

**PJN**

ISSN 1680-5194

PAKISTAN JOURNAL OF  
**NUTRITION**

**ANSI***net*

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## Isolation and Identification of *Staphylococcus* spp. in Fresh Beef

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**Abstract:** Forty samples of fresh meat (beef) were randomly sampled from Khartoum, Omdurman and Bahri in Khartoum State, Sudan and analyzed microbiologically for the bacterial load and *Staphylococcus* (*Staph.*) count and identify *Staph.* spp. present in fresh meat. Total viable count ranged from  $4.78 \times 10^4$  to  $3.39 \times 10^5$  cfu/g and *Staph.* count ranged from  $3.23 \times 10^3$  to  $8.7 \times 10^3$ . A total of 58 *Staph.* isolates belonging to 19 species of *Staphylococcus* genus were grouped as follows: 1) coagulase-positive species, was *Staphylococcus aureus*, 2) coagulase-negative species (Novobiocin-sensitive): ten isolates, *Staph. epidermidis*, *Staph. caseolyticus*, *Staph. lugdunensis*, *Staph. chromogenes*, *Staph. capitis*, *Staph. felis*, *Staph. warneri*, *Staph. haemolyticus*, *Staph. capitis* ssp. *urealyticus* and *Staph. hycius.*, 3) coagulase-negative species (Novobiocin-resistant): eight isolates, *Staph. saprophyticus*, *Staph. xylosus*, *Staph. Kloosii*, *Staph. lentus*, *Staph. cohnii*, *Staph. sciuri*, *Staph. gallinarum* and *Staph. cohnii* ssp. *urealyticus*. The frequency of isolation of staphylococci in Khartoum State was higher in Omdurman City 23(39.7 %) followed by Bahri City 18 (31%) and Khartoum City 17(29.3%). Among these isolates of staphylococci, *Staph. epidermidis*, *Staph. aureus*, *Staph. caseolyticus* and *Staph. saprophyticus* were the most abundant isolates. Statistical analysis of the microbial load and total staphylococci count showed no significant difference between the Cities ( $P > 0.05$ ).

**Key words:** Khartoum, *Staphylococcus* spp., fresh meat, total viable count, coagulase test

### INTRODUCTION

Meat is a major constituent of the human diet in Sudan. It is an essential food item and one of the main sources of protein, fats, minerals and vitamins. Most meat have high water content corresponding to the water activity approximately 0.99 which is suitable for microbial growth (Rao *et al.*, 2009). Meat is subjected to changes by its own enzyme, by microbial action and its fat may be oxidized chemically microorganisms grow on meat causing visual, textural and organoleptic change when they release metabolites (Jackson *et al.*, 2001).

Meat is a good material for bacterial growth; its quality depends on the initial bacterial contamination. This contamination causes meat deterioration, lowers quality and sometimes illness may be caused by bacterial pathogens or their toxins through meat and meat products.

Generally, animal proteins such as meats, meat products, fish and fishery products are generally regarded as high risk commodity in respect of pathogen contents, natural toxins and other possible contaminants and adulterants (Yousuf *et al.*, 2008). In fact, tissue from healthy animal are sterile however, it

has been pointed that during slaughter, dressing and cutting, microorganisms came chiefly from the exterior of the animal and its intestinal tract but that more added from knives, cloths, air, carts and equipment in general. External contamination of meat is a constant possibility from the moment of bleeding unit consumption (Lawrie, 1984). Among the factors that affect microbial growth in meat are intrinsic properties (physical and chemical properties of meat) and extrinsic (environmental factors) (Rombout and Nout, 1994), however the factors having the greatest influence on the growth of microorganisms in meat and meat products are the storage temperatures, moisture and oxygen availability (Forest *et al.*, 1985). The possible sources of these bacteria are likely to come from the skin of the animal from which the meat was obtained. Other potential sources of microbial contaminations are the equipment used for each operation that is performed until the final product is eaten, the clothing and hands of personnel and the physical facilities themselves are all implicated (Rombouts and Nouts, 1994). Retail cut could also result in greater microbial load because of the large amount of exposed surface area (Forest *et al.*, 1985).

However in Sudan; there are studies on the genera of aerobic bacteria in fresh meat (Hussein, 1987, Mohammed, 2000). *Bacillus* spp., *Micrococcus* spp., *Staphylococcus* spp., *Pseudomonas* spp., *Acinetobacter* spp. and *Proteus* spp. were isolated from beef. But additional research is needed to know the species of each specific genus. The present work was done to assess the total viable count, isolate and identify the *Staphylococcus* spp. in fresh meat sold in Khartoum State and investigate the presence of staphylococcal pathogens that may constitute a public health hazard from consumption of meat.

## MATERIALS AND METHODS

**Samples collection:** A total of 40 fresh beef samples were collected from different markets in Khartoum State: the samples were wrapped with sterile aluminum foil, labeled and stored in sterile containers in ice water and transferred immediately to the laboratory for further bacteriological analysis as described by the methods of (Harrigan, 1998).

**Samples preparation:** Under aseptic conditions the samples were opened, 10 gram of sample was taken, pounded with sterile mortar and pestle and suspended in 90 ml of normal saline. Ten fold dilutions of the homogenates mixer were made using sterile pipettes as described by the methods of (Harrigan, 1998).

**Culturing, isolation, purification and preservation:** Media used in this study included: Nutrient Agar, Peptone Water, Blood agar medium, Nutrient broth, Glucose phosphate medium and Cooked meat medium as general and enriched media. Other media with selective and differential characteristics used were Baird-Parker medium and Mannitol salt agar medium. All media were prepared according to the manufacturer's specification and sterilized at 121°C for 15 min. From the 10-fold dilutions of the homogenates; 0.1ml of 10<sup>-2</sup>, 10<sup>-3</sup>, 10<sup>-4</sup> and 10<sup>-5</sup> dilutions of the homogenate was plated in replicate on different media (in triplicates), using pour plate method. The plates were then incubated at 37°C for 24-48 h. Baird-Parker medium and Mannitol salt agar were used for *staphylococcus* spp. count while the Plate count agar was used for the total viable aerobic bacteria count. At end of the incubation periods, colonies were counted using the colony counter (Gallenkamp, England). The counts for each plate were expressed as colony forming unit of the suspension (cfu/g). Staphylococcal colonies obtained from selective media were subjected to gram staining and catalase test. Purification was done by several sub-culturing on corresponding media. Pure colonies were used for biochemical test according to Prof Elsanousi scheme (Appendix) for identification of *Staphylococcus* species. The pure cultures were inoculated on cooked-meat medium and then incubated at 37°C for 24-48 hrs, then stored at 4°C in refrigerator.

**Identification of staphylococci isolates:** *Staphylococcus* spp. Were identified based on coagulase test (slide test and tube test) and Novobiocin sensitivity as well as biochemical tests for example Urea test, Voges-Proskauer (VP) test and Sugar fermentation as described by (Harrigan, 1998) and (Barrow and Gelthan, 1993) were carried out.

## RESULTS AND DISCUSSION

Forty samples of fresh meat, 14 samples from Khartoum, 13 samples from Omdurman and 13 samples from Bahri were analyzed microbiologically for incidence of *Staphylococcus* spp. and bacterial load.

The *Staphylococcus* count of fresh meat samples was found to range from 3.23 x 10<sup>3</sup> to 8.7 x 10<sup>3</sup>. In Khartoum City the range was 4.46 x 10<sup>3</sup> to 8.71 x 10<sup>3</sup>, Bahri 3.81 x 10<sup>3</sup> to 4.46 x 10<sup>3</sup> and Omdurman 3.23 x 10<sup>3</sup> to 5.01 x 10<sup>3</sup>. Total viable count was ranged 4.78 x 10<sup>4</sup> to 3.39 x 10<sup>5</sup>, whereas in Khartoum it was 4.78 x 10<sup>4</sup> to 9.55 x 10<sup>4</sup>, Bahri 6.31 x 10<sup>4</sup> to 3.39 x 10<sup>5</sup> and Omdurman 7.07 x 10<sup>4</sup> to 1.41 x 10<sup>5</sup> as shown in Table 1. The results show that *Staphylococcus* count was highest in Omdurman and lowest in Khartoum. However, the total viable count was highest in Bahri.

The result obtained in this study was in agreement with Canadian Government guidelines which specified that the Aerobic Plate Count (APC) at 35°C for non-frozen ground beef should be less than 1.0 x 10<sup>7</sup> CFU/g. Our finding differed from the finding that, in meat, APC ranged between 10<sup>2</sup> and 10<sup>3</sup> cfu/g (Alalla, 1990). This difference will be due to different factors such as different locations. In this last work (Alalla, 1990) the samples were taken from abattoirs, while the fresh meat in this study was taken from butchereries from different markets where additional contamination may have taken place during transportation.

Nineteen species of the staphylococci genus were isolated as shown in Table 2. It was appeared at different frequencies in different cities, some of them appeared more than once and others appeared once, as individual it's observed that, the highest prevalence of staphylococci species was that of *Staph. epidermidis* (13.8%). This finding is in agreement with other workers who found that *Staph. epidermidis* was the most frequently recovered organism isolated from clinical infection and also in agreement with others who reported that *Staph. epidermidis* was found in large numbers all over human skin and mucous membrane (Allen *et al.*, 1997; Lamb *et al.*, 1990; Duerden *et al.*, 1992). *Staphylococcus aureus* ranks as a second. The presence of this organism could be indicative of contamination of meat from skin, mouth and nose of butchers and this is in agreement with others who reported that *Staph. aureus* was found on the anterior of nasal mucosa of 40-50% of healthy adults and in the throats of many of them (Duerden *et al.*, 1992). This may contaminate food during handling and cutting but contamination may also come from other sources.

Table 1: Total viable count and *Staphylococcus* count of fresh beef collected from Khartoum State

Location	Total viable count			Std. Dev.	Staph. count			Std. Dev.
	Minimum	Mean	Maximum		Minimum	Mean	Maximum	
Khartoum	4.78 x 10 <sup>4</sup>	6.31 x 10 <sup>4</sup>	9.55 x 10 <sup>4</sup>	0.15	4.46 x 10 <sup>3</sup>	6.02 x 10 <sup>3</sup>	8.71 x 10 <sup>3</sup>	0.1473
Bahri	6.31 x 10 <sup>4</sup>	1.39 x 10 <sup>5</sup>	3.39 x 10 <sup>5</sup>	0.36	3.81 x 10 <sup>3</sup>	4.16 x 10 <sup>3</sup>	4.46 x 10 <sup>3</sup>	3.61
Omdurman	7.07 x 10 <sup>4</sup>	5.0 x 10 <sup>5</sup>	1.41 x 10 <sup>5</sup>	0.15	3.23 x 10 <sup>3</sup>	3.98 x 10 <sup>3</sup>	5.01 x 10 <sup>3</sup>	9.50
Total	4.78 x 10 <sup>4</sup>	9.55 x 10 <sup>4</sup>	3.39 x 10 <sup>5</sup>	0.25	3.23 x 10 <sup>3</sup>	4.57 x 10 <sup>3</sup>	8.7 x 10 <sup>3</sup>	0.12

Table 2: Percentage of *Staphylococcus* spp. isolated from fresh beef collected from Khartoum State

Staphylococcus species	Total No. of isolates	% of individual Staph. spp. to the total No. of isolates*	Percentage		
			Khartoum	Omdurman	Bahri
<i>Staph. epidermidis</i>	8	13.8	25	37.5	37.5
<i>Staph. aureus</i>	7	12.0	28.6	42.9	28.6
<i>Staph. caseolyticus</i>	7	12.0	42.9	28.6	28.6
<i>Staph. saprophyticus</i>	6	10.3	33.3	50	16.7
<i>Staph. sciuri</i>	5	8.6	40	0	60
<i>Staph. cpitis</i>	4	6.9	0	50	50
<i>Staph. lugdunensis</i>	4	6.9	25	25	50
<i>Staph. cohnii</i>	3	5.2	0	33.3	66.7
<i>Staph. xylosus</i>	2	3.4	100	0	0
<i>Staph. kloosii</i>	2	3.4	0	100	0
<i>Staph. capitis ssp. Urealyticus</i>	2	3.4	50	50	0
<i>Staph. cohnii ssp. Urealyticus</i>	1	1.7	0	100	0
<i>Staph. felis</i>	1	1.7	0	100	0
<i>Staph. warneri</i>	1	1.7	0	100	0
<i>Staph. gallinarum</i>	1	1.7	0	100	0
<i>Staph. hycius</i>	1	1.7	0	0	100
<i>Staph. chromognes</i>	1	1.7	100	0	0
<i>Staph. lentus</i>	1	1.7	100	0	0
<i>Staph. haemolyticus</i>	1	1.7	0	100	0

1\Coagulase-positive, *Staph. aureus*.

Table 3: Coagulase-positive and coagulase-negative *Staphylococcus* species isolated from fresh beef samples collected from Khartoum State

Coagulase- negative <i>Staphylococcus</i> species		Coagulase-positive <i>Staph. spp.</i>	Location
Novobiocin- resistant	Novobiocin-sensitive		
<i>Staph. xylosus</i>			
<i>Staph. saprophyticus</i>			
<i>Staph. lentus</i>			
<i>Staph. sciuri</i>	<i>Staph. epidermidis</i> <i>Staph. caseolyticus</i> <i>Staph. lugdunensis</i>		
	<i>Staph. chromogenes</i> <i>Staph. capitis ssp. Urealyticus</i>	<i>Staphylococcus aureus</i>	Khartoum
<i>Staph. sciuri</i>			
<i>Staph. cohnii</i>			
<i>Staph. saprophyticus</i>	<i>Staph. epidermidis</i> <i>Staph. caseolyticus</i> <i>Staph. lugdunensis</i> <i>Staph. hycius</i> <i>Staph. cpitis</i>	<i>Staphylococcus aureus</i>	Bahri
<i>Staph. saprophyticus</i>			
<i>Staph. kloosii</i>			
<i>Staph. cohnii</i>			
<i>Staph. cohnii ssp. urealyticus</i>			
<i>Staph. gallinarum</i>	<i>Staph. epidermidis</i> <i>Staph. caseolyticus</i> <i>Staph. cpitis</i>		
	<i>Staph. felis</i> <i>Staph. lugdunensis</i> <i>Staph. warneri</i>		
	<i>Staph. haemolyticus</i> <i>Staph. capitis ssp. Urealyticus</i>	<i>Staphylococcus aureus</i>	Omdurman

<sup>2</sup>Coagulase-negative (novobiocin-sensitive), *Staph. epidermidis*, *Staph. caseolyticus*, *Staph. lugdunensis*, *Staph. capitis*, *Staph. capitis ssp. urealyticus*, *Staph. felis*, *Staph. warneri*, *Staph. hycius*, *Staph. chromogenes* and *Staph. haemolyticus*.

<sup>3</sup>Coagulase-negative (novobiocin-resistance), *Staph. saprophyticus* *Staph. sciuri*, *Staph. cohnii*, *Staph. cohnii ssp. urealyticus*, *Staph. xylosus*, *Staph. kloosii* and *Staph. gallinarum*.

In the present study, the isolates of staphylococci were divided into two groups according to the coagulase test, as shown in Table 3

These results show that most of staphylococci isolates were coagulase-negative and this was in agreement with other researchers who reported that most of

Table 4: Prevalence of staphylococci in Khartoum, Bahri and Omdurman

<i>Staphylococcus species</i>	Khartoum	Bahri	Omdurman
<i>Staph. epidermidis</i>	+	+	+
<i>Staph. Aureus</i>	+	+	+
<i>Staph. caseolyticus</i>	+	+	+
<i>Staph. saprophyticus</i>	+	+	+
<i>Staph. sciuri</i>	+	+	-
<i>Staph. cpitis</i>	-	+	+
<i>Staph. lugdunensis</i>	+	+	+
<i>Staph. cohni</i>	-	+	+
<i>Staph. xylosus</i>	+	-	-
<i>Staph. kloosii</i>	-	-	+
<i>Staph. capitis ssp. Urealyticus</i>	+	-	+
<i>Staph. cohni ssp. Urealyticus</i>	-	-	+
<i>Staph. felis</i>	-	-	+
<i>Staph. wameri</i>	-	-	+
<i>Staph. gallinarum</i>	-	-	+
<i>Staph. hycius</i>	-	+	-
<i>Staph. chromogenes</i>	+	-	-
<i>Staph. lentus</i>	+	-	-
<i>Staph. haemolyticus</i>	-	-	+

Table 5: % Isolates of the first four predominant species (*Staph. epidermidis*, *Staph. aureus*, *Staph. caseolyticus* and *Staph. saprophyticus*) from fresh beef collected from Khartoum, Bahri and Omdurman

Isolation	<i>Staphylococcus species</i>			
	<i>Staph. epidermidis</i> (%)	<i>Staph. aureus</i> (%)	<i>Staph. caseolyticus</i> (%)	<i>Staph. saprophyticus</i> (%)
Khartoum	25	28.6	42.9	33.3
Bahri	37.5	28.6	28.6	16.7
Omdurman	37.5	42.9	28.6	50

staphylococci found in the skin and respiratory tract are coagulase-negative (Duerden *et al.*, 1992). This could be due to the presence of isolates which come from human skin by contact.

Figure 1 illustrates that *Staphylococcus* spp. isolated from Khartoum beef samples (17 isolates, 29.3% of staphylococci) were identified during this study to belong to 10 species, *Staph. caseolyticus*, *Staph. epidermidis*, *Staph. aureus*, *Staph. saprophyticus*, *Staph. sciuri*, *Staph. xylosus*, *Staph. lugdunensis*, *Staph. chromogenes*, *Staph. lentus* and *Staph. capitis ssp. urealyticus*.

The number of staphylococci species isolated in Khartoum City was lower than that in Bahri and Omdurman. Among the 10 species isolated from Khartoum City, *Staph. caseolyticus* was representing the most common species 17.6% compared to 11.8% for each of *Staph. epidermidis*, *Staph. aureus*, *Staph. saprophyticus*, *Staph. Sciuri* and *Staph. xylosus* and 5.9% for each of *Staph. lugdunensis*, *Staph. chromogenes*, *Staph. lentus* and *Staph. capitis ssp. urealyticus*. These results are in agreement with those who found that the *Staph. caseolyticus* is the most frequently isolated species from meat (Igimi *et al.*, 1989).

In Bahri City 18 isolates (31% of staphylococci) belonged to 9 species of the genus *Staphylococcus*, *Staph. epidermidis*, *Staph. aureus*, *Staph. saprophyticus*,

*Staph. sciuri*, *Staph. cohni*, *Staph. caseolyticus*, *Staph. lugdunensis*, *Staph. hycius* and *Staph. capitis* as shown in Fig. 2.

*Staph. epidermidis* and *Staph. Sciuri* rank first with 16.7% for each, followed by *Staph. aureus*, *Staph. cohni*, *Staph caseolyticus* and *Staph. capitis* (11.1% each ) and *Staph. saprophyticus* and *Staph. hycius* (5.6% each). Most of the isolates were coagulase-negative. This is due to the normal flora found in human and animals and this was in agreement with others who stated that the coagulase-negative staphlococci are endogenous to human and certain animals (Lennette *et al.*, 1985).

The isolated species are in agreement with studies done in Sudan where these species were isolated from various organs of man and animals (Saeed, 1995; Hussein, 1997).

In Omdurman 23 isolates (39.7% of staphylococci) belonged to 14 species of the genus *Staphylococcus* as shown in Fig. 3. *Staph. epidermidis*, *Staph. saprophyticus* and *Staph. aureus*, were the top isolates (13% each). *Staph. caseolyticus*, *Staph. capitis* and *Staph. kloosii* came second (8.7% each). *Staph. cohni*, *Staph. felis*, *Staph. lugdunensis*, *Staph. cohni ssp. urealyticus*, *Staph. gallinarum*, *Staph. warneri*, *Staph. haemolyticus* and *Staph. capitis ssp. urealyticus* came as the last isolates (4.3% each). The first isolates were considered to be well- known pathogens to humans and animals, specially *Staph. aureus*. Their presence could

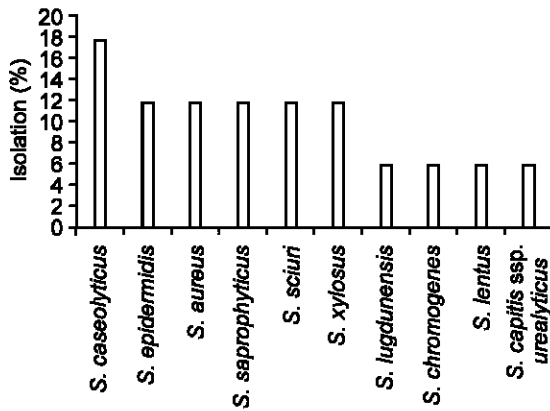


Fig. 1: *Staphylococcus* spp. Isolated from fresh beef in Khartoum city

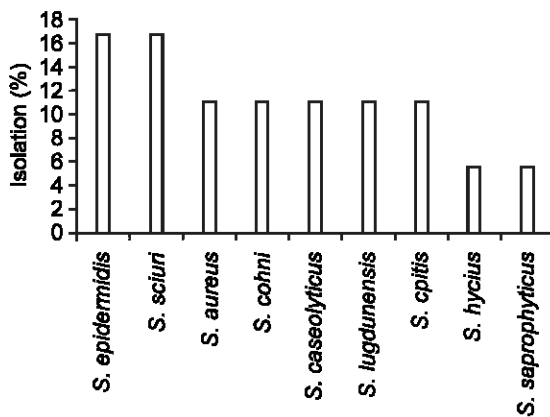


Fig. 2: *Staphylococcus* spp. Isolated from fresh beef in Bahri city

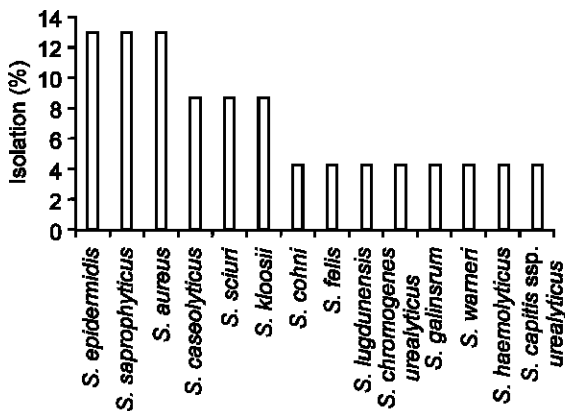


Fig. 3: *Staphylococcus* spp. Isolated from fresh beef in Omdurman city

condition of the butcher and absence of the health services in butcheries. This was in agreement with others who reported that *Staph. aureus* should be considered a potential pathogen (Lennette *et al.*, 1985). However, *Staph. epidermidis* and *Staph. saprophyticus* have low pathogenicity (Cruickshank *et al.*, 1975).

Generally, in this study, the prevalence rate did vary with different places under studies, for example in Omdurman, where the number of isolates were higher and also in their species, that is to say 23 isolates from 13 fresh beef samples contained 14 species. This means that more contamination takes place during the handling and preparation of the meat and also from air dust and personal contact during selling and this is in agreement with others who reported that the contamination of meat came from external sources during bleeding, handling, skinning and cutting and additional contamination took place in the retail markets, chopping blocks, sawdust and containers (Harrigan and McCance, 1976). According to the results some *Staphylococcus* species appeared in some places and were absent in others. As shown in Table 4, *Staph. kloosii*, *Staph. cohnii* ssp. *urealyticus*, *Staph. felis*, *Staph. warneri*, *Staph. gallinarum* and *Staph. haemolyticus* were isolated from Omdurman samples and were absent in both Khartoum and Bahri. *Staph. lentus*, *Staph. chromogenes* and *Staph. xylosus* were found in Khartoum samples but were absent in both Bahri and Omdurman. *Staph. sciuri* was found in Bahri samples and *Staph. capitis* and *Staph. cohnii* were found in Bahri and Omdurman and were not found in Khartoum. These differences may be attributed to the differences in cities, location and handling, selling way and methods of meat exposure in markets and whether the meat was sold on the same table as the viscera.

Data in Table 5 show that the first four predominant species with their percent appearance in Khartoum, Bahri, and Omdurman respectively, in brackets): *Staph. epidermidis* (25, 37.5 and 37.5), *Staph. aureus*, (28.6, 28.6 and 42.), *Staph. caseolyticus* (42.9, 28.6 and 28.6) and *Staph. saprophyticus* (33.3, 16.7 and 50). All these species are associated with human, especially in the skin and mucosa membrane and are considered as normal human flora. In the Sudan, these species were isolated by others (Esmail, 1997; Saeed, 1995; A/Elkarim, 1997; Hussein, 1997) at various rates.

Fresh meats sold to the public in open markets are grossly contaminated with staphylococcus as well as other bacterial forms. The finding of this study revealed that fresh meat sold at Khartoum, Omdurman and Bahri are contaminated with pathogenic staphylococci bacteria (*Staph. aureus*). The possible source of contaminants, are due to the unhygienic manner of handling meat from the slaughters to the markets (Okonko *et al.*, 2008b,c,d, 2009a,b). This study also reveals that the highest load of bacteria and staph. count were  $39 \times 10^5$  and  $8.7 \times 10^3$

be due to the insanitary condition of the butcher and absence of the health services in butcheries. The first isolates were considered to be well-known pathogens to humans and animals, specially *Staph. aureus*. Their presence could be due to the insanitary

cuf/g., respectively and the higher isolation percent of staphylococci was in Omdurman (39.7%) followed by Bahri (31%) and Khartoum (29.3%). Among these isolation of staphylococci, coagulase negative staphylococci were the major isolates. This mean that all these species were associated with human specially on the skin and mucous membrane and considered as normal human flora, some of these isolates (*Staph. epidermidis*, *Staph. saprophyticus*, *Staph. warneri*, *Staph. lugdunensis* and *Staph. heamolyticus*) have become a major problem in human infection. They cause nosocomial infection in neonatal and urinary tract infections, particularly in young women. Moreover, staphylococcal food poisoning, toxic shock syndrome and scaled skin syndrome are caused by *Staph. aureus*. (Kabir, 2009), it is therefore necessary that we also make the following recommendations from the findings of this study, that: 1.) Meat handlers and sellers should be educated on the adverse effect of lack of proper personal and environmental hygiene and sanitation; 2.) Veterinary doctors should inspect the animals to be slaughter before the meat is sold to the general public; 3.) The slaughter houses should be routine investigated for hygiene; 4.) Workers in connection with meat production and distribution must be routinely medical examined.; 5) Meat must be transported by special refrigerator cars and stored at low temperature to prevent staphylococcal growth, because food poisoning is caused by ingestion of improperly stored food in which *Staphylococcus aureus* has grow.; 6.) Meat must be stored in refrigerated cases at selling point, so as to avoid contamination resulting from an, dust...etc.; 7). Meat should be sold in big cuts as further cutting increases contamination.; 8.) Cutting boards and utensils must be kept clean and butchers should use gloves during work. and 9.) Meat must be kept away from viscera during the transportation and selling, especially in remote area.

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