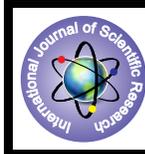


## The Prevalence of Staphylococci in Sausages at Khartoum State



### Food Microbiology

**KEYWORDS :** Sausages, Coagulase positive staphylococci, Coagulase negative staphylococci, Characterization.

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### ABSTRACT

*Foods free of Staphylococcal food poisoning organisms have a great concern by consumers nowadays. The aim of this study is to characterize the population of Staphylococci in sausages obtained from different sources in Khartoum State. A total of 40 fresh sausage samples were collected randomly from (a) butcheries in Khartoum, Khartoum North, and Omdurman, (b) factory-processed sausages at retail outlets and (c) home-made sausages obtained from households in Khartoum. Enumeration and characterization of different species of Staphylococci were carried out. The identification of the isolates was mainly based on morphological properties and biochemical tests. Staphylococcal mean counts in all samples ranged from  $1.48 \times 10^5$  cfu/g to  $7.66 \times 10^6$  cfu/g. The coagulase positive *Staphylococcus aureus* was identified in all sausage samples that were investigated, making 51.06% of the total isolates, while the coagulase negative isolates were identified as *Staphylococcus epidermidis* (31.91%), *Staphylococcus capitis* (12.77%), *Staphylococcus simulans* (2.13%), and *Staphylococcus hominis* (2.13%). These results indicate that sausage samples are highly contaminated with *Staphylococcus* species particularly *Staphylococcus aureus*. The high incidence of this bacterium in butcheries, processed and home-made sausages is a potential health hazard which indicate improper processing procedure, handling and bad personal habits. Utilization of high quality meat for sausage production and careful handling during processing is necessary to produce sausage free of pathogens.*

### Introduction

Meat and meat products are highly perishable and are susceptible to contamination by spoilage and pathogenic microorganisms. Different types of microorganisms may be introduced into sausages from various sources due to improper storage temperature and manipulation and poor hygienic condition in non-industrial establishments especially in butcheries (Cordoba & Jordano, 1998). Some of these microorganisms can cause food poisoning and infections when they are introduced in high numbers due to direct contact with food handlers (Byrant, Javis & Gilbet, 1988). Children and elderly people are the more susceptible group to food infections (Patricia Blas & Ana, 2006).

Staphylococcal food poisoning is gastrointestinal illness caused by food contaminated with *Staphylococcus aureus*. *Staphylococcus aureus* is normally found in different parts of human beings and other animals as in skin, the mucosal membrane, mouth, nose, pimples, boils, different environments, and in a wide range of food stuff (Acco, Ferreira, Henriques, & Tondo, 2003). *Staphylococcus aureus* is usually present in small numbers in raw meat and in food handlers. Human beings, different types of foods and environment are considered as favorable environment for *Staphylococcus aureus* transmission (Lateef, 2004). Angela Cheryl and Charles (2005) reported that up to 25% of healthy people have *Staphylococcus aureus* in their skin and in nose. Keeping of meat and meat products free from contamination with *Staphylococcus* species is difficult, therefore the food processor should keep meat and meat products at temperature that prevent the growth of *Staphylococcus* spp. Meat and meat products safety depends on quality of raw materials used, their adequate handling, processing, preparation, transportation, marketing, and storage. Generally staphylococcal food poisoning strains come from human sources and 95% of food poisoning outbreaks are caused by enterotoxin A, B, C, D and E (Letetre, Perelle, Dilasser & Fach 2003). The typical symptoms of food poisoning required very low amount of enterotoxin ranging from 20 µg to 1 µg (US Food and Drug Administration, 2001) which corresponding to  $10^5$  Staphylococci-colony forming units per gram of foods (Normanno Salandra, Dambrosio, Quaglia, Corrente, Parisi, Santagada, Firinu, Crisetti & Celano, 2007). Nadia (2010) found that sausages

have the highest count of *Staphylococcus aureus* with the rate of 72% when compared with the other Sudanese meat products (burger, minced meat and kofta....) sold in Khartoum State. As reported by Rabba Mohammed, Mashair, Sulieman and Elgasim (2013) the highest *Staphylococcus aureus* count (log 4 cfu/g) was recorded for prepared Sudanese fresh beef sausages without bee honey, while the lowest count (log 1.5 cfu/g) was recorded for sausages with 7.5% bee honey. Nowadays sausages are becoming an important food item in Sudanese meals. They are usually not fully cooked when sold at retail points as cafeterias and restaurants which make this product a health hazard. The objective of this piece of work is to study the prevalence of *Staphylococcus* species in fresh Sudanese sausages sold in Khartoum State.

### Material and Methods

#### Collection of samples

A total of 40 fresh sausage samples were collected from eight different sources (5 samples each). These included (a) butcheries in Khartoum, Khartoum North, and Omdurman, (b) Factory-processed sausage at retail outlets (F1, F2, F3, and F4), (c) homemade sausages obtained from households in Khartoum. Samples were collected aseptically and kept in sterile insulated ice containers and immediately transferred to the laboratory for analysis.

#### Microbiological analysis

Thirty grams of each sample of fresh sausages were weighed aseptically, added into sterile conical flask containing 270 ml sterile peptone water and then blended for 30 sec in a sterilized electric blender. Serial ten-fold dilutions were prepared following the method described by Harrigan (1998). From suitable dilutions, 0.1 ml was drawn aseptically and spread onto the surface of dried Baird-Parker agar medium using sterile glass rod then the plates were incubated at 37°C for 48 hours. Counting of *Staphylococcus* sp. was carried out using a colony counter (Quebec Colony Counter) and results were expressed as colony forming unit per gram (cfu/g) of the sample.

#### Purification and identification of isolates

Typical colonies of *Staphylococcus* sp. were purified and identi-

fied following the method described in Bergey's Manual (Sneath, Mair, Sharp & Holt. 1986), Barrow & Felthman (1993) and Harigan (1998).

## Results and Discussion

The pH values and total Staph count are presented in Table 1. The mean pH values of the sausage samples ranged between 5.83 and 6.23. The highest mean pH values were recorded in samples obtained from butchereries. Cocolin *et al.* (2004) reported that the pH values for fresh sausages is not lower than 5.5. Results showed that the pH of most samples investigated were higher than this value. The high mean pH values for the sampled sausages revealed that these sausages were produced from low quality meat with pH values higher than 6.00, which is referred to as dark, firm and dry meat (DFD). Newton and Gill (1981) claimed that DFD meat is characterized by high ultimate pH > 6.00 and deficiencies in glucose because of exercise or stress. These factors can result in bacterial spoilage becoming evident at an earlier stage of growth of the meat flora. Newton and Gill (1981) found that the pH value is an important indicator for microbial growth and high pH resulted in high microbial count and short shelf life. The ultimate pH of meat is significant for its resistance to spoilage because most bacteria grow optimally at about pH 7.0 and not below pH 4.0 or above pH 9 (Jamilah, Abbas, and Abdoul 2008).

Staphylococcal mean counts ranged from  $1.48 \times 10^5$  to  $7.66 \times 10^6$  cfu/g. Factory 2 recorded the highest count ( $7.66 \times 10^6$  cfu/g), while the lowest mean count was reported for samples obtained from factory 3 (Table 1). Similar results were obtained by Oluwafemi and Simisaye (2006) who found that the *S. aureus* counts ranged from  $1.30 \times 10^5$  cfu/g to  $2.20 \times 10^7$  cfu/g for sausage samples obtained from markets in Nigeria. The mean values of Staphylococcal counts of the investigated sausage samples were higher than the standard limits ( $5 \times 10^2$  cells) established by the Sudanese Microbiological Standards for Foods (SSMO, 2001). The number of these bacteria in sausages is usually low as obtained in Sudan by Ali (2004), who found that *Staphylococcus* count of sausages at zero day of storage at refrigerator temperature was  $1.17 \times 10^2$  cfu/g, reached  $1.19 \times 10^2$  cfu/g after 5 days of storage and  $2.8 \times 10^2$  cfu/g at the end of 10 days of storage. Samappito, Leenanon, and Robert. (2011) recorded a lower count of staphylococci ( $3.3 \times 10^3$ – $2.7 \times 10^6$  cfu/g) for "Mhom", a traditional meat sausage in Thailand, than that obtained in this study.

The high counts of this bacteria in the sampled sausages may be attributed to cross-contamination during preparation, processing, transportation and packaging as has been observed by Abbar & Mohammed (1989). In addition, some bad habits of butchers such as wiping and dusting of displayed sausage with dirty pieces of cloth that are used for cleaning hands and tables, sneezing and snuffing play an important source of contamination. Humans are the principal source or reservoir of these organisms. Staphylococci are found in the nose, throat and skin of up to 60% of healthy humans. Staphylococcal food poisoning is a persistent cause of gastroenteritis worldwide, especially in the developing countries (Vora, Senecal, and Schaffner, 2003). Concerning public health Staphylococci are important organisms that may occur in cooked comminuted meat products. They are primarily found in processed meat and dairy products; survive in the salted medium of hams and sausages (Jay, 1996).

The identified isolates of the *Staphylococcus* sp. are presented in Table 2. Results showed that the coagulase-positive *Staphylococcus aureus* was identified in all sausage samples that were investigated, making 51.06% of the total isolates. Most of the isolates from Khartoum, Khartoum North and Omdurman butchereries were identified as *Staphylococcus aureus* (25.53%). *Staphylococcus aureus* is considered the third most important cause of disease in the world amongst the reported food-borne illnesses

(Anonou, Maqueda, Martine, Bueno, Galvez and Valdivia, 2007). It is a saprophyte and commensal of the skin mucus membrane in both animal and humans. As a pathogen it can cause a number of diseases ranging from minor skin infection to fetal sepsis. Toxigenic strains of *S. aureus* are currently among the leading causes of food borne intoxication.

The coagulase negative staphylococci were identified as *Staphylococcus epidermidis* (31.91%), *S. capitis* (12.77%), *St. simulans* (2.13%), and *S. hominis* (2.13%). Presence of staphylococci in Sudanese sausages is not unique as other authors identified *Staphylococcus* spp. in fresh sausages samples stored at 4°C for 10 days (Rantsiou Iacumin, Urso, Cantoni, Comi and Cocolin, 2005). They found that most of the isolates were *Staphylococcus xylosum* (50%), strains of *S. pasteurii*, *S. warneri*, *S. equorum* and *S. succinus*, were also identified. Cocolin Rantsiou, Iacumin, Lürso, Cantoni, and Comi, (2004) reported that *Staphylococcus xylosum* was isolated from fresh sausages stored at 4°C for 10 days.

The identified *S. epidermidis* nowadays is seen as an opportunistic pathogen. It is now the most frequent cause of nosocomial infections at a rate about as high as that due to its more virulence cause *S. aureus*. The fact that they are extremely difficult to treat represent a serious burden for the public health system (Otto, 2009). *S. capitis* is part of normal flora of the skin of the human scalp, face, neck and ears and has been associated with prosthetic valve of endocarditis (D'mello Daley, Rahman, Qu, Garland, Pearce and Deighton, 2008).

The identified *S. simulans* is frequently acquired through contact with domestic animals. It is an authentic pathogenic agent of osteoarticular infection (Mallet, Loiez, Melliez, Yazdanpanah, Senneville, Lemaire, 2011). *S. huminus* may occasionally cause infection in patients whose immune system are compromised for example by chemotherapy (Kloos & Schleifer, 1975). Poor hygiene practices such as negligence to wash hands after visiting the bath room may result in up to  $10^7$  pathogens under the fingernails of the handlers (Nel Lues, Buys and Venter, 1993). The high prevalence rate of *Staphylococcus aureus* and the presence of coagulase negative staphylococci in the sausage samples under study indicates the poor hygienic conditions during sausages production in Sudan. Also it reflects the poor personal hygiene of people dealing with food processing. Lack of standards hygiene measures among workers in factories processing sausage may lead serious health hazards to consumers. These type of research make a critical step towards understanding the serious prevalence of staphylococci in sausage produced under poor processing conditions.

**Table 1. Counts of *Staphylococcus* spp. in sausage samples per gram fresh weight**

| Sample No | Sample source                    | *Mean pH | *Mean (cfu/g)      | TVBC |
|-----------|----------------------------------|----------|--------------------|------|
| 1         | Khartoum butchereries (Kh)       | 6.00     | $2.68 \times 10^6$ |      |
| 2         | Khartoum North butchereries (KN) | 6.03     | $3.44 \times 10^6$ |      |
| 3         | Omdurman butchereries (O)        | 6.23     | $4.53 \times 10^6$ |      |
| 4         | **Factory 1                      | 5.85     | $2.48 \times 10^6$ |      |
| 5         | **Factory 2                      | 5.87     | $7.66 \times 10^6$ |      |
| 6         | **Factory 3                      | 5.83     | $1.48 \times 10^5$ |      |
| 7         | **Factory 4                      | 5.99     | $6.16 \times 10^5$ |      |
| 8         | Homemade (H)                     | 5.91     | $1.59 \times 10^6$ |      |

\* Mean of 5 replicates.

\*\* Factory samples at retail outlet.



|                            |                       |                         |                      |              |
|----------------------------|-----------------------|-------------------------|----------------------|--------------|
| Legend:                    | (d) Delayed reaction. | 1-----10: Sugars tested | (*) another isolate. |              |
|                            | (w) Weak reaction.    | 1-Lactose               |                      | 6-Trehalose  |
|                            | (F) Fermentative      | 2-Maltose               |                      | 7-Xylose     |
|                            | (+) Positive reaction | 3-Mannitol              |                      | 8-Cellobiose |
|                            | (+) Negative reaction | 4-Fructose              |                      | 9-Raffinose  |
| (St) <i>Staphylococcus</i> | 5-Sucrose             | 10-Mannose              |                      |              |

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