1997). The safety of cheese with respect to food borne diseases is of great concern around the world (Yilma *et al*., 2007).

This is especially true in Ethiopia where the consumption of Ethiopian cottage cheese (Ayib) which is typically manufactured in small dairy farms under poor hygienic conditions are common practices (Wubete, 2004).

In spite of the aforementioned prevailing situation and the presence of a number of public health problems due to food borne diseases resulting from the consumption of different food items in Ethiopia there is paucity of well-documented information on the occurrence of Staphylococcus in Ethiopian cottage cheese (Ayib). Therefore, this study was designed to investigate the presence of Staphylococcus and identify *Staphylococcus* species and determine their prevalence and distribution in Ethiopian cottage cheese (Ayib) as well as to characterize the isolates in order to determine their ability in synthesizing coagulase.

MATERIALS AND METHODS

Origin of samples and description of open market establishments: Ethiopian cottage cheese (Ayib) samples were collected from retail outlets by purchasing from retailers in Debre Zeit, Ethiopia, open market establishments. Samples were taken from cottage cheese that originated from the various vicinity of the town and sold at open market establishments.

The vendors displayed cottage cheese in plastic buckets, polyethylene bags and clay pots covered with clothes, leaves or plastics. Based on visual assessment the hygienic status of the containers, retailing outlets and the vendors themselves was unsatisfactory. In the course of this study, it has been observed that there was lack of hygienic measures in the handling of cheese for sale. In addition, the method of their retailing was entirely based on tradition and the selling outlets of cheese were dusty. Moreover, the vendors frequently took a slice of cheese

Corresponding Author: Mekonnen Addis, College of Agriculture and Veterinary Medicine, Jimma University, Jimma, Ethiopia

with hands or unclean laden for organoleptic test such as colour, taste and smell to the customers. The customers returned the extra cheese to the retailers and put back to the original container.

Study type: A cross-sectional study was conducted from October 2008-April 2009. Sampling was carried out repeatedly at retail outlets in Debre Zeit, Ethiopia open market establishments.

Sample collection and transportation: Samples were collected at retail outlets in Debre Zeit open market every Saturday when farmers often offer their products. After removing the external surface of approximately 2-2.5 cm depth, about 100 g of cheese was sampled from each vendor selected for the study. Samples were aseptically collected and put into a sterile screw capped bottles and kept in an icebox containing ice packs and taken immediately to the laboratory of Microbiology at the Faculty of Veterinary Medicine, Addis Ababa University, Debre Zeit, Ethiopia. Upon arrival, the samples were stored overnight in a refrigerator at 4°C until analyzed the next day.

Study methodology: Samples which were kept for overnight in a refrigerator at 4°C were thawed for 3-5 h at room temperature. About 25 g of each cottage cheese was stirred separately into 225 mL of sterile Buffered Peptone Water (BPW) in a sterile stomacher bag.

The pre-enriched samples were homogenized in a stomacher (Lab-Blender 400) for 2 min and incubated aerobically at 37°C for 24 h. Following this, 0.1 mL or a loopful of the pre-enriched broth of the various dilutions were streaked (seeded) aseptically onto sterile Blood Agar Plates (BAP) enriched with 7% heparinized sheep blood and incubated at 37°C for 24-48 h under aerobic culture conditions. The plates were examined for the presence of Staphylococcus colonies. Isolates supposed to belong to *Staphylococcus* species on the basis of their morphological aspects (creamy, greyish, white or yellow colonies) and haemolytic pattern on the surface of BAP were collected. Presumed staphylococcal colonies were then sub-cultured on Nutrient Agar Plates (NAP) and incubated at 37°C for 24-48 h to get a pure culture (clone of cells derived from a single cell). The pure isolates in the NAP were preserved and maintained for biochemical differentiation tests and characterizing the isolates.

Isolation and identification of *Staphylococcus* species: Final identification of staphylococci organisms and species assignment were done based on gram staining, catalase test, sugar fermentation and coagulase test.

Gram's staining: All suspected cultures of *Staphylococcus* 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.

Catalase test: Pure culture of the isolates were picked using a sterile loop from the agar slant and mixed with a drop of 3% H_2O_2 on a clean glass slide. If the organism was positive, bubbles of oxygen were liberated within a few seconds and the catalase negative isolates did not produce bubbles. The catalase positive cocci were considered as staphylococci.

Mannitol salt agar: The colonies that were identified by gram-staining and catalase test as Staphylococcus were 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 presence of growth and change of pH in the media (red to yellow colour) were regarded as confirmative identification of the salt tolerant staphylococci. Phenol red pH indicator detected the acidic metabolic product of mannitol. Fermentation of mannitol by *S. aureus* causes yellow discolouration of the medium. Colonies that develop weak or delayed yellow colour after 24 h of incubation were taken as *S. intermedius* and colonies that failed to produce any change on the medium were considered as *S. hyicus* and CNS (Fig. 1).

Coagulase test: 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°C for 24 h to 0.5 mL of citrated rabbit plasma. After mixing by gentle rotation, the tubes were incubated at

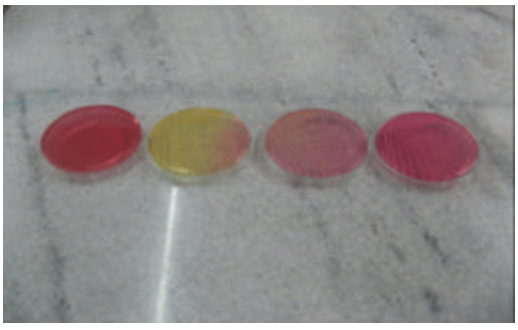

Fig. 1: Original mannitol salt agar, *S. aureus*, *S. intermedius and S. hyicus* and coagulase negative staphylococci (left to right)

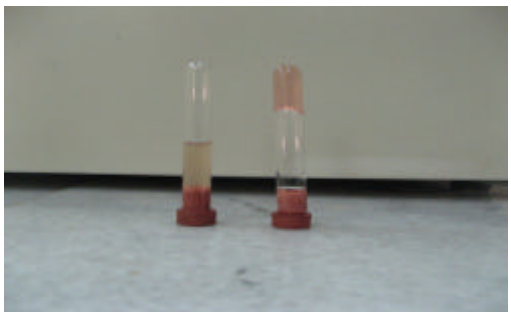

Fig. 2: Coagulase negative reaction showing no degree of clotting (left) and coagulase positive reaction showing a solid clot (right)

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 was 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) was visible within the tube and no degree of clotting would be taken as negative (Fig. 2).

Purple agar base: The suspected culture was inoculated on Purple Agar Base (PAB) media plate with 1% of maltose and incubated at 37°C for 24-48 h to differentiate pathogenic staphylococci, particularly coagulase-positive isolates. 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.

Data management and analysis: Prevalence of *Staphylococcus* and *Staphylococcus* species were computed as the number of each food items positive for *Staphylococcus* and *Staphylococcus* species divided by the sample sizes of food items examined. The 95% Confidence Interval (CI) of a proportion was used to calculate the lower and upper limits of the proportion of *Staphylococcus* and *Staphylococcus* species in the samples examined.

RESULTS

Prevalence and distribution of *Staphylococcus* in cottage cheese: About 200 Ethiopian cottage cheese (Ayib) samples were cultured for Staphylococcus. About 1 or more *Staphylococcus* species were detected by routine bacteriological investigation. The identification results proved 48 (24%) positive isolates of *Staphylococcus* species. All the isolates identified as staphylococci were tested for species assignment using biochemical characteristics. They were grouped into *S. aureus* with 10 (5%) isolates, *S. intermedius* with 11(5.5%) isolates, *S. hycius* with 8 (4%) isolates and CNS with 19 (9.5%) isolates (Table 1).

Table 1: Proportional distribution of *Staphylococcus* species from 200 cottage cheese samples

Species	Prevalence (%)	95% CI
S. aureus	5.0	2.74-8.960
S. intemidius	5.5	3.10-9.580
S. hicus	4.0	2.04-7.690
Coagulase negative staphylococci	9.5	6.17-14.36

Prevalence and distribution of coagulase positive staphylococci isolates: All isolates were characterized in order to determine their ability in synthesizing coagulase. The overall prevalence of CPS from cottage cheese samples was 14.5% (29/200).

Coagulase-positive staphylococci that were identified were divided into 3 groups comprising *S. aureus* (5%), *S. intermedius* (5.5%) and *S. hyicus* (4%) in the 200 samples examined.

DISSCUSSION

Food-borne diseases are of major concern, worldwide. *Staphylococcus* species are prevalent food-borne bacterial pathogens that cause food poisoning in humans when ingested in contaminated foods, including dairy products such as cheese. They cause Staphylococcus food poisoning by toxin production (Salandra *et al*., 2008). The organisms can gain access to raw milk and milk products either by direct excretion from udders having clinical and subclinical staphylococcal mastitis or by contamination from food handlers (Yilma *et al*., 2007). Proper sanitary measures are needed to improve the hygienic conditions during milking, storage, transport and manufacturing of cheese in order to guarantee the quality of this dairy product (Kaloreu *et al*., 2007). In this study, it was observed that there was lack of hygienic measures in the manufacturing, preparation, handling and storage of Ethiopian cottage cheese (Ayib).

In addition, the method of retailing was entirely based on tradition and the selling outlets of cheese were dusty. Bautista *et al*. (1988) found out that of 124 staphylococcal strains isolated from sheep milk, 78 (62.9%) produced enterotoxin, Martin *et al*. (2004) found that 15% of 157 Staphylococcus isolates from dairy products were enterotoxigenic whereas Morandi *et al*. (2007) reported

that 28.6% of staphylococci isolated from milk and dairy products were enterotoxin producers. The different rates of enterotoxin production found in these reports could be explained by the different techniques used in these studies, differences in the origin of the isolates or by geographical differences. Based on the above information, out of the 48 *Staphylococcus* species isolated and identified in this study some could be enterotoxigenic and the Ethiopian cottage cheese (Ayib) sold in open market establishments in Debre Zeit might be potential sources of food poisonings due to Staphylococcus to the public. *Staphylococcus aureus* was detected in 5% (10/200) of the Ethiopian cottage cheese (Ayib).

The present study revealed a lower prevalence rate than that reported by Lamprell *et al.* (2004) as 82.2% (852/1036) from cheeses made from raw cow's milk. *Staphylococcus intermidius* was detected in 5.5% (11/200) of the samples.

The prevalence of *S. intermidius* in the Ethiopian cottage cheese (Ayib) in the present research was lower than that reported by Lamprell *et al.* (2004) of 13.3% (138/1036) of cheese samples but >2% of 81 milk and milk product samples by Bendahou *et al.* (2008). *Staphylococcus hicus* was isolated in 4% (8/200) of the cheese. Bendahou *et al.* (2008) and Lamprell *et al.* (2004) recorded almost similar findings of 4% in 81 milk and milk product samples and 4.5% (46/1036) from cheeses, respectively.

CONCLUSION

The results showed that Coagulase Negative *Staphylococcus* (CNS) species more frequently occurred in the Ethiopian cottage cheese (Ayib), 19 (9.5%). The high number of CNS isolated in the current study may be due to lack of hygienic measures in the manufacturing, preparation, handling, storage of cottage and commercialization of cottage cheese and unhygienic milking. Also, the method of their sale is entirely based on tradition. Because CNS are a part of the normal teat skin flora and mucosa of humans and animals, some species are also found free-living in the environment (Kloos and Bannerman, 1994).

Coagulase production was described as one of the most reliable criteria for the identification of pathogenic *Staphylococcus* species (Quinn *et al*., 2002). In the present study, Coagulase Positive *Staphylococcus* (CPS) species were identified in 14.5% of the samples. The prevalence of CPS species in this study were found to be lower than Hamid and Owni (2007) who obtained prevalence of 38% of CPS in cheese collected in Sudan.

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