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Evaluation of Methicillin Resistance among *Staphylococcus aureus* Isolated from some Cream Field Bakery Products in Jimma Town

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ABSTRACT

Bakery products are moracious and staple foods consumed among most of the world's population. These products are prepared from cream, nut, nougat. These delicious foods can cause food borne diseases particularly halophile pathogenic microbes such as *Staphylococcus aureus* in case of poor hygienity. This study was aimed to evaluate the extent of cream filled bakery products contaminated with *Staphylococcus aureus*. A total of 30 samples were purchased from randomly selected cafeterias in Jimma town and transported to research and postgraduate laboratory of Biology department. A serial dilution was made accordingly and 0.1 mL aliquot sample was plated on pre-solidified Mannitol Salt Agar (MSA) and incubated at 35°C for 48 h. Samples were collected from three sites (Merkato, Agip and Kochi). The result of the study showed that the mean count (CFU g⁻¹) of *S. aureus* was dominant (6.3±0.1) in cream cake followed by donut (4.5±0.2) while the lowest mean count was recorded in zebib (4.3±0.2). Most (73.3%) of the food samples were positive for *S. aureus*. The isolates of *S. aureus* were resistant to penicillin G, methicillin and oxacillin, however; 75% of isolates were sensitive to erythromycin. Generally, the microbial safety of cream filled bakery products was poor and needs special attention.

Key words: Bakery products, cream filled, food poisoning, microbial load, *S. aureus*

INTRODUCTION

Currently, bakery products are getting attention day to day in the world, since they are delicious and endowed with high amount of calories. Most of cream filled bakery products like pastries generate sufficient amount of calories, providing good flavor and hence they are loved among children and youth, however, they are susceptible to microbial contamination during process and poor handling (Sami *et al.*, 2012).

Foods commonly contaminated with *S. aureus* include meat and its products, salads, cream filled bakery (Siriken *et al.*, 2009; Giannatale *et al.*, 2011) and dairy products. These items can be contaminated during preparation and subsequent mishandling or poor hygienity prior to consumption (Tasci *et al.*, 2011). The microbial safety of cream filled bakery products is influenced by raw materials by which they are being prepared, misprocessing and storage conditions (Hozova *et al.*, 2002). Food borne pathogens including *S. aureus*, *Salmonella* spp., *Bacillus cereus* and molds are a group of potential microorganisms that are able to cause food borne disease in human. Food poisoning is commonly caused by microbial toxins produced by *S. aureus*, *Clostridium botulinum* and etc., which are thermostable and fatal to human beings (Oonmetta-Aree, 2005).

S. aureus is a microflora of human skins which affect human health by causing diseases ranging from superficial infection to more severe one and hence humans and animals are the major reservoirs even though this bacterium is ubiquitous and found everywhere (Stewart *et al.*, 2003; Argudin *et al.*, 2010). Up to 30-50% of the human populations are carriers of *S. aureus* and this bacterium can cause skin and soft tissue infections such as abscesses, bloodstream infections, pneumonia, or bone and joint infections (Schmitt *et al.*, 1990). Bacteria are the leading cause of food borne disease and appear to be causative agents of more than two third of recorded infection (James, 2004).

The disease caused by *S. aureus* depends on the ability of the strain to survive and to multiply under extreme environment and the organism can produce extracellular substance like haemolysins, nuclease, protease, coagulase, lipase and enterotoxin (Temilade, 2009). *S. aureus* produces several extracellular proteases, including serine and cysteine proteases (Dubin, 2002).

S. aureus infection is difficult to treat because it is able to resist beta-lactam antibiotics (including, methicillin, dicloxacillin, nafcillin, oxacillin). *Staphylococcal* related infections are enhanced by its ability of biofilm production. *S. aureus* can avoid phagocytosis by producing cell wall capsule, or by aggregating in to biofilm (Szweda *et al.*, 2012).

Even though bakery products such as cream filled cake, pizza, donut, zebib and other locally prepared products are highly consumed among Ethiopian youngsters and females, for the best of our knowledge microbial safety (especially pathogenic microorganisms like *S. aureus*) of these products is still not documented yet. Moreover, some of the consumers of these staple foods emerging to complain the hygienity of these products due to discomfortness when using the bakery products which are currently appeared in Jimma town. To this effect, the present study was designed to evaluate *S. aureus* load on cream filled bakery products.

MATERIALS AND METHODS

Study area and period: The study was conducted in Jimma town, located 353 km south west of Addis Ababa and the capital city of Ethiopia (Fig. 1). The town is located at 7°41'N latitude, 36° 50' longitudes with average altitude of 1,780 m above sea level. The climatic condition of Jimma town is locally known as Weyna Dega which is favorable for agriculture and human settlement. The mean annual maximum and minimum temperature of the town is about 30 and 14°C, respectively. The annual rain fall ranges from 1138 to 1690 mm (Alemu *et al.*, 2011). The study period covered from February 2013 to May 2013.

Sample collection: A total of 102 samples were collected from cream filled bakery product selling cafeterias of Jimma town from three sites (Merkato, Agip and Kochi) starting from February to April 2013. The purchased samples were added in to sterile poly ethylene bag and transported to research and post graduate laboratory, department of Biology, College of Natural Science and Jimma University. The microbial analysis was done within 1-3 h of sample collection. The food samples were kept in refrigerator 4°C till microbial analysis done.

Sample preparation and microbial numeration: A 10 g cream filled bakery product sample was suspended in 90 mL of saline solution and homogenized in the Erlenmeyer flask for about 5 min using shaker at 160 rpm. The homogenized sample was serially diluted 10^{-1} to 10^{-6} . A 0.1 mL of aliquot was spread plated on MSA (Mannitol salt agar) from appropriate dilutions and incubated at 35°C for 36 to 48 h (Acco *et al.*, 2003). The colonies were counted from countable plate containing microbial colonies from 30 to 300 and expressed in colony forming unit per gram (CFU g⁻¹).

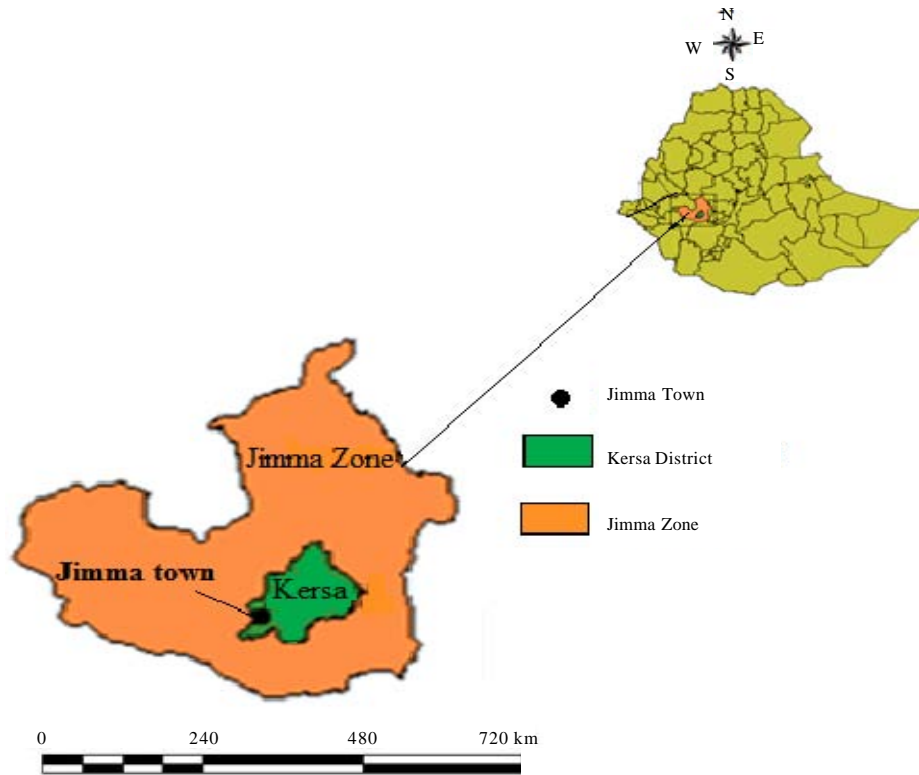


Fig. 1: Study area map: The figure shows that Ethiopia and study area, Jimma town where microbiological study took place for this study

Mannitol Salt Agar (MSA) Oxoid, Nutrient Agar, Muller-Hinton Agar (Oxoid) and Nutrient Broth supplemented with or without 7.5% Sodium Chloride were used for culturing of samples at different stages of the experiment. The sterility of these media was checked by incubating the sterilized media overnight before use. Standard reference strains of *S. aureus*, ATCC 25923, *E. coli*, ATCC 25922 and *Streptococcus* spp. were kindly obtained Jimma university medical science college.

Microbial analysis and confirmation of bacterial isolates: From appropriate young culture plate, 10 to 15 colonies with distinct morphological differences were picked and transferred to test tube containing 5 mL of nutrient broth and then after it were incubated at 35°C for 24 to 48 h. After incubation, the bacteria colonies obtained were purified on nutrient agar. The culture plates were examined by recording the appearance, size, colour and morphology of the colonies. The characteristic colonies (rounded, convex, golden yellow mannitol fermenting colonies on MSA plates) were aseptically picked, further purified by repeated streaking and characterized using established microbiological methods that included colonial morphology, cell shape and grouping, Gram reaction, catalase and coagulase tests. The identification and confirmation procedures used were Gram staining (Gram, 1884) and biochemical tests such as KOH test according to Gregersen (1978), catalase (Mac Faddin, 1980) and coagulase (Uwaezuoke and Aririatu, 2004).

Antimicrobial susceptibility testing for identified pathogens: The antimicrobial susceptibility of the isolates were tested using disks diffusion method and the microbial cell centrifugation was adjusted to the standardize turbidity of 0.5 McFarland which is equivalent to 10^7 to 10^8 CFU g^{-1} (Bauer *et al.*, 1966). The 0.5 McFarland was obtained by mixing 0.5 mL of 1% BaCl₂ solution and 99.5 mL of 1% H₂SO₄ in cap umbel bottle. The standardize Cell suspension was swabbed by cotton swab on the Muller-Hinton Agar and allow to dry. Thereafter the antibiotic discs were dispensed on the medium and incubated at 37°C for 18 h and the zones of inhibition was measured using transparent ruler. The result of antimicrobial susceptibility was interpreted based on the guidance on of National Committee for Clinical Laboratory Standards (NCCLS, 2007).

RESULTS AND DISCUSSION

Now a day, cream filled bakery products are consumed worldwide as they are tasty and provide high amount of calories (Sami *et al.*, 2012). These products could be spoiled as a result of improper handling and inadequate refrigeration before consumption (Abdussalam and Kaferstein, 1993; Tasci *et al.*, 2011). The mean count (CFU g^{-1}) of *S. aureus* was highest (6.3) in cream cake followed by donut (4.5) whereas the lowest was recorded in zebib (4.3) which is closer to donut (Table 1). The percentage of coefficient of variation (%CV) ranged from 1.6 to 4.7%. The highest (4.7%) was observed from zebib count of *S. aureus* while the lowest was (1.6%) was observed in cream cake (Table 2).

Lues and Van Tonder (2007) in their study obtained about 88 and 48% *S. aureus* from the hands aprons of food handlers, respectively. *S. aureus* was the main pathogenic bacteria responsible for Staphylococcal Food Poisoning (SFP) since it produces enterotoxin by its nature (Pundir and Jain, 2011). According to Ogundare and Adetuyi (2003), *Staphylococcus* spp. has also been found in bread baked with wheat flour.

Microbial analysis: A total of 282 isolates of (226 from cream cake, 35 from donut and 21 from zebib) (Fig. 2) were characterized based on colony and cellular morphological and by biochemical test and identified as *S. aureus* based on Barrow and Feltham (2004) bacterial classification. All of the isolates had a golden yellow color with grape like cluster shape. In addition, the isolates were positive for gram reaction, catalase and couagulase tests.

The results of this study was tried to assess the metheciline resistant *S. aureus* on some selected cream filled bakery products in Jimma town south west Ethiopia. It also investigated that the filling (cream) could be favored for the growth of *S. aureus*. In Nebraska, Sumner *et al.* (1993) isolated

Table 1: Mean counts of *S. aureus* in (log CFU g^{-1}) in Jimma town, south west Ethiopia

Food item	No. of food items	Average colony (%)	log CFU g^{-1}	log CFU g^{-1} ±SD	CV (%)
Cream cake	10	226 (80.2)	6.3	6.3±0.1	1.6
Donut	10	35 (12.4)	4.5	4.5±0.2	4.4
Zebib	10	21 (7.4)	4.3	4.3±0.2	4.7
Total	30	282 (100)	15.1	-	10.7

Table 2: Prevalence of *S. aureus* in some selected cream filled bakery products

Food sample	Sample size	No. of <i>S. aureus</i> positive samples
Cream cake	10	10 (100%)
Donut	10	7 (70%)
Zebib	10	5 (50%)
Total	30	22

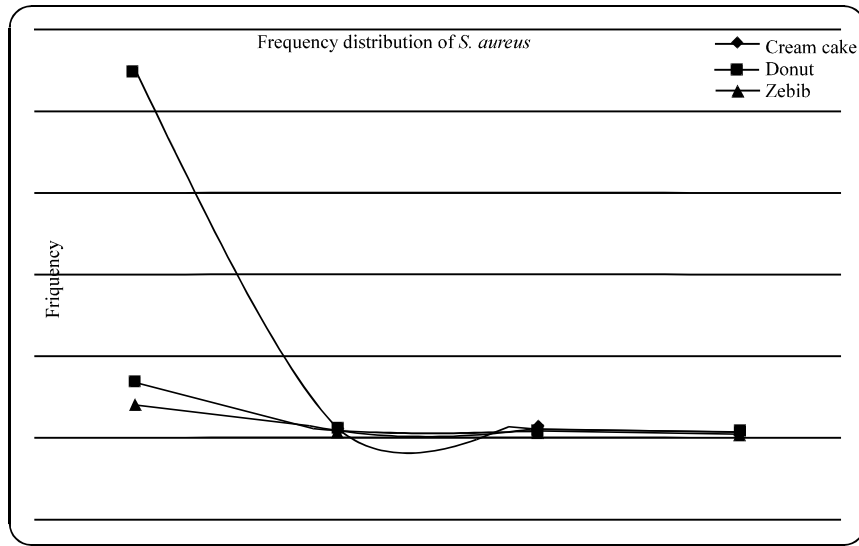


Fig. 2: Frequency distribution of *S. aureus* in some selected cream filled bakery products in Jimma zone south west Ethiopia

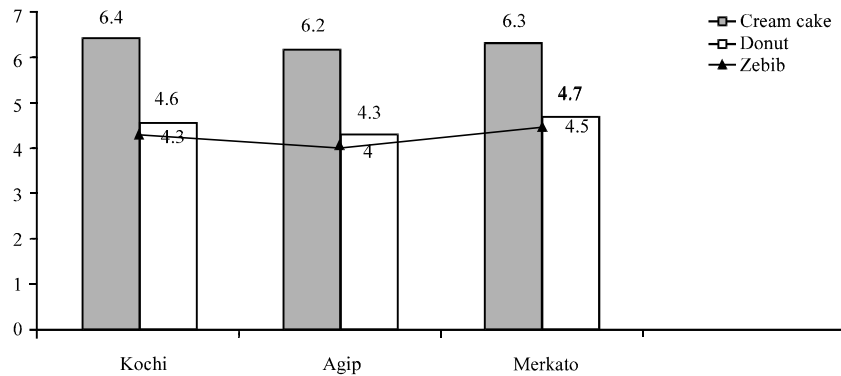


Fig. 3: Mean count of *S. aureus* isolate from cream cake, donut and zebib collected from Agip, Merkato and Kochi, Jimma town

S. aureus from 30% of cream puffs (12 cream puffs). The growth of *S. aureus* was observed in a significant number in cream cake as compared to donut and zebib. Hugh and Leifson (1953) reported, Cream cake is an excellent growth medium for many kinds of microorganisms, as it provides rich nutrient for microorganism and it is high in moisture and has neutral pH. The ingredients of cream cake such as chocolate, fruit, milk and butter cream has also perishable food. For this reason cream cake are not shelf stable and pose a potential public health risk if subjected to temperature abuse at any stage of the product's production, storage, distribution and marketing as well as production under unhygienic condition (Cogan *et al.*, 2002; Soriano *et al.*, 2002).

The mean count (CFUg⁻¹) of *S. aureus* was highest (6.4) in cream cake collected from Kochi followed by Mekato (6.3) while the lowest mean count was recorded in Agip (6.2). In case of donut, the highest incidence (4.7) was observed in Merkato followed by Kochi (4.6) and the least (4.3) was in Agip. In other cases, the frequency of *S. aureus* in zebib is higher (4.5) in Merkato followed by Kochi (4.3) whereas the lowest (4) was recorded in Agip (Fig. 3).

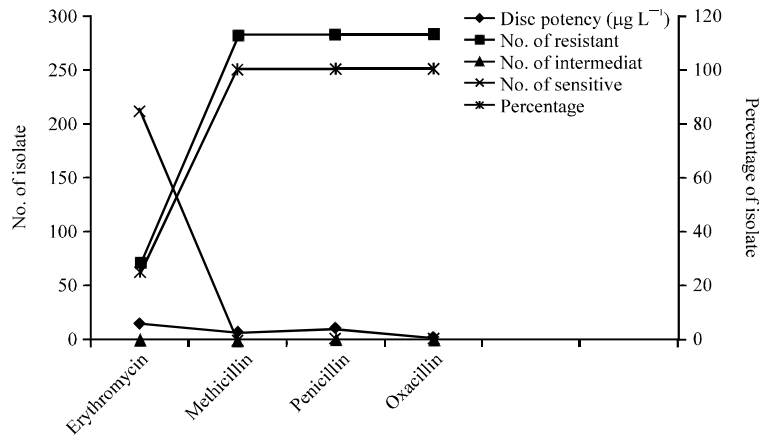


Fig. 4: Antimicrobial susceptibility pattern of *S. aureus* isolated from cream cake, donut and zebib collected from Agip, Merkato and Kochi, Jimma town, south western Ethiopia

Prevalence of *S. aureus*: In the present study, the overall food samples were *S. aureus* positive through the degree of distribution varied from sample to sample. Accordingly it was prevalent among 80.2% in cream cake, 12.4% in donut and the lowest (7.4%) prevalence was observed in zebib. Regarding to the sites, the prevalence of *S. aureus* was highest in cream cake collected from Kochi, Merkato and Agip that accounted about 82.3, 86.8 and 72%, respectively relative to donut and zebib (Table 2).

The present study also revealed that consumption of products which had high amount of *S. aureus* load could cause so many food borne diseases such as diarrhea, abdominal pain, vomiting and headache. Behling *et al.* (2010) reported that in foods *S. aureus* can cause an estimated 1,513,000 cases of illness and 1,210 deaths annually in United States. The bacterium *S. aureus* caused food poisoning as it transformed from contaminated hands, equipments or while the handlers respire on foods (Soto *et al.*, 1996). Adams and Moss (1995) revealed that work surface and equipment used are important sources of indirect food contamination.

Antimicrobial susceptibility pattern of *S. aureus*: Antibiotic susceptibility testing data for erythromycin, penicillin G, methicillin and oxacillin, were compiled. There was low resistance documented against erythromycin. Besides that, all the isolates were totally resistant to three antibiotics including penicillin G, Methicillin and Oxacillin (Fig. 4).

Furthermore there is no intermediate in susceptibility in all of the isolate but zero sensitivity in the three antibiotics, i.e., methicillin, penicillin and oxacillin respectively. Among the total of 282 isolates, 211 (75%) were susceptible to the antibiotics erythromycin whereas the remaining 71 (25%) were resistant to erythromycin. In other words, all of the isolates were resistant to penicillin G (DeLeo and Chambers, 2009; Alexandra *et al.*, 2011), Methicillin and Oxacillin.

CONCLUSION

The overall microbial safety of cream filled bakery products assessed in the current study was poor due to poor personal hygiene practice such as handling with barehanded. The storage place, the garment, cervices material and the nature of the cake was also the reason for high number *S. aurous*. The nature of the cream cake i.e., moisture content and its sensitivity to contact

with materials and human skin creates a chance for the increments of the number of *S. aureus* on the cream cake. Consumption of miss prepared or handled foods leads to dissemination of drug resistant bacteria like *S. aureus*. In all the food samples, the pathogenic bacterium *S. aureus* could grow with 24 h. In the present study, *S. aureus* predominate in cream cake as compared to donut and zebib. The presence of this pathogenic bacterium could cause food borne disease like diarrhea, nausea, abdominal pain, vomiting and headache. *S. aureus* isolates were susceptible to antibiotics like erythromycin but resistant to antibiotics like penicillin G, Methicillin and Oxacillin.

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