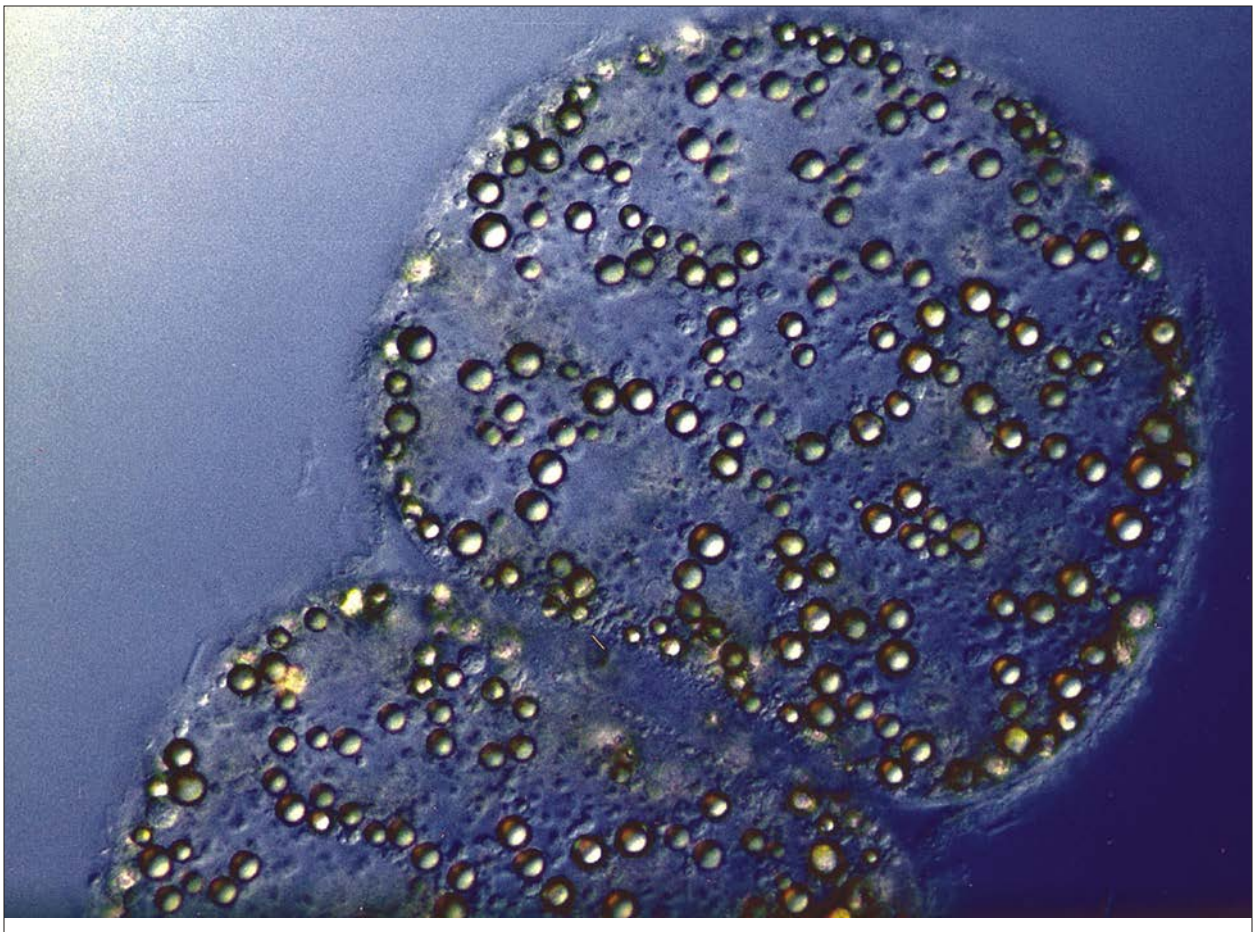

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# PCR Detection of Staphylococcal Enterotoxin A and B Genes in *Staphylococcus aureus* Isolated from Salted Fermented Fish

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## ABSTRACT

One hundred samples from salted fermented fish (faseik) were collected from Khartoum state markets or out lets. These were examined microbiologically for isolation of *Staphylococcus aureus* (*S. aureus*) using recommended general and selective media as well as recommended tests. The results show presence of *S. aureus* in faseik in 72%, also we found *Bacillus* sp. 19% and *Yeast* sp. 19%. Staphylococcal enterotoxins (SEs) SEA and SEB were found in *S. aureus* strains isolated from salted fermented fish. *S. aureus* was found in 72 (72%) out of 100 specimen collected from salted fermented fish. Of the 72 isolates studied 42 isolate were positive for DNA extraction, both SEs A and B were identified using polymerase chain reaction. The gene coding for Staphylococcal Enterotoxin A (SEA) were 38.1 and 35.7% were positive for genes coding the Staphylococcal Enterotoxin B (SEB).

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

## INTRODUCTION

Food borne pathogens are the leading causes of illness and death in less developed countries, killing approximately 1.8 million people annually. In developed countries, food borne pathogens are responsible for millions of cases of infectious gastrointestinal disease each year, costing billions of dollars in medical care and lost productivity. New food borne pathogens and food borne diseases are likely to emerge, driven by factors such as pathogen evolution, changes in agricultural and food manufacturing practices and changes to the human host status. There are growing concerns that terrorists could use pathogens to contaminate food and water supplies in attempts to incapacitate thousands of people and disrupt economic growth (Fratamico and Bayles, 2005).

*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), it is a common cause of bacterial food borne disease worldwide. 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). As a result of ingestion of *S. aureus* toxin-contaminated food. The symptoms arise from ingestion of preformed enterotoxin, which accounts for the short incubation time. Staphylococcal enterotoxins are superantigens and, as such, have adverse effects on the

immune system. The enterotoxin genes are accessory genetic elements in *S. aureus*, meaning not all strains of this organism are enterotoxin-producing. The enterotoxin genes are found on prophages, plasmids and pathogenicity in different strains of *S. aureus*. Expression of the enterotoxin genes is often under the control of global virulence gene regulatory systems (Stewart, 2008).

In United States, among the staphylococcal food poisoning cases reported between 1975 and 1982, 36% were due to red meat, 12.3% to salads, 11.3% to poultry, 5.1% to pastries and only 1.4% to milk products and seafood. In 17.1% of the cases, the food involved was unknown 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 (Genigeorgis, 1989). Many types of 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. (Bergdoll, 1989). Staphylococcal food poisoning differs widely from one country to another. In the United Kingdom, for example, 53% of the staphylococcal food poisonings reported between 1969 and 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% to fish and shellfish and 3.5% to eggs (Wieneke *et al.*, 1993). In France, things are different. Among the staphylococcal food poisonings reported in a two-year period (1999-2000), among the cases in which the food involved had been identified as milk products and especially cheeses which were responsible for 32% of the cases, meats for 22%, sausages and pies for 15%, fish and seafood for 11%, eggs and egg products for 11% and poultry for 9.5% (Haeghebaert *et al.*, 2002).

Meat and meat products are highly perishable and easily spoiled and contaminated by bacterial from different sources of contamination before, during and after processing and soon become unfit and possibly dangerous to health. *Staphylococcus aureus* is regarded as the most common causes of meat poisoning in Sudan (Hamid, 2010).

## MATERIALS AND METHODS

**Semi preserved food samples:** One hundred semi preserved sudanese food salted fermented fish (faseik) samples were collected from different markets located in Khartoum, Khartoum North and Omdurman and analyzed.

***Staphylococcus aureus* isolation and identification:** For *S. aureus* isolation was performed by plating on Mannitol Salt Agar (Oxoid) and incubated at 37°C for 24-48 h. Characteristic colonies were subcultured in Nutreient Agar (Oxoid) and incubated at 37°C for 18-24 h. The pure colonies were tested by Gram staining. DNase test was performed using DNase medium (Oxoid), catalase was using hydrogen peroxide 3% and coagulase production. The coagulase positive species were submitted to the Voges-Proskauer (VP) test.

**Detection of staphylococcal enterotoxins by PCR (polymerase chain reaction):** DNA isolation was performed using the phenol chloroform procedure as described by Ausubel *et al.* (2003). Each PCR contained Taq DNA Polymerase (50 mL $U^{-1}$) 2.5 U, dNTPs mM, reaction buffer (10x)1x and gel loading buffer1x (PCR PreMix Kit) for 20 mL reaction, template

Table 1: Primers and annealing temperature used for the detection of *Staphylococcus aureus* Enterotoxin (SE) genes

| Gene | Primer sequence | Base pair | Annealing temperature (°C) | References |
|---|---|---|---|---|
| SEA-1 | ttggaaacggttaaaacgaa | 120 | 50 | Johnson *et al*. (1991) |
| SEA-2 | gaaccttcccatcaaaaaca | | | |
| SEB-1 | tcgcatcaaactgacaaacg | 478 | 50 | Johnson *et al*. (1991) |
| SEB-2 | gcaggtactctataagtgcc | | | |

Table 2: Percentages of microorganisms isolated from salted fermented fish

| Microorganisms | Isolation from salted fermented fish (%) |
|---|---|
| *Staphylococcus aureus* | 72 |
| *Bacillus* sp. | 19 |
| *Yeast* sp. | 19 |

DNA 2 mL, primer F 1 mL. Primer R 1 mL, the primers used for the detection of SE genes are listed in Table 1, and 18 mL distilled water. DNA amplification was performed in a thermal cycler machine under the conditions of initial denaturation for 5 min at 94°C followed by 30 cycles of denaturation (94°C for 2 min), annealing (50°C for 1 min) and extension (72°C for 1 min). A final extension step (72°C for 5 min) was performed after the completion the PCR products, with a 3000, 2000 and (100-1000) base pair DNA ladder (Solis BioDyne), were loaded into 1.5% agarose gel containing ethidium bromide and submitted to electrophoresis in TBE buffer for 20 min. The amplified DNA fragments were visualized with an image analyzer software.

## RESULTS

*S. aureus* on Mannitol Salt Agar gave yellow colonies due to mannitol fermentation, on nutrient agar this bacteria gave white to yellow colonies, *S. aureus* colonies tested for gram's stain *S. aureus* was gram positive cocci in cluster, this bacteria was catalase, Dnase, coagulase and Voges-Proskauer (VP) positive.

*Staphylococcus aureus* isolated from 72 specimens (with *Bacillus* sp. *Yeast* sp. or both) out of 100 specimens of the salted fermented fish (faseik). The rest of 100 specimens (28) Bacillus sp. and *Yeast* sp. were only founded, isolation percentages were shown in Table 2 and Fig. 1.

Out 72 isolates 42 (58.3%) were positive for DNA extraction and 30 isolate (41.7%) were negative, both SEs A and B were identified. The gene encoding for enterotoxin A, sea was detected from 16 specimens (38.1%), 26 specimens (61.9%) were negative for sea, Fig. 2 explain that and *Staphylococcus aureus* Enterotoxins A (SEA) molecular weight 120 base pair as shown in Fig. 3.

While the gene encoding for enterotoxin B was detected in 15 specimens (35.7%), 27 specimens (64.3%) were negative for seb, as explained in Fig. 4 and *Staphylococcus aureus* Enterotoxins B (SEB) molecular weight 478 base pair as shown in Fig. 5.

## DISCUSSION

The prevalence of enterotoxigenic *S. aureus* isolates, which is attributable to different types of foods and biovars involved (Mathieu *et al*., 1991), According to Tranter (1996), the minimal amount of enterotoxin that is required to cause the disease is not known, but the ingestion of at least 1 g of toxin per 100 g of food is enough to induce the symptoms. Asao *et al*. (2003) reported an outbreak of food borne disease in Kansai, Japan. SEA is the most frequently observed enterotoxin in enterotoxigenic strains of *S. aureus* (Normanno *et al*., 2005).

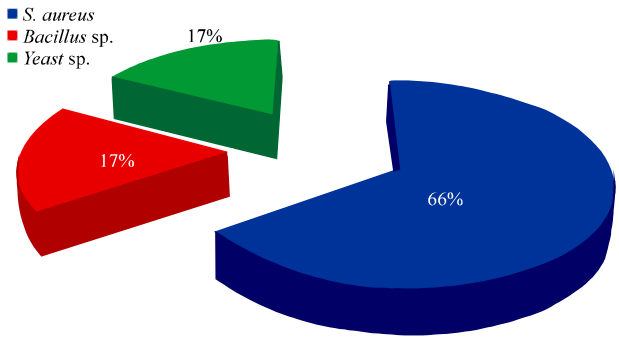


Fig. 1: Percentages of microorganisms which have been isolated from salted fermented fish (Faseik)

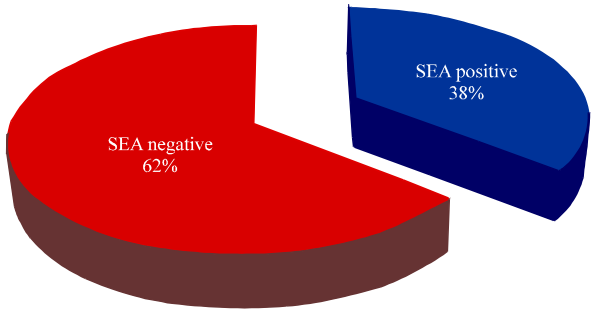


Fig. 2: *Staphylococcus aureus* Enterotoxins A (SEA) percentages which had been detected in 44 out of 72 specimens

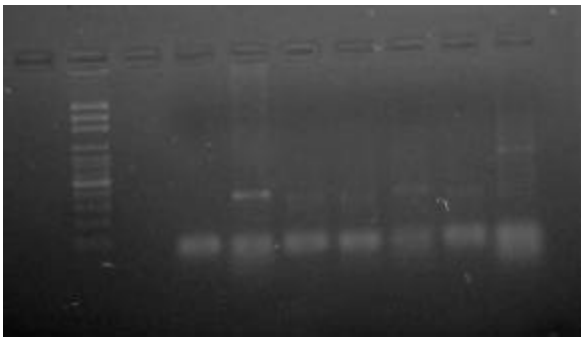

Fig. 3: *Staphylococcus aureus* Enterotoxins A (SEA) molecular weight 120 base pair

In this study, genes encoding *Staphylococcus aureus* enterotoxins (SEs) both A and B were detected. The genes encoding for enterotoxin A, was positive in 36.4 while enterotoxin B was positive for 34.1%. The same result was repeated by (Rall *et al*., 2008) who tested that gene encoding *S. aureus* entrotoxins were 39 were positive for at least one enterotoxin gene. The most frequently observed gene was sea, in 16 (41%) of the isolates, followed by sec (8 strains, 20.5%), sed (5 strains, 12.8%), seb (3 strains, 7.7%) and see (2 strains, 5.1%).

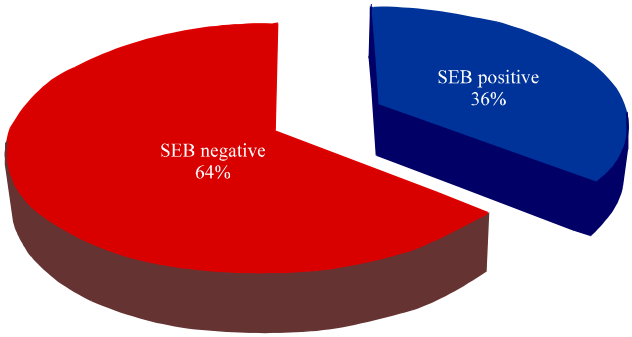


Fig. 4: *Staphylococcus aureus* Enterotoxins B (SEB) percentages which had been detected in 44 out of 72 specimens

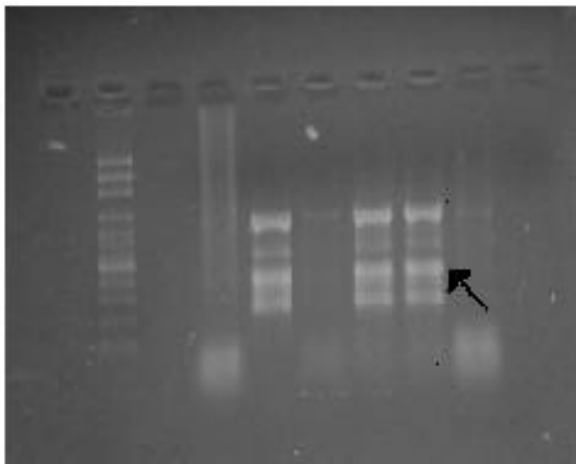

Fig. 5: *Staphylococcus aureus* Enterotoxins B (SEA) molecular weight 478 base pair

In conclusion, we detected genes encoding the classic SEA and SEB in *S. aureus* strains isolated from salted fermented fish (faseik). The recent identification of new SEs has considerably increased the perceived frequency of enterotoxigenic staphylococcal isolates, indicating that the pathogenic potential of *S. aureus* may be greater than previously thought. Further studies are needed to confirm the expression of these new enterotoxins by *S. aureus* and to assess their significance for food borne disease.

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