# Isolation and Identification of Microorganisms in Processed Meats in Khartoum State

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***Abstract***
*A study was conducted at the University of Khartoum Faculty of Agriculture, To evaluate the microbiological characteristics of meat products samples obtained from four different factories in Khartoum State. Microorganisms from the different samples were isolated and identified to the genus level. Results indicated high Total Viable Bacterial Count (TVBC) in all samples, and frankfurter showed the highest mean TVBC, followed by a burger and then Pastrami. All samples showed Staphylococcus species' presence, and Salmonella was detected in 4 samples, while Coliforms were detected in Pastrami samples. Coliforms and E. coli were undetected in Frankfurter samples. Staphylococcus aureus was identified in Burger and Frankfurter samples, and Salmonella typhi was found in two samples of Frankfurter and Pastrami. Salmonella paratyphi A and Salmonella arizonae were placed in two samples of the burger.*

**Keywords**: *Microorganism; Processed meat; Khartoum State*

## I. INTRODUCTION

Nowadays, there is an increasing demand worldwide for a safe, constant supply of animal protein, and a gradual increase is being witnessed in the consumption of processed meats. There is a global interest in preserving processed meat to ensure its safety and bioavailability for a longer time. Meat is the most perishable of all-important foods since it contains sufficient nutrients needed to support microorganisms' growth [1]. Microbial contamination can lower the quality of fresh minced meat, shorten its shelf-life and result in economic loss and probably health hazards. The practically unavoidable infection causes spoilage and subsequent decomposition of meat by bacteria and fungi, borne by the animal itself, by the people handling the meat, and by their implements. Among the factors that affect microbial growth in meat are intrinsic properties (physical and chemical properties of meat) and extrinsic (environmental factors) [2]; however, the elements having the most significant influence on the growth of microorganisms in meat and meat products are the storage temperatures, moisture, and oxygen availability [3]. This study aimed to evaluate and identify the microorganism in the processed meat from different meat processing factories in Khartoum State-Sudan.

## II . MATERIALS AND METHODS

### *A. Collection of samples*

Eleven samples of frozen beef meat products in plastic packaged {4frankfurter (F), 4burger (B), and 3pastrami (P)} from four different meat processing factories in Khartoum-Sudan were collected from the markets and subjected to microbiological analysis. Microorganisms from different samples were isolated and identified to the genus level according to [4]

### *B. Preparation of serial dilutions of the meat samples*

Sterile 0.1% peptone water of pH 6.8-7.0 [5] was used for aerobic and anaerobic bacteria. The 1:10 dilution of the sample was prepared by suspending 10g of the whole meat products or homogenizing in 90ml of the peptone water diluents (W\V). This same procedure was followed for the preparation of the sequential dilutions up to $10^{-6}$.

### *C. Total Viable Bacterial Count (TVBC)*

The total viable bacterial count (TCBC) was carried out using the pour plate method described by [6]. One ml from $10^{-1}$ to $10^{-6}$ dilutions were aseptically transferred into sterile Petri dishes, and Nutrient Agar (NA) was added. The inoculum was mixed with the medium and allowed to solidify and was then incubated at 37°C for 2 days. Then plates with 30-300 colonies were counted using a colony counter (Quebec colony counter). Results were expressed as colony-forming units (CFU) per gram of the sample. Also, colonies of the dominant groups as judged roughly by colony and cell shapes were separately counted.

### *D. Presumptive coliform test*

For the presumptive test, one ml of each of the $10^{-1}$, $10^{-2}$, and $10^{-3}$ dilutions was added to 9ml of MacConkey Broth using the five-tube technique with

Durham tubes as described by [6]. The tubes were incubated at 37°C for 48 hours. The accumulation of gas in Durham tubes and acid production (change of color to yellow) indicated positive results.

***E. Confirmed coliform test***

To confirmed the coliform test, all tubes of the two highest dilutions showing gas fermentation in 24 hours were submitted to the confirmation test using Brilliant Green Lactose Bile (BGLB) broth fermentation tubes. All tubes of all dilutions, in which gas was produced only at the end of 48 hours, were submitted to the confirmed test. A loopful from the positive tubes was inoculated into BGLB lactose broth and then incubated at 37°C for 48 hours. The most probable number (MPN) was recorded using the most probable number tables used to record the coliform numbers [7]

***F. Fecal coliform test***

For the fecal coliform test, at least 3 loopfuls of each confirmed positive tube were sub-cultured into EC broth medium and then incubated at 44.5°C for 48 hours. Tubes showing any amount of gas production were considered positive. The MPN was recorded from tables.

***G. The differential fecal coliform test***

For further confirmation of fecal coliforms, EC broth tubes giving positive reaction at 44.5°C after 24 hours were streaked onto MacConkey agar and incubated at 37°C. Primary Escherichia coli isolated from MacConkey agar (pink colonies) was examined using Eosin Methylene Blue Agar plates (EMB) and incubated at 37°C for 48 hours. Colonies with green metallic sheen indicated a positive test for *Escherichia coli* [8].

***H. Staphylococcus aureus* count**

For determination of *Staphylococcus aureus* counts, Aliquots of 0.1 ml of the prepared dilutions of test samples were transferred to each of 3 Baird Parker Agar plates, distributed over the surface using a sterile bent glass rod. Inoculums were absorbed by the medium before inverting the plates and incubating at 35°C for 48 hours. Colonies in Plates typical of *Staphylococcus aureus* were counted.

***I. Detection of Salmonella***

For detection of *Salmonella* Ten grams of the samples of meat products were mixed with 90ml of Nutrient Broth medium, incubated overnight at 35°C, then 1.0 ml of the mixture was transferred to 10 ml Selenite-F broth medium, incubated at 35°C for 24 hours, mixed and aloopefull of a sample from Selenite- F broth was streaked onto Bismuth Sulfite Agar and incubated for 18-24 hours at 35°C. Plates were examined for the presence of colonies typical for *Salmonella spp.* A confirmatory test was carried out by taking discrete black colonies and sub-culturing them onto Triple Sugar Iron Agar slopes. The slopes were incubated at 37oC for 24 h, and the production of black color at the bottom of the tube confirmed Salmonella's presence. Another confirmatory test was carried out [4] [6] onto slants of Kligler Iron Agar medium, black color at the bottom of the tube again confirmed Salmonella's presence.

***J. Purification of bacterial isolates***

Predominant bacteria from morphologically different colony types were isolated from plate count agar done by pour plate method [4]; these isolates were purified by streaking twice on nutrient agar. The cultures were then kept in a refrigerator at 4°C, and then further identification was made through biochemical tests according to [4].

## III . RESULTS AND DISCUSSION

Table (1) all samples showed high total viable bacterial counts (TVBC) as determined by the pour plate method. The TVBC ranged from 1.0 $x10^8$ to 2.1$x10^9$ CFU/g in burger samples. This result is above the total viable counts of chilled and unfrozen uncooked meats set by the Sudanese Standard and Metrology Organization [9]. In this respect, [10] reported that in processed meats, the median numbers (CFU/g) of bacteria, anaerobic bacteria, and yeasts and molds were 4.70 x $10^7$(CFU/g), 6.15x $10^4$ CFU/g, and 6.60$x10^3$ CFU/g respectively in a beef burger. In frankfurter samples, the TVBC ranged from 2.1$x10^8$ to 2.7$x10^9$ CFU/g, and in pastrami samples, it went from 2.5$x10^6$ to 2.0$x10^8$CFU/g. Samples of frankfurter showed the highest mean TVBC (1.5$x10^9$ CFU/g), followed by burger samples (1.0$x10^9$ CFU/g) then Pastrami (7.5$x10^7$CFU/g). Similar results were obtained by [11] who reported that the aerobic plate count of fresh meat before processing was $10^2$-$10^3$ CFU/g, which increased after processing to $10^7$-$10^8$ CFU/g. The increase in TVBC of Frankfurter may be attributed to the effect of cold water used in the preparation after smoking to separate cellulose from the product. In this respect, [12] reported that cold water washes provide no perspective for effective microbial loads reduction. The high TVBC values reported in this study may be attributed to various factors, including inadequate cooling of cooked meat, bad time and/or temperature during cooking or heal processing of meat products such as frankfurters. Also, the display of marketed meat products uncovered for sale at ambient temperature and sometimes at refrigeration

temperatures unsuitable for storage, due to fluctuating and inadequate electricity supply. The inadequacy and fluctuation in electric supply have been reported by [13] and by [14] as the major factor contributing to meat product spoilage and increase in TVBC. The microbiological quality of meat depends on the animal's physiological status at slaughter, the spread of contamination during slaughter and processing, the temperature, and other conditions of storage and distribution are important factors that will determine the microbiological quality of the meat [16]. In addition to that, re-mincing and preparing these products increases the surface area of meat and eventually distributes microorganisms throughout the product, thus increasing the microbial load of the final product [17]. Additional sources of microorganisms that can be introduced into the cooked meat products are the seasoning and formulation ingredients used in the recipes for products. [18] reported that spices and hot seasoning to the ground beef and fresh products significantly increased the count of bacterial flora. Several studies have shown that spices contain various microorganisms such as pathogenic bacteria and toxogenic molds [19]; [20].

The comparatively low TVBC values of the pastrami samples compared with the other sampled meat products could be due to the effect of some additional ingredients such as salts and nitrite, in addition to the reduction of the water content during the hanging of the product for two days after coating with salt and spices during preparation.

Table (1) showed that *Salmonella spp.* was detected in four meat products (samples B1 and B2 of burger, sample F1 of frankfurter, and sample P1 of Pastrami). Two were identified as *Salmonella typhi*, one from frankfurter samples (S5), and the other (S7) was obtained from pastrami samples. The other two isolates (S2 and S1) were obtained from burger samples. They were identified as *Salmonella paratyphi* A and *Salmonella arizonae* (Table 2) so that these meat products were unsuitable for human consumption. The high incidence of *Salmonella* in the meat product samples may be due to the

*Table* (I): Total viable bacterial count, plate counts of *Salmonella typhi*, *Staphylococcus aureus*, coliforms, and *E. coli* in the investigated processed meat samples

| Sample code | Source | TVBC (CFU/g) | *Salmonella* presence | *Staphylococcus* (CFU/g) | Coliform (MPN/g) | *E. coli* (MPN/g) |
|---|---|---|---|---|---|---|
| B1 | Burger-factory 1 | $2.9x10^8$ | + | $1.7x10^4$ | 2400 | 95.0 |
| B2 | Burger-factory 2 | $2.1x10^9$ | + | $0.7x10^5$ | 2400 | 27.0 |
| B3 | Burger-factory 3 | $1.0\ x10^8$ | - | $2.5x10^3$ | 2400 | 79.0 |
| B4 | Burger-factory 4 | $1.6x10^9$ | - | $2.7x10^5$ | 920 | 920 |
| Mean | | $1.0x10^9$ | | $9.0x10^4$ | 2030 | 280.25 |
| F1 | Frankfurter-factory 1 | $2.3x10^9$ | + | $3.5x10^4$ | 0.00 | 0.00 |
| F2 | Frankfurter-factory 2 | $2.7x10^9$ | - | $2.4x10^3$ | 0.00 | 0.00 |
| F3 | Frankfurter-factory 3 | $9.2x10^8$ | - | $3.0x10^4$ | 0.00 | 0.00 |
| F4 | Frankfurter-factory 4 | $2.1x10^8$ | - | $1.9x10^4$ | 0.00 | 0.00 |
| Mean | | $1.5x10^9$ | | $2.2x10^4$ | 0.00 | 0.00 |
| P1 | Pastrami-factory 1 | $2.0x10^8$ | + | 0.00 | 6.1 | 0.00 |
| P2 | Pastrami-factory_2 | $2.1x10^7$ | - | 0.00 | 13 | 0.00 |
| P3 | Pastrami-factory_3 | $2.5x10^6$ | - | $4.5x10^2$ | 23 | 0.00 |
| Mean | | $7.5x10^7$ | | $4.5x10^2$ | 14 | 0.00 |

*n=3

*Table* (II): Identification of *Salmonella* isolates from the processed meat samples.

| Isolate code / Biochemical Test | S2 | S1 | S5 | S7 |
|---|---|---|---|---|
| Gram staining | - | - | - | - |
| Shape | Rod | Rod | Rod | Rod |
| Endospore staining | - | - | - | - |
| Catalase test | + | + | + | + |
| Oxidase test | - | - | - | - |
| O/F test | F | F | F | F |
| Motility test | + | + | + | + |
| Growth in air | + | + | + | + |
| Growth anaerobically | - | - | - | - |
| Starch | - | - | - | - |
| acid from glucose) | + | + | + | + |
| gas from glucose) | + | + | _ | _ |
| Indole test | - | - | - | - |
| H2S in (TSI test) | _ | + | D | D |
| Methyl red | + | + | + | + |
| Urease test | - | - | - | - |
| Arginine dihydrolase | - | - | - | - |
| V.P. test | - | - | - | - |
| Casein hydrolysis | - | - | - | - |
| Lactose | + | + | + | + |
| Sucrose | d | d | D | D |
| D- Manitol | + | + | + | + |
| Adonitol | - | - | - | - |
| Arabinose | + | + | _ | _ |
| Cellobiose | - | - | - | - |
| Glycerol | d | _ | D | D |
| Inositol | _ | _ | _ | _ |
| Maltose | d | + | + | + |
| Raffinose | - | - | - | - |
| Salicin | - | - | - | - |
| Sorbitol | - | - | - | - |
| Trehalose | + | + | + | + |
| Xylose | - | - | - | - |
| MacConkey growth | + | + | + | + |
| Species | paratyphi A | arizonae | Typhi | Typhi |

Contamination of minced meat is used for production by feces, contaminated water, environment, hides, and poor personal hygiene during processing, handling, and marketing of sausage (Lefoka, 2009). On the other hand, *Salmonella* was not detected in the seven remaining samples. This is in accordance with the results of Selvan *et al.* (2007), who did not recover *Salmonella* from samples of retail meat products. Also, Vazgecer *et al.* (2004) failed to detect *Salmonella* in meat products. The absence of Salmonella from Pastrami was investigated by Genigeorgis and Lindroth (1984), and it was found that the product is generally safe.

*Staphylococcus spp*. Showed higher counts in all samples of burger and frankfurter. The *Staphylococcus aureus* was identified in burger and frankfurter samples, making 33% of the Staphylococcus isolates. While the rest of the isolates were *Staphylococcus epidermidis* (33.3%), *Staphylococcus simulans* (22.2%), and *Staphylococcus xylosus* (11.1%), which was obtained from the pastrami sample (Table 3). The Staphylo-coccal counts ranged from $1.7x10^4$ CFU/g to $2.7x10^5$ CFU/g in burger samples, and from $2.4x10^3$ CFU/g to $3.5x10^4$ CFU/g in frankfurter samples, while on the Pastrami only one sample showed Staphylococcal growth, with a count of $4.5x10^2$ CFU/g (Table 1). The mean values of Staphylococcal counts of all collected samples were $4.5x10^2$, $2.2x10^4$, and $9.0x10^4$ CFU/g on Pastrami, frankfurter, and burger, respectively. The Sudanese Microbiological Standards for Foods [9] indicate that the acceptable Staphylo-coccus limits are $5x10^2$cell/g and the level of the maximum count is $1x10^3$ CFU/g. Thus, the burger and frankfurter samples were spoiled and unsuitable for human consumption except for the samples of Pastrami, which its count was acceptable as it falls within the permissible limits.

The high counts of *Staphylococcus* in the sampled meat products may be attributed to cross-contamination during preparation, processing, transportation, and packaging, as observed by [25], who reported that chopped meat, spices, or the environment could also have contributed to product contamination. Also [26]reported that *Staphylococcus*' presence in food indicates human contact, such as personal hygiene and poor food vendor's poor manufacturing practices. In connection to this [27] reported that *Staphylococcus* are primarily found in processed meat and dairy products, survive in the salted medium of hams and sausages, and are known to multiply in custard, potato, salads, and ice cream. As shown in Table 1, the total coliforms and *Escherichia coli* were detected in all the investigated burger samples. There were four isolates of *E. coli* from burger samples. Two (E1, E4 ) were identified as *E. coli* type I, and the other (E2, E3) were identified as *E. coli* type II, the difference between the two classes on the indol test which was positive of *E. coli* type I and negative for *E. coli* type II (Table 4). While in pastrami samples, only coliforms were detected, but on frankfurter samples, coliforms and *E.coli* were not detected, revealing that the smoking and cooking were done in preparation of frankfurter, in addition to salt and nitrite added were effective. In this respect, [28] showed that the heating step in the production of cooked cured meats destroyed the typical raw meat

flora except for the spore, which is the case in this study.

The highest coliforms mean counts were showed in burger samples (2030 MPN/g) while the least mean counts were in pastrami samples (14 MPN/g). Also, the mean count of *Escherichia coli* was higher in burger samples (280 MPN/g) while they were not detected in pastrami samples (Table 1). Sudanese Standard and Meteorology Organization SSMO [9] reported that the acceptable microbiological limit is 50 CFU/g, and the level of the maximum count is $5 \times 10^2$ CFU/g and 0.00 for *E. coli*. The increase of coliforms count may be correlated with processing, post-processing contamination, and handling, which may enhance their growth. Pathogenic coliforms may also exist on the fingertips and cannot be washed off simply by handwashing. These pathogens may, along with other organisms, be able to be transferred to the food.

This study's results agree with finding [29] who reported that foods of animal origin (minced meat), either cooked or uncooked, were predominantly contaminated with *E. coli*. Also, [30] reported.

*Table* (III): Identification of *Staphylococcus* isolates from processed meat Samples

| Biochemical | Staphylococcus isolates | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | B1 | B2 | B3 | B4 | F1 | F2 | F3 | F4 | P1 |
| Gram staining | + | + | + | + | + | + | + | + | + |
| Shape | coccus | Coccus | coccus | Coccus | coccus | Coccus | coccus | Coccus | coccus |
| Endospore staining | - | - | - | - | - | - | - | - | - |
| Catalase test | + | + | + | + | + | + | + | + | + |
| Oxidase test | - | - | - | - | - | - | - | - | - |
| O/F test | F | F | F | F | F | F | F | F | F |
| Motility | - | - | - | - | - | - | - | - | - |
| Growth in air | + | + | + | + | + | + | + | + | + |
| Growth anaerobically | + | + | + | + | + | + | + | + | Weak |
| Glucose (acid) | + | + | + | + | + | + | + | + | + |
| Urease test | D | + | + | + | d | + | d | + | + |
| Coagulase | + | - | - | - | + | - | + | - | - |
| Nitrate reduction | + | + | + | + | + | + | + | + | + |
| V.P. test | + | + | - | + | + | - | + | + | - |
| Arginine | - | - | - | - | - | - | - | - | - |
| Indole test | - | - | - | - | - | - | - | - | - |
| Lactose | + | - | + | - | + | + | + | - | + |
| Maltose | + | + | - | + | + | - | + | + | + |
| Mannitol | + | - | + | - | + | + | + | - | + |
| Fructose | + | + | + | + | + | + | + | + | + |
| Sucrose | + | + | + | + | + | + | + | + | + |
| Trehalose | + | - | + | - | + | + | + | - | + |
| Xylose | - | - | - | - | - | - | - | - | + |
| Cellobiose | - | - | - | - | - | - | - | - | - |
| Raffinose | - | - | - | - | - | - | - | - | - |
| Species | aureus | Epidermis | simulans | epidermis | Aureus | simulans | aureus | epidermis | xylosus |

Legend:

(d) = Delayed reaction. (B1-B4) isolated from burger
(+) = Positive reaction. (P1) isolated from pastrami
(-) = Negative reaction. (F1- F4) isolated from frankfurter

*Table* (IV): Identification of *E. coli* isolates from the processed meat samples

| Isolate code / Biochemical Test | E1 | E2 | E3 | E4 |
|---|---|---|---|---|
| Gram staining | - | - | - | - |
| Shape | Rod | Rod | Rod | Rod |
| Endospore staining | - | - | - | - |
| Catalase test | + | + | + | + |
| Oxidase test | - | - | - | - |
| O/F test | F | F | F | F |
| Motility test | + | + | + | + |
| Growth in air | + | + | + | + |
| Growth anaerobically | + | + | + | + |
| Citrate utilization | - | - | - | - |
| acid from glucose | + | + | + | + |
| gas from glucose | + | + | + | + |
| Indole test | + | - | - | + |
| H2S in (TSI) | - | - | - | - |
| Methyl red | + | + | + | + |
| Urease | - | - | - | - |
| Arginine dihydrolase | - | - | - | - |
| V.P test | - | - | - | - |
| Gelatine liquefaction at 22°C | - | - | - | - |
| Lactose | + | + | + | + |
| Sucrose | d | d | d | D |
| D- Manitol | + | + | + | + |
| Salicin | d | d | d | D |
| D-Sorbitol | + | + | + | + |
| Arabinose | + | + | + | + |
| Raffinose | d | d | d | D |
| L-Rhamnose | + | + | + | + |
| Maltose | + | + | + | + |
| D-Xylose | + | + | + | + |
| Trehalose | + | + | + | + |
| Cellobiose | _ | _ | _ | _ |
| Escalin hydrolysis | d | d | d | D |
| Melibiose | + | + | + | + |
| Species | Type 1 | Type 2 | Type 3 | Type1 |

Legend:

(E1, E2, E3, E4) = different isolates of E. coli
(d) Delayed reaction.
(+) Positive reaction.
(-) Negative reaction.
(F) Fermentative

that *E. coli* O157 was detected in 2.8% (43/1,533) of raw minced beef and beef burger samples on retail sales in Ireland. In connection to this, [31] [32] reported that improper handling and improper hygiene might lead to the contamination of ready-to-eat foods, which might eventually affect the health of the consumers.

The results of this study may suggest that burger samples contain the highest level of pathogenic bacteria, which are potential health threats to the consumer, then frankfurter, and the least count was reported in Pastrami. [33] and [31] isolated similar organisms from sausages, hamburgers, and seafood. This result is attributed to the preparation of the meat product, and the ingredients added. It is noted that burger and sausage are uncooked meat products, while frankfurter and mortadella are cooked products, and Pastrami is a cured meat product. [34] reported that in raw meat products with higher salt concentrations like salami or raw ham, aw falls to 0.93 and that bacteria do not grow any longer; only molds can cope with such low aw values. Also, the garlic in Pastrami's paste improves the hygienic properties of this product, since other raw hams easily become moldy and may contain mycotoxins [35]. To prevent the health risk, different methods are used to reduce or eliminate pathogens from spices such as the use of ultraviolet (UV), infrared or gamma rays, microwave treatments, or the use of ethylene oxide. Also, the proper procedure of meat products making, handling, and avoiding bad habits will minimize or reduce the number of pathogens in the final product.

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