Isolation of *Staphylococcus aureus* from some Semi Preserved Sudanese Food

Thuraya Ahmed Mohammed, Amna Elsubki Khalid and Abdulmoniem Mohamed Saadabi

Department of Microbiology and Molecular Biology, Faculty of Science and Technology, El Neelain University, P.O. Box 12702, Khartoum, Sudan

Corresponding Author: Thuraya Ahmed Mohammed, Department of Microbiology and Molecular Biology, Faculty of Science and Technology, El Neelain University, P.O. Box 12702, Khartoum, Sudan

ABSTRACT

One hundred samples from cheese (white cheese Jebna baladi) and salted fermented fish (faseik) were collected from Khartoum state markets or outlets. These were examined microbiologically for isolation of *Staphylococcus aureus* using recommended general and selective media as well as recommended tests. The results showed presence of *S. aureus* in faseik in 72%, *Bacillus* sp. 19% and Yeast sp. 19%. While no *S. aureus* was isolated from examined cheese samples, *Bacillus* sp. (52%), *Lactobacillus* sp. (19%) and Yeast sp. (93%).

Key words: *S. aureus*, isolation, semi preserved foods, Sudan

INTRODUCTION

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. There are various examples of staphylococcal food poisoning (Bergdoll, 1989). In one case, cheese was involved in an outbreak because it had been made from milk contaminated after pasteurization and before inoculation with lactic starter culture. In this particular case, the starter culture did not grow properly resulting in a fermentation accident that allowed the *S. aureus* strain to develop and produce SE (Bergdoll, 1989).

*Staphylococcus aureus* is a significant bacterial pathogen producing a variety of proteins and toxins that contribute to its ability to colonize and cause diseases (Dinges et al., 2000). Some *S. aureus* strains are able to produce Staphylococcal Enterotoxins (SEs) in food matrices and are responsible for food poisoning characterized by such symptoms as nausea, vomiting, abdominal cramps and diarrhea (Balaban and Rasooly, 2000). In France, *S. aureus* is reported as the most frequent pathogen involved in food-borne diseases associated with raw milk cheeses (De Buyser et al., 2001) and dairy products (Delmas et al., 2006).

It is generally accepted that SE production constitutes a risk when *S. aureus* bacteria exceed a threshold of 105 *S. aureus* CFU g⁻¹ of cheese during manufacture. Numerous studies have reported on *S. aureus* behavior during cheese manufacturing, focusing only the *S. aureus* growth and enterotoxin production (Tatini et al., 1971; Tatini et al., 1978; Cords and Tatini, 1973; Gayat et al., 1988; Nunez et al., 1988; Otero et al., 1988; Gomez-Lucia et al., 1992; Otero et al., 1993; Vernozay-Rozand et al., 1998; Meyrand et al., 1998; Meyrand and Vernozay-Rozand, 1999; Hamama et al., 2002; Delbes et al., 2006; Aoyama et al., 2008).
No study has investigated the expression of the genes encoding SEs in foods or during food production. Numerous parameters such as pH, aeration or temperature could indeed affect the expression of these genes. During the cheese-making process, natural staphylococcal contamination is minor in the total microbial population. The initial \textit{S. aureus} contamination is usually below 103 CFU mL\(^{-1}\) of raw milk, while bacterial starters are inoculated at least at 106 CFU mL\(^{-1}\) of milk. Analysis of SE gene expression, thus, requires an efficient method of extracting bacterial RNA from cheese to ensure recovery of quantifiable amounts of staphylococcal RNA, a direct method (Bonaïti et al., 2006), Monnet et al. (2008). A precise method is also needed to quantify minor transcripts in the extracted bacterial RNA. Two main strategies were initially used to extract bacterial RNA from cheese, one based on separation of cells from the cheese matrix prior to RNA extraction (Makhzami et al., 2008; Monnet et al., 2008).

The method of Bonaïti et al. (2006), which was developed to analyze the surface of cheese curd, is not directly applicable for all cheese fractions. Two methods were developed on model semi fat cheeses and these either have not been applied to cheese with standard fat content (Monnet et al., 2008) or gave bad results with commercial cheese (Monnet et al., 2008). Makhzami et al. (2008) separated cells from the cheese matrix using a Nycodenz density gradient but cheeses contained more than 108 CFU g\(^{-1}\) of cheese (Makhzami et al., 2008) which is not always the case at the beginning of cheese processing. In the absence of a reliable method, there was still a need to develop an efficient bacterial RNA extraction method usable at any time in the cheese-making processing and applicable to any fraction of the cheese.

**MATERIALS AND METHODS**

\textbf{Semi preserved food samples:} One hundred of each of semi preserved Sudanese foods (white cheese Jebna baladja) and salted fermented fish (faseik) samples were collected from different markets located in Khartoum, Khartoum North and Omdurman. The samples were analyzed immediately after collection.

\textbf{Staphylococcus aureus isolation and identification:} From each specimen, 1 g was taken then suspended in 9 mL of sterile distilled water (10\(^{-1}\)), this dilution was used for \textit{S. aureus} isolation.

\textit{S. aureus} plated on mannitol salt agar (oxoid) and incubated at 37\(^\circ\)C for 24-48 h. Characteristic colonies were subcultured in nutrient agar (oxoid) and incubated at 37\(^\circ\)C for 18-24 h. The pure colonies were tested by gram stain. DNase test by subculturing on DNase medium (oxoid) incubated at 37\(^\circ\)C for 24 h. Catalase test using hydrogen peroxide 3% and coagulase test using human plasma. The coagulase positive specie were subjected to the Voges-Proskauer (VP) test.

**RESULTS**

\textit{S. aureus} on mannitol salt agar gave yellow colonies due to mannitol fermentation, on nutrient agar this bacteria gave white to yellow colonies, suspected \textit{S. aureus} colonies were gram positive cocci in clusters, catalase test positive, coagulase positive, DNase and Voges-Proskauer (VP) test were positive.

\textit{Staphylococcus aureus} isolated from 100 specimen collected from salted fermented were fish (72%), \textit{Bacillus} sp. (19%), \textit{Lactobacillus} sp. (0%) and Yeast sp. (19%). Isolates from cheese specimens resulted in 0% \textit{S. aureus}, \textit{Bacillus} sp. (52%), \textit{Lactobacillus} sp. (19%) and Yeast sp. (39%) (Table 1).
Table 1: Percentage of microorganisms isolated from semi preserved foods (salted fermented fish and cheese)

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Salted fermented fish</th>
<th>Cheese</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus aureus</td>
<td>72</td>
<td>0</td>
</tr>
<tr>
<td>Bacillus sp.</td>
<td>19</td>
<td>52</td>
</tr>
<tr>
<td>Lactobacillus sp.</td>
<td>0</td>
<td>19</td>
</tr>
<tr>
<td>Yeast sp.</td>
<td>19</td>
<td>93</td>
</tr>
</tbody>
</table>

DISCUSSION

The foods that are most often involved in staphylococcal food poisoning differ widely from one country to another. In the United Kingdom, for example, 53% of the staphylococcal food poisonings reported between 1969-1990 were due to meat products, meat-based dishes and especially ham; 22% of the cases were due to poultry and poultry-based meals, 8% were due to milk products, 7% due to fish and shellfish and 3.5% due to eggs (Wieneke et al., 1993). In the United States, the staphylococcal food poisoning cases reported between 1975-1982, 36% were due to red meat, 12.3% due to salads, 11.3% due to poultry, 5.1% due to pastries and only 1.4% to milk products and seafoods. In 17.1% of the cases, the food involved was unknown (Genigeorgis, 1989). Thus, the origins of staphylococcal food poisoning differ widely among countries, this may be due to differences in the consumption and food habits in each of the countries. In France, for example, the consumption of raw milk cheeses is much higher than in Anglo-Saxon countries. This may explain the relative importance of milk products involved in staphylococcal food poisoning. In France, things are different. Staphylococcal food poisonings reported in a two-year period (1999-2000), the food involved identified, the milk products especially cheeses were responsible for 82% of the cases, meats 22%, sausages and pies 15%, fish and seafood 11%, eggs and egg products 11% and poultry 9.5% (Haeghebaert et al., 2002).

In this study, S. aureus is not isolated from cheese, this disagree (Ertas et al., 2010) with the report that S. aureus were isolated from 60 (60%), 26 (52%) of sheep cheese and from dairy desserts, respectively. S. aureus were isolated from salted fermented fish (70%). This result agreed with Wieneke et al. (1993) who reported that 7% S. aureus were isolated from fish and shellfish, where most probably collected from sea water.

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REFERENCES


Staphylococcus aureus strains, producers of enterotoxins C₁ or C₂, during the manufacture and
Behaviour and enterotoxin production by Staphylococcus aureus during the manufacture and