**Journal of Agri-Food and Applied Sciences**
Available online at jaas.blue-ap.org
©2014 JAAS Journal. Vol. 2(7), pp. 206-219, 31 July, 2014
E-ISSN: 2311-6730

# Microbial Profile of Sausages in Khartoum State

**Nagwa B. Elhag[1*] El Rakha B. Babiker[2] and Ahmed A. Mahdi[3]**

1-Deparment of Food Hygiene and Safety, College of Public & Environmental Health, Bahri University, Khartoum North, Sudan.
2-Food Research Centre, Microbiology & Biotechnology Department, Agricultural Research Corporation, Shambat, Sudan.
3-Department of Botany and Agricultural Biotechnology, Faculty of Agriculture, University of Khartoum, Shambat, Sudan

**Corresponding Author:** Nagwa B. Elhag

*Received: 15 June, 2014* *Accepted: 10 July, 2014* *Published: 31 July, 2014*

**ABSTRACT**

In the last ten years meat industry has expanded widely in Sudan. Several sausages factories have bean established in Khartoum State. This study was carried out to investigate and identify different types of microorganisms associated with fresh Sudanese sausage samples sold in Khartoum State, Sudan. The Microbiological parameters investigated were total viable bacterial count, *Salmonella*, aerobic and facultative anaerobes, coliform, *E. coli*, and yeasts and moulds. Enumeration of different microorganisms was carried out on selective media and the Identification of microbial isolates was determined using conventional methods. Forty fresh sausage samples were collected from (a) butcheries, (b) 4 factories and (c) home-made. All samples showed high mean total viable bacterial count of which the butchery samples recorded the highest mean ($2.81x10^7$- $6.87x10^7$cfu/g). *Salmonella* was almost detected in all samples. The mean counts for aerobic spore-formers ranged between 9.0x10 and $1.21x10^3$cfu/g, while for facultative anaerobes ranged between 1.70 x10 and $7.50x\ 10^3$cfu/g. Only four samples were free of coliforms. The highest mean count for coliforms was reported in F1 factory samples (mean 5124 MPN/g). *E. coli* was detected in 27 of the 40 sausage samples examined. The highest counts were recorded in Khartoum North butchery samples (mean 143MPN/g).Yeasts were found in 36 samples with mean counts between 4.00x10 and $1.49x10^4$cfu/g, while moulds were detected in 23 samples ranging between 0.10x and $4.40x10^2$cfu/g. The dominant bacterial species isolated from the investigated sausage samples belonged to the genera *Bacillus*, *Staphylococcus*, *Streptococcus* and *Micrococcus*. *Bacillus cereus* represented the highest percentage (22.92%) of all isolates. The aerobic spore-formers were dominated by *Bacillus subtilis* (60%), while the facultative anaerobic spore-formers were dominated by *Bacillus cereus* (80%). The predominant mould flora was identified as *Rhizopus nigricans* and *Aspergillus niger*. Fresh Sudanese sausages as offered for sale suffer from high microbiological loads of bacteria including probable bacterial pathogens, yeasts and moulds. To produce high quality sausage, it is necessary to follow high technology procedures for sausage production and safety measures during processing, handling, transportation and marketing

***Keywords:*** *coliforms, Identification, moulds & yeasts, sausages, salmonella, spore-formers.*


## INTRODUCTION

The expansion of meat industry in Sudan has resulted in the production of different types of meat products e.g. sausages , kufta ,burger ,shawrma……etc. Nowadays sausage is becoming the most popular meat product in Sudan. The storage of this product is usually inadequate which make the product prone to early spoilage by spoilage and pathogenic microorganisms. Meat

and meat products cause many diseases and therefore imply a risk for human health. Recently several food-borne diseases occurred in many African countries such as Salmonellosis ,cholera, entero-haemorragic *Escherichia coli*) EHEC), hepatitis A and acute aflatoxins .Approximately 34000 cases of cholera occurred due to intake of contaminated water and foods which have been reported in 30 countries with more than 1000 death (FAO news release, 2005). Contaminated food is responsible for high percent of infant diarrhea. There are 1000 million cases of acute diarrhea in children under 5 years of age in Africa, Asia except China, and Latin America (Zden ěk and Matyáš, 1992). Many factors contribute to cause Food-borne diseases in developing countries; these factors include polluted or unsatisfactory water supplies, inadequate waste disposal, contaminated production and processing area, and transportation of food in unhygienic containers. Raw meat and poultry are usually contaminated by hazard microorganisms such as *Salmonella*, *Campylobacter*, *Listeria* and *Escherichia coli* .These products play an important role in the transmission of these microorganisms to other foods during preparation and storage (Lin, 1996; Uyttendaele, 1999, Chapman, 2001. As pointed by the World Factbook (2013) the degree of risk due to food or waterborne disease is very high and the infectious diseases are bacterial and protozoa diarrhea, hepatitis A and typhoid fever. Typhoid fever is transmitted by food or water contaminated with faecal materials or sewage. The mortality death by typhoid can reach 20% in Sudan (The World factbook, 2013) .

Statistical analysis of food-borne diseases in Sudan are not well analyzed and the laboratories facilities for food hazards inspection is not enough and equipments are relatively old (Codex Alimenaris, 2013). Ibrahim, (2013) stated that 10-30% of food-borne diseases in food service operations can be caused by food handlers in Sudan. Several factors play an important role in food poisoning broadcasting and these include improper holding temperatures, inadequate cooking, contaminated equipments, purchase and receipt of food from unsafe sources, and poor personal hygiene (Pingar and Cooke, 1985; Ibrahim, 2013). The most common diseases are those caused by *Salmonella*, *Staphylococcus*, *Bacillus* and *Clostridium* (Pringer and Cooke, 1985). A total of 30.1% of food handlers in Omdurman area of Sudan were found to be carriers of pathogenc organisms (Humodi and Hatim, 2010). These pathogenic organisms include *Staphylococcu aureus*, *Salmonella typhi*, *Sheglla boydii* and intestinal parasites *Giardia lamblia* and *Entamoeba hostilitica*/ dispar. Elfaki and Abdalla (2011) investigated Shawerma which is one of the popular meat products in Sudan. They found that the total bacterial count (TBC) was found to be $5.3\times10^3$, $8.4\times10^3$ and $5.3\times10^4$ cfu/g in Albait Alssory, Collage of Agricultural Studies- Sudan University of Science and Technology and Omdurman market. Samples from Omdurman market were found highly contaminated with pathogenic bacteria and coliforms. The prevalence of diarrhea and gastroenteritis diseases are considered as one of the most 10 diseases leading to hospital admission either in Khartoum State or other different states in Sudan. Khartoum State represents high prevalence compared with other states representing 39/1000 population (outpatients) for the year 2010. The prevalence rate per 1000 population is 18, while for children age (0-4) is 10.6%. The percent of death by diarrhea and gastroenteritis between children age 0-4 is 5% of total deaths. Total cases of patients for typhoid, dysentery, diarrhea and gastroenteritis diseases were 10221, 8059, 73396 respectively. The rate death for typhoid was 64, 196 for dysentery, and 591 for diarrhea and gastroenteritis (National Health Information Center, 2010). This research is carried out to determine the degree of safety in Sudanese sausages and to study the pathogenic microorganisms associated with it in Khartoum State .

## MATERIALS AND METHODS

### *Sample Collections*

Forty fresh sausage samples were collected from eight sources (5 samples each) which included (a) butcheries in Khartoum, Khartoum North, and Omdurman, (b) Factory-processed sausages at retail outlets ( include F1, F2, F3, and F4), (c) homemade sausages obtained from households in Khartoum, Sudan. Samples were kept in sterile insulated ice container and were immediately transferred to the laboratory for analysis.

### *Microbiological analysis*

Thirty grams of each sample of fresh sausages were weighed aseptically in sterile bottles and then blended with 270 ml sterile peptone water for 30 sec in a sterilized electric blender. Serial ten-fold dilutions were prepared following the method described by Harrigan (1998)Total viable bacterial count was determined by the pour plate method using Nutrient Agar medium (NA). Counting was carried out using a colony counter (Quebec Colony Counter) and then the results were expressed as colony forming unit) cfu/g) of the sample .

For *Salmonella* presence 25 grams of each sample were weighed, added aseptically and mixed well with 250 ml of sterile Nutrient Broth (NB) and incubated at 37ºC for 24 hours. Ten ml of the enriched mixture were drawn aseptically into 100 ml Selenite Broth, and then incubated at 37ºC for 24 hours. A loopful from the broth was streaked onto dried Bismuth Sulphite Agar plates which were then incubated at 37$^{O}$C for 24 hours. Confirmatory tests were carried out onto Triple Sugar Iron Agar and Kligler Iron Agar tubes respectively and incubated at 37ºC for 24 hours (Harrigan and MacCance, 1976; Harrigan, 1998).

Total coliforms group were determined using the Most Probable Number (MPN) technique. MacConkey and Brilliant Green Bile Lactose Broth (BGB) were used for presumptive and confirmed tests for coliforms respectively (FAO, 1992). For faecal colifrom presence, positive tubes from Brilliant Green Bile Lactose Broth (BGB) medium were subcultured into *Escherichia*

*coli* broth medium and then incubated at 44.5°C for 48 hours. The Most Probable Number (MPN) for both coliforms and faecal colifroms was recorded using the MPN table (FAO, 1992). For the confirmation of faecal coliforms, a loop full from EC broth giving positive results was streaked onto Eosin Methelene Blue agar medium (EMB) and incubated at 37°C for 48 hours. Colonies with green metallic sheen indicated a positive test for *Escherichia coli*. Extra confirmatory tests of *E coli* were carried out by the IMVEC test (Harrigan, 1998). Aerobic spore-forming and facultative anaerobic bacteria were investigated by the surface plate method using Starch Milk Agar medium (Harrigan, 1998).

For spore-formers, ten ml of a 1/10 dilution of the sample were heated in a water bath at 80°C for 15 minutes as described by Harrigan (1998). Several dilutions were made and suitable dilutions were plated in Starch Milk agar plates and incubated aerobically at 37°C for 48 hours for aerobic spore formers, and also in anaerobic jar for facultative anaerobic spore formers.

Yeasts and moulds were detected by spreading 0.1 ml of each sample from suitable dilutions onto Malt-extract Agar (MEA) containing 0.1g chloramphenicol to suppress bacterial growth. Plates were incubated at 30°C for 5 days (Harrigan, 1998; Andrews, 1992). The results were represented as cfu/g for each sample. Identification of bacterial isolates was done by convential methods based on cultural, morphological, and biochemical tests (Sneath, 1968; Barrow and Feltham, 1993; Harrigan, 1998). Mould isolates were identified according to Ellis (1976), Pitt and Hoking (1985), Kulwant, (1992) and Andrews (1992), while yeasts isolates were not identified.

## RESULTS AND DISCUSSION

The mean total viable bacterial counts (TVBC) of the sampled sausages were high, ranging from $2.1210^5$cfu/g to $6.87x10^7$cfu/g. Samples obtained from butcheries in Khartoum, Khartoum North and Omdurman exhibited the highest mean ($2.81x10^7$- 6.87x $10^7$ cfu/g) (Table 1). Oluwafemi and Simisaye (2006) found that the total aerobic bacterial counts of sausage samples sold in Nigeria ranged between $2.06x10^6$ cfu/g and $4.0x10^8$cfu/g. British fresh sausages from shops had been found to contain 1- $5000x10^5$ viable organisms/g (Dowdell and Board, 1968). Similar results were obtained in Sudan by Musa (2004) who found that the total viable bacterial counts for sausages during and after processing were $1.1x10^7$cfu/g and $8.2x10^7$cfu/g, respectively. The high TVBC values reported in this study may be attributed to various factors. One major factor is the display of sausages uncovered for sale at ambient temperature and sometimes at refrigeration temperatures unsuitable for storage, due to fluctuating and inadequate electricity supply (Abugroun et al., 1993 and Pearson and Tuber (1984). Additional handling by butchers may lead to the increase of microbial load, as butchers have a habit of re-mincing the displayed and stored sausages from the previous batches to be refilled into new casings to be sold as fresh product for the consumers as in Khartoum butcheries sausages. Re-mincing increase the microbial load of sausages (Jay, 2005). Humodi and Hatim (2010) stated that 30.1% of food handlers were carriers of pathogenic microorganisms in Omdurman area in Sudan. The addition of vegetables, fruits, beans, other plant ingredients and spices plays an important role in the characteristics of meat products. This addition may contribute to the increase of bacterial flora (Colak, 2006; Cohen 2008; Hampikyan, 2009). Adams and Moss (2008) found that animal hides carry a mixed microbial population of different microorganisms and contribute to the contamination of meat products. Also contaminated hooks used for displaying sausages play an important role in increasing the microbial load of sausages (FAO, 1990). Factories-processed samples (F3, F1) showed lower TVBC, this may be referred to the processing under better hygienic conditions, high quality meat used in sausage production, and also the effect of salts and spices added to sausage batter (Snyder, 1997). The mean pH of the sausage samples ranged between 5.83 and 6.23 (Table.1). The mean pH range of the samples obtained from butcheries in Khartoum, Khartoum North and Omdurman was 6.00-6.23, while factory-processed sausage in retail outlets showed a mean pH range of 5.83 - 5.99 and home-made samples had a mean pH of 5.91. The high mean pH values observed in this study may be attributed to the use of dark, firm and dry meat (DFD), which makes the meat prone to early spoilage (Dharmaveer, 2007; Jamilah, 2008).

With regard to Salmonella species presences, results revealed that Salmonella spp. were detected in all investigated sausage samples (97.5%) except one (Table. 2). Many studies revealed that Salmonella spp. can be isolated from different types of sausages (Abrahim, 1998; Mattick, 2002; Özbey, 2007). The high incidence of Salmonella in the sausage samples may be attributed to the contamination of minced meat used for sausage production by faeces, contaminated water, environment, hides, and poor personal hygiene.

Table 1. Sausage sample sources, pH, and total viable bacterial counts

| Sample No | Sample source | * Mean TVBC (cfu/g) | * Mean pH |
|---|---|---|---|
| 1 | Khartoum butcheries | $6.57x10^7$ | 6.00 |
| 2 | Khartoum North butcheries | $6.87x10^7$ | 6.03 |
| 3 | Omdurman butcheries | $2.81x10^7$ | 6.23 |
| 4 | **Factory1 | $7.02x10^5$ | 5.85 |
| 5 | **Factory2 | $3.97x10^6$ | 5.87 |
| 6 | **Factory3 | $2.12x10^5$ | 5.83 |
| 7 | **Factory4 | $2.23x10^7$ | 5.99 |
| 8 | ***Homemade | $8.63x10^6$ | 5.91 |

* Mean of 5 replicates. ** Factory samples at retail outlets
*** Homemade samples at Khartoum State

Table 2. Presence of *Salmonella*, aerobic and facultative anearobic bacteria associated with sausages samples

| Sample No | Sample source | *Salmonella* presence | * Mean of aerobic spore-formers cfu/g | * Mean of facultative anaerobic spore-formers (cfu/g( |
|---|---|---|---|---|
| 1 | Khartoum butcheries | except one | $9.2x10^2$ | $5.70x10^2$ |
| 2 | Khartoum North butcheries | + | $1.21x10^3$ | 1.76x10 |
| 3 | Omdurman butcheries | + | $7.20x10^2$ | $2.9x10^2$ |
| 4 | **Factory 1 | + | 9.0x10 | $1.80x10^2$ |
| 5 | **Factory 2 | + | $3.60x10^2$ | $3.50x10^2$ |
| 6 | **Factory 3 | + | $6.41x10^2$ | $2.37x10^2$ |
| 7 | **Factory 4 | + | $7.04x10^2$ | $7.50x10^3$ |
| 8 | Homemade | + | $2.2x10^2$ | $1.10x10^2$ |

*Mean of 5 replicate.
** Factory samples at retail outlets

during processing, handling, marketing of sausage and from domestic animals and poultry in markets. (Podpecan *et al.*, 2007; Lefoka, 2009). Özbey, (2007) reported that the potential risk of acquiring salmonellosis is due to the consumption of camel sausages.

The highest mean counts of the aerobic spore-formers were recorded in samples collected from butcheries ranging between $7.20x10^2$ and $1.21x10^3$cfu/g, while facultative anaerobic spore-formers were detected in 34 of the 40 sausages samples. The mean counts ranged between 1.76x10 and $7.50x10^3$cfu/g (Table 2.). Many studies investigated the presence of spore-formers in meat products, fresh fish meat and other types of foods (Saleh, 1993; Gamal El-Deen, 2010). High counts of aerobic and facultative anaerobic spore-formers may originate from the addition of spices. The addition of contaminated spices to meat products increases the risk of early spoilage, food-borne infections and intoxications (Banerjee and Sarkar, 2003).

Coliforms were detected in 36 of the 40 sausages samples investigated. The highest mean counts were reported in the samples obtained from F1 (5124 MPN/g). The least mean count was found in samples from F3 sausages (150 MPN/g) as shown in Table.3. *Escherichia coli* was found in 27 of the 40 sausage samples. The butcheries samples showed the highest *E coli* count compared to factory and homemade samples. However the highest mean count was recorded in samples from Khartoum North butcheries It is quite clear that the total coliform and faecal coliform counts are higher than the acceptable limits (50 for coliforms and 0.00 for *E. coli*) established by the Sudanese Standard Metrology

Table 3. .Most probable numbers of coliforms and *E. coli* associated with sausage samples

| Sample No | Sample source | * Mean coliform MPN/g | * Mean *E. coli* MPN/g |
|---|---|---|---|
| 1 | Khartoum butcheries | 1392 | 130 |
| 2 | Khartoum North butcheries | 163 | 143 |
| 3 | Omdurman butcheries | 1098 | 46.2 |
| 4 | **Factory 1 | 5124 | 68 |
| 5 | **Factory 2 | 403.4 | 14.5 |
| 6 | **Factory 3 | 150 | 22 |
| 7 | **Factory 4 | 1486.8 | 73.4 |
| 8 | Homemade | 900 | 52 |

* Mean of 5 replicates.
** Factory samples at retail outlets

Organization (SSMO, 2001) and lower than those obtained by Abd ELaziz (1996). The high incidence of coliform and faecal coliform in this study may be due to the poor hygiene conditions during processing, handling, marketing and storage, and due to the lack of preventive measures to reduce the chance of dust falling on the finished product, and addition of spices, (Al-Mutairi, 2011).

Yeasts were detected in 36 of the 40 investigated samples. Factory-processed sausages exhibited high counts in the range of $6.02x10^2$ and $1.49x10^4$cfu/g, however butcheries samples showed the least counts (Table 4). Seventeen of the forty sausage samples were found free of moulds. Other samples showed mould loads that ranged from as low as 0.10x10cfu/g (F4 samples) to as high as $4.40x10^2$cfu/g (Home-made samples) (Table. 4). Many studies revealed the presence of yeasts and moulds in different types of sausages (Drosinos, 2005; Samappito, 2011). Some authors stated that yeasts and moulds were not detected in meat products at the end of the ripening period (Rebecchi, 1998). Moulds have been used as an indicator of sanitary quality in food processing plants since they can grow rapidly on food remaining and adhering to the surfaces. As the Sudanese Standard Metrology Organization (SSMO, 2001) did not establish sausage safety limits for yeasts and moulds, it can not be stated that if these values for yeast and mould counts can imply a risk for human health or not.

Affiliation of microorganisms in this study revealed that most of the isolates (81.24%) were Gram-positive. *Bacillus cereus* accounted for 22.92% of the

Table 4 .Yeasts and moulds associated with sausage samples

| Sample No | Sample source | * Mean Yeasts cfu/g | * Mean Moulds cfu/g |
|---|---|---|---|
| 1 | Khartoum butcheries | $3.10x10^2$ | 0.00 |
| 2 | Khartoum North butcheries | 4.00x10 | 1.86x10 |
| 3 | Omdurman butcheries | $9.40x10^2$ | $1.06x10^2$ |
| 4 | **Factory 1 | $2.40x10^2$ | $1.56x10^2$ |
| 5 | **Factory 2 | $1.49x10^4$ | $1.50x10^2$ |
| 6 | **Factory 3 | $6.02x10^2$ | 3.20x10 |
| 7 | **Factory 4 | $1.12x10^3$ | 0.10x10 |
| 8 | Homemade | $1.72x10^3$ | $4.40x10^2$ |

* Mean of 5 replicates.
** Factory samples at retail outlets

isolates, while 18.75% were assigned to Staphylococcus aureus, 14.58% to Streptococcus faecalis, 10.42% to Staphylococcus epidermidis, 6.25% to Bacillus coagulans, and 4.17% each to Micrococcus roseus and Bacillus firmus. Bacillus subtilis, Bacillus licheniformis, Streptococcus facium, Streptococcus pyogenes, Streptococcus bovis, Staphylococcus capitis, Bacillus mycoides, Micrococcus varians and Micrococcus luteus each accounted for 2.08% of the isolates (Table. 5). The presence of spoilage and pathogenic microorganisms in sausage samples indicated that the hygiene is poor. Contamination of sausages may be introduced from different sources as reported by Selvan, (2007) and Hampikyan, (2009). The type of spoilage and pathogenic microorganisms are primarily related to the type of initial contamination which is affected by the resident factory microflora, the manufacturing site and the processing hygiene.

Salmonella typhi represented 40% of all Salmonella isolates (80 isolates), followed by Salmonella pullorum (17%), Salmonella entirtidis (15%), Salmonella gallinarum (12.5%), Salmonella choleraesuis (7.5%), Salmonella paratyphi (5%) and Salmonella arizonae (2.5%) (Table 6).Non-typhoidal Salmonella represented 55% and typhoidal Salmonella represented 45%. The presence of Salmonella typhi and Salmonella paratyphi in the sampled sausages indicates contamination of human origin which reveals poor hygienic conditions in the handling of sausages during processing, distribution, displaying and marketing (Mrema, 2006).

With respect to spore-formers, the identified isolates of aerobic spore-formers included, Bacillus subtilis (60%), Bacillus brevis (14.3 %); Bacillus firmus.

Table 5. Identification of bacterial isolates of sausage samples collected from different sources

| Isolates No | Isolates Code | Shape | Gram staining | Endo-spore | Motility | Aerobic growth | Anaerobic | Growth at 45°C | Growth in 10% | PH 9.5 | Catalase | Oxidase | Acid from | O/F | Urease | Indole | VP | Nitrate reduction | Arginine | Coagulase | Haemolysis | Acid from tested sugars 1 | 2 | 3 | 4 | 5 | 6 | 7 | Species |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | KhB1 | rod | + | + | + | + | + | + | d | + | + | - | + | F | d | - | + | + | + | - | - | + | d | - | - | - | - | + | - | B. cereus |
| 2 | KhB2 | rod | + | + | + | + | + | + | d | + | + | - | + | F | d | - | + | + | + | - | - | + | d | - | - | - | - | + | - | B. cereus |
| 3 | KhB3 | rod | + | + | + | + | + | d | + | + | + | - | + | F | d | - | + | + | + | - | - | + | + | - | - | - | - | + | - | B. mycosis |
| 4 | KhB4 | cocci | + | - | - | + | + | + | + | + | + | - | + | F | + | + | + | + | + | + | + | + | - | + | + | + | - | - | - | St. aureus |
| 5 | KhB5 | cocci | + | - | - | + | + | + | w | + | + | - | + | F | + | + | + | + | - | - | - | + | - | + | + | + | - | - | - | St. epidirmidis |
| 6 | KhNB1 | cocci | + | - | - | - | + | + | + | + | - | - | + | F | + | - | + | + | + | - | - | + | - | + | + | + | - | + | - | S. faecalis |
| 7 | KhNB2 | cocci | + | - | - | + | - | - | - | - | + | + | + | O | - | + | - | d | - | - | - | d | - | - | - | + | - | - | - | M. roseus |
| 8 | KhNB3 | rod | + | + | + | + | + | + | d | + | + | - | + | F | d | - | + | + | + | - | - | + | d | - | - | - | - | + | - | B. cerseu |

| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 9 | KhNB4 | rod | + | + | + | + | + | + | - | - | + | - | + | F | + | - | - | + | + | - | - | + | + | d | + | + | + | + | d | B. subtilis |
| 10 | KhNB5 | cocci | + | - | - | + | + | + | + | + | + | - | + | F | + | + | + | + | + | + | + | + | - | + | + | + | - | - | - | St. aureus |
| 11 | OB1 | cocci | + | - | - | - | + | + | + | + | - | - | + | F | + | - | + | + | + | - | - | + | - | + | + | + | - | + | - | S. faecalis |
| 12 | OB2 | rod | + | + | + | + | + | + | d | + | + | - | + | F | d | - | + | + | + | - | - | + | d | - | - | - | - | + | - | B.cereus |
| 13 | OB*2 | cocci | + | - | - | - | + | + | + | + | - | - | + | F | + | - | + | + | + | - | - | + | - | + | + | + | - | + | - | S. faecalis |
| 14 | OB3 | rod | + | + | + | + | + | + | d | + | + | - | + | F | d | - | + | + | + | - | - | + | d | - | - | - | - | + | - | B. cereus |
| 15 | OB*3 | cocci | + | - | - | + | + | + | + | + | + | - | + | F | + | + | + | + | + | + | + | + | - | + | + | + | - | - | - | St. aureus |
| 16 | OB4 | cocci | + | - | - | + | + | + | + | + | + | - | + | F | + | + | + | + | + | + | + | + | - | + | + | + | - | - | - | St. aureus |
| 17 | OB5 | cocci | + | - | - | + | + | + | + | + | + | - | + | F | + | + | + | + | + | + | + | + | - | + | + | + | - | - | - | St. aureus |

Legend:

(d)Delayed reaction.
(+)Positive Reaction.
(-)Negative Reaction.
(F)Fermentative.
(O) Oxidative
(*) another isolate

1----------8: Sugars tested
1-Sucrose. 5-Mannitol.
2-Cellobiose. 6-Raffinose.
3-Galactose. 7-Salicin.
4-Mannose 8-Xylose

Table 5 (Cont.). Identification of bacterial isolates of sausage samples collected from different sources

| Isolates No | Isolates Code | Shape | Gram staining | Endo-spore | Motility | Aerobic growth | Anaerobic | Growth at 45ºC | Growth in 10% | pH 9.5 | Catalase | Oxidase | Acid from | O/F | Urease | Indole | VP | Nitrate reduction | Arginine | Coagulase | Haemolysis | Acid from tested sugars 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | Species |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 18 | LB1 | Rod | + | + | + | + | + | + | + | + | + | - | + | F | + | - | - | + | + | - | - | + | - | + | + | + | - | - | - | B. firmus |
| 19 | LB2 | Cocci | + | - | - | + | + | + | w | + | + | - | + | F | + | + | + | + | - | - | - | + | - | + | + | + | - | - | - | St. epidirmidis |
| 20 | LB3 | Cocci | + | - | - | - | + | + | + | + | - | - | + | F | + | - | + | + | + | - | - | + | - | + | + | + | - | + | - | S. faecalis |
| 21 | LB*3 | Cocci | + | - | - | + | - | - | d | - | + | + | - | O | + | - | - | - | - | - | - | - | - | - | - | - | - | - | - | M. luteus |
| 22 | LB4 | Rod | + | + | + | + | + | + | - | - | + | - | + | F | + | - | + | + | + | - | - | + | + | + | + | - | d | + | + | B. licheniformis |
| 23 | LB5 | Cocci | + | - | - | + | + | + | + | + | + | - | + | F | - | | + | + | + | - | - | + | - | + | + | + | - | - | - | St. capitis |
| 24 | LB*5 | Cocci | + | - | - | + | - | - | - | - | + | + | + | O | - | + | - | d | - | - | - | d | - | - | - | + | - | - | - | M. roseus |
| 25 | MB1 | Rod | + | + | + | + | + | + | d | + | + | - | + | F | d | - | + | + | + | - | - | + | d | - | - | - | - | + | - | B. cereus |
| 26 | MB2 | Cocci | + | - | - | + | + | + | + | + | + | - | + | F | + | + | + | + | + | + | + | + | - | + | + | + | - | - | - | St. aureus |
| 27 | MB*2 | Cocci | + | - | - | - | + | + | + | + | - | - | + | F | + | - | + | + | + | - | - | + | - | + | + | + | - | + | - | S. faecalis |
| 28 | MB3 | Rod | + | + | + | + | + | + | - | - | + | - | + | F | - | - | + | d | + | - | - | + | d | d | + | d | + | d | d | B. coagulans |

| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 29 | MB*3 | Cocci | + | + | + | + | + | + | + | + | - | - | + | F | + | + | - | - | - | - | - | + | + | + | + | + | d | + | + | S. faecium |
| 30 | MB4 | Rod | + | + | + | + | + | + | d | + | + | - | + | F | D | - | + | + | + | - | - | + | d | - | - | - | - | + | - | B. cereus |
| 31 | MB5 | Rod | + | + | + | + | + | + | - | - | + | - | + | F | - | - | + | d | + | - | - | + | d | d | + | d | + | d | d | B. coagulans |
| 32 | GB1 | Rod | + | + | + | + | + | + | + | + | + | - | + | F | + | - | - | + | + | - | - | + | - | + | + | + | - | - | - | B. firmus |
| 33 | GB2 | Cocci | + | + | - | + | + | + | + | + | + | - | + | F | + | + | + | + | + | + | + | + | - | + | + | + | - | - | - | St. aureus |

Legend:

(d)Delayed reaction.
(+)Positive Reaction.
(-)Negative Reaction.
(F)Fermentative.
(O) Oxidative
(*) another isolate

1----------8: Sugars tested
1-Sucrose. 5-Mannitol.
2-Cellobiose. 6-Raffinose.
3-Galactose. 7-Salicin.
4-Mannose 8-Xylose

Table 5 (Cont.). Identification of bacterial isolates of sausage samples collected from different sources

| Isolates No | Isolates Code | Shape | Gram staining | Endo-spore | Motility | Aerobic | Anaerobic | Growth at | Growth in | pH 9.5 | Catalase | Oxidase | Acid from | O/F | Urease | Indole | VP | Nitrate | Arginine | Coagulase | Haemolysis | Acid from tested sugars 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | Species |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 34 | GB3 | cocci | + | - | - | + | + | + | W | + | + | - | + | F | + | + | + | + | - | - | - | + | - | + | + | + | - | - | - | St.. epidermidis |
| 35 | GB4 | cocci | + | - | - | + | - | - | - | + | + | - | + | F | + | - | - | - | + | - | + | + | - | + | + | d | - | + | - | S. pyogenes |
| 36 | GB5 | Rod | + | + | + | + | + | + | d | + | + | - | + | F | d | - | + | + | + | - | - | + | d | - | - | - | - | + | - | B. cereus |
| 37 | WB1 | Rod | + | + | + | + | + | + | d | + | + | - | + | F | d | - | + | + | + | - | - | + | d | - | - | - | - | + | - | B. cereus |
| 38 | WB2 | cocci | + | - | - | + | - | + | - | + | - | - | + | F | + | - | + | + | - | - | - | + | + | + | + | d | + | + | + | S. bovis |
| 39 | WB3 | cocci | + | - | - | + | - | + | - | - | - | - | - | O | + | | - | - | - | - | - | d | - | + | - | - | - | - | - | M. varians |
| 40 | WB*3 | Rod | + | + | + | + | + | + | d | + | + | - | + | F | d | - | + | + | + | - | - | + | d | - | - | - | - | + | - | B. cereus |
| 41 | WB4 | Rod | + | + | + | + | + | + | - | - | + | - | + | F | - | - | + | d | + | - | - | + | d | d | + | d | + | d | d | B. coagulans |
| 42 | WB5 | cocci | + | - | - | - | + | + | + | + | - | - | + | F | + | - | + | + | + | - | - | + | - | + | + | + | - | + | - | S. faecalis |
| 43 | HB1 | cocci | + | - | - | + | + | + | w | + | + | - | + | F | + | + | + | + | - | - | - | + | - | + | + | + | - | - | - | St. .epidirmidis |
| 44 | HB2 | cocci | + | - | - | + | + | + | + | + | + | - | + | F | + | + | + | + | + | + | + | + | - | + | + | + | - | - | - | St. aureus |
| 45 | HB3 | Rod | + | + | + | + | + | + | d | + | + | - | + | F | d | - | + | + | + | - | - | + | d | - | - | - | - | + | - | B. cereus |
| 46 | HB4 | cocci | + | - | - | - | + | + | + | + | - | - | + | F | + | - | + | + | + | - | - | + | - | + | + | + | - | + | - | S. faecalis |
| 47 | HB5 | cocci | + | - | - | + | + | + | w | + | + | - | + | F | + | + | + | + | - | - | - | + | - | + | + | + | - | - | - | St.epidirmidis |
| 48 | HB*5 | cocci | + | - | - | + | + | + | + | + | + | - | + | F | + | + | + | + | + | + | + | + | - | + | + | + | - | - | - | St. aureus |

Legend:

(d)Delayed reaction.
(+)Positive Reaction.
(-)Negative Reaction.
(F)Fermentative.
(O) Oxidative

1----------8: Sugars tested
1-Sucrose. 5-Mannitol.
2-Cellobiose. 6-Raffinose.
3-Galactose. 7-Salicin.
4-Mannose 8-Xylose

(*) another isolate.

Table 6. Specific identification of Salmonella isolated from sausage samples from different sources

| Isolate No | Isolates Code | Shape | Gram staining | Endo | Motility | Growth in | Catalase | Oxidase | Acid from | O/F | Citrate | Gelatin | VP | Urease | Arginine | Indole | Acid from tested sugar 1 | 2 | 3 | 4 | 5 | 6 | 7 | | Species |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | KhS1 | Rod | - | - | + | + | + | - | + | F | - | - | - | - | + | - | - | - | - | - | + | + | - | S. typhi |
| 2 | khS2 | Rod | - | - | + | + | + | - | + | F | - | - | - | - | + | - | - | - | - | - | + | + | - | S. typhi |
| 3 | KhS3 | Rod | - | - | + | + | + | - | + | F | + | - | - | - | + | - | + | d | - | - | d | + | d | S. entritidis |
| 4 | KhS4 | Rod | - | - | + | + | + | - | + | F | - | - | - | - | + | - | - | - | - | - | + | + | - | S. typhi |
| 5 | KhS5 | Rod | - | - | + | + | + | - | + | F | - | - | - | - | + | - | - | - | - | - | + | + | - | S. typhi |
| 6 | KhNS1 | Rod | - | - | + | + | + | - | + | F | + | - | - | - | + | + | d | - | - | d | + | - | - | S. choleraesuls |
| 7 | KhNS2 | Rod | - | - | + | + | + | - | + | F | + | - | - | - | + | - | - | - | - | + | + | - | - | S. typhi |
| 8 | KhNS3 | Rod | - | - | + | + | + | - | + | F | + | - | - | - | + | - | - | - | - | + | + | - | - | S. typhi |
| 9 | KhNS4 | Rod | - | - | + | + | + | - | + | F | + | - | - | - | + | + | - | - | - | + | + | - | - | S. paratyphi A |
| 10 | KhNS5 | Rod | - | - | + | + | + | - | + | F | + | - | - | - | + | + | - | - | + | + | + | - | - | S. arizone |
| 11 | OS1 | Rod | - | - | + | + | + | - | + | F | + | - | - | - | + | + | + | d | - | - | d | + | d | S. entritidis |
| 12 | OS2 | Rod | - | - | + | + | + | - | + | F | + | - | - | - | + | + | + | D | - | - | d | + | d | S. entritidis |
| 13 | OS3 | Rod | - | - | + | + | + | - | + | F | - | - | - | - | + | - | - | - | - | + | + | - | - | S. typhi |
| 14 | OS4 | Rod | - | - | + | + | + | - | + | F | - | - | - | - | + | - | - | - | - | + | + | - | - | S. typhi |
| 15 | OS5 | Rod | - | - | + | + | + | - | + | F | - | - | - | - | + | - | - | - | - | + | + | - | - | S. typhi |

Legend:

(d)Delayed reaction.
(F) Fermentative.
(+) Positive reaction.
(-) Negative reaction .
4-Adonitol

1----------7: Sugars tested
1-Arabinose. 5-Dulcitol.
2-Lactose. 6-Mannitol.
3-Sucrose. 7-Inositol.

Table 6 (cont.). Specific identification of Salmonella isolated from sausage samples from different sources

| Isolate No | Isolates code | Shape | Gram staining | Endo | Motility | Growth in | Catalase | Oxidase | Acid from | O/F | Citrate | Gelatin | VP | Urease | Arginine | Indole | Acid from tested sugar 1 | 2 | 3 | 4 | 5 | 6 | 7 | | Species |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 16 | LS1 | Rod | - | - | + | + | + | - | + | F | + | - | - | - | + | - | + | - | - | - | + | + | - | S. paratyphiA |
| 17 | LS2 | Rod | - | - | + | + | + | - | + | F | + | - | - | - | + | - | + | - | - | - | - | + | - | S. pulloirum |
| 18 | LS3 | Rod | - | - | + | + | + | - | + | F | + | - | - | - | + | - | - | - | - | - | - | - | - | S. choleraesuls |
| 19 | LS4 | Rod | - | - | + | + | + | - | + | F | + | - | - | - | + | - | + | - | - | - | - | + | - | S. pulloirum |
| 20 | LS5 | Rod | - | - | + | + | + | - | + | F | + | - | - | - | + | - | + | - | - | - | - | + | - | S. pulloirum |
| 21 | MS1 | Rod | - | - | + | + | + | - | + | F | + | - | - | - | + | - | + | - | - | - | + | + | - | S. pulloirum |
| 22 | MS2 | Rod | - | - | + | + | + | - | + | F | + | - | - | - | + | - | + | - | - | - | + | + | - | S. pulloirum |
| 23 | MS3 | Rod | - | - | + | + | + | - | + | F | - | - | - | - | + | - | + | d | - | - | d | + | d | S. entriditis |
| 24 | MS4 | Rod | - | - | + | + | + | - | + | F | + | - | - | - | + | - | + | - | - | - | + | + | - | S. typhi |
| 25 | MS5 | Rod | - | - | + | + | + | - | + | F | - | - | - | - | + | - | - | - | - | - | + | + | - | S. typhi |

| | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 26 | GS1 | Rod | - | - | + | + | + | - | + | F | - | - | - | - | + | - | - | - | - | - | + | + | - | S. typhi |
| 27 | GS2 | Rod | - | - | + | + | + | - | + | F | - | - | - | - | + | - | - | - | - | - | + | + | - | S. typhi |
| 28 | GS3 | Rod | - | - | + | + | + | - | + | F | + | - | - | - | + | - | - | - | - | - | - | - | - | S. choleraesus |
| 29 | GS4 | Rod | - | - | + | + | + | - | + | F | + | - | - | - | + | - | + | - | - | - | - | + | - | S. pulloirum |
| 30 | GS5 | Rod | - | - | + | + | + | - | + | F | - | - | - | - | + | - | + | - | - | - | - | + | - | S. pulloirum |

Legend:

(d)Delayed reaction.
(F) Fermentative.
(+) Positive reaction.
(-) Negative reaction .

1----------7: Sugars tested
1-Arabinose. 5-Dulcitol.
2-Lactose. 6-Mannitol.
3-Sucrose. 7-Inositol.
4-Adonitol

Table 6 (cont.). Specific identification of Salmonella isolated from sausage samples from different sources

| Isolate No | Isolates code | Shape | Gram staining | Endo | Motility | Growth in | Catalase | Oxidase | Acid from | O/F | Citrate | Gelatin | VP | Urease | Arginine | Indole | Acid from tested sugar 1 | 2 | 3 | 4 | 5 | 6 | 7 | Species |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 31 | WS1 | Rod | - | - | + | + | + | - | + | F | + | - | - | - | - | - | + | - | - | - | + | + | - | S. gallinarum |
| 32 | WS2 | Rod | - | - | + | + | + | - | + | F | + | - | - | - | - | - | + | - | - | - | + | + | - | S. gallinarum |
| 33 | WS3 | Rod | - | - | + | + | + | - | + | F | + | - | - | - | - | - | + | - | - | - | + | + | - | S. gallinarum |
| 34 | WS4 | Rod | - | - | + | + | + | - | + | F | + | - | - | - | + | - | + | d | - | - | D | + | d | S. entritidis |
| 35 | WS5 | Rod | - | - | + | + | + | - | + | F | - | - | - | - | + | - | - | - | - | - | + | + | - | S. typhi |
| 36 | HS1 | Rod | - | - | + | + | + | - | + | F | + | - | - | - | - | - | + | - | - | - | + | + | - | S. gallinarum |
| 37 | HS2 | Rod | - | - | + | + | + | - | + | F | + | - | - | - | - | - | + | - | - | - | + | + | - | S. gallinarum |
| 38 | HS3 | Rod | - | - | + | + | + | - | + | F | - | - | - | - | + | - | - | - | - | - | + | + | - | S. typhi |
| 39 | HS4 | Rod | - | - | + | + | + | - | + | F | - | - | - | - | + | - | + | - | - | - | + | + | - | S. typhi |
| 40 | HS5 | Rod | - | - | + | + | + | - | + | F | + | - | - | - | + | - | + | d | - | - | D | + | d | S. entritidis |

Legend:

(d)Delayed reaction.
(F) Fermentative.
(+) Positive reaction.
(-) Negative reaction .

1----------7: Sugars tested
1-Arabinose. 5-Dulcitol.
2-Lactose. 6-Mannitol.
3-Sucrose. 7-Inositol.
4-Adonitol

(11.4%); Bacillus mycoides (8.6%) and Bacillus pumilus (5.7%). Facultatively anaerobic spore-formers, on the other hand, were identified as Bacillus cereus

(80%), Bacillus licheniformis (15.6%), Bacillus coagulans (2.2%), and Bacillus circulans (2.2%). Bacillus cereus showed high percentage (22.92%) of all isolates followed by Bacillus subtilis, B. licheniformis and B. brevis (Table 7). Bacillus cereus could be isolated from a variety of foods including vegetables, dairy and meat products causing vomiting or diarrhea illness that is becoming increasingly important in the industrialized world (Granum, 2005). There are many sources that play an important role in introduce spore-formers into sausages such as soil, personnel, spices, other raw materials and additives (Granum, 2005;). Contamination with Bacillus sp. is rather great in this study. The presence of Bacillus sp. in meat products is rather a dangerous phenomenon. Moreover, these food-borne pathogens are able to grow at refrigeration temperature (Schmidl and Kaya, 1990), thus it is very important to use different methods to eliminate this genus from raw materials and other sources of contamination. Bacillus cereus is probably the second best known mammalian pathogen in the genus Bacillus. This organism causes two types of food poisoning: an emetic type and a diarrheal type. The presence of such pathogens in any type of meat products will enhance its spoilage and consequent food poisoning if eaten by humans.

All moulds isolates were found to belong to the genera: Rhizopus and Aspergillus. The predominant moulds isolated were identified as Rhizopus

Table 7. Specific identification of aerobic and facultative anaerobic spore-formers isolated from sausage samples from different sources

| Isolates No | Isolates Code | Aerobic | Facultative | Gram staining | Endo-spore | Motility | Growth in air | Anaerobic | Growth at 50 | Growth in | Catalase | Oxidase | O/F | Citrate | Urease | Indole | VP | Nitrate | Starch | Acid from tested sugars 1 | 2 | 3 | 4 | 5 | 6 | 7 | Species |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | KhSP | | 4 | + | + | + | - | + | + | - | + | - | F | + | d | - | + | - | + | + | d | - | - | - | - | + | - | B. cereus |
| 2 | KhSP | 1 | | + | + | + | + | - | + | - | + | - | F | d | d | - | + | + | + | + | d | d | - | - | - | d | - | B. mycoids |
| 3 | KhSP | | 5 | + | + | + | - | + | + | d | + | - | F | + | d | - | + | - | + | + | d | - | - | - | - | + | - | B. cereus |
| 4 | KNSP | | 2 | + | + | + | - | + | + | - | + | - | F | + | d | - | + | - | + | + | d | - | - | - | - | + | - | B. cereus |
| 5 | KhNSP | 1 | | + | + | + | + | - | + | - | + | - | F | + | - | - | + | - | + | + | + | + | + | + | d | + | + | B. pumilus |
| 6 | KhNSP | | 2 | + | + | + | - | + | + | - | + | - | F | + | + | - | + | + | + | + | + | + | + | d | d | + | + | B. licheniformis |
| 7 | KhNSP | 2 | | + | + | + | + | - | + | + | + | - | F | + | - | - | + | + | + | + | + | d | + | + | + | + | d | B. subtilis |
| 8 | KhNSP | | 1 | + | + | + | - | + | + | - | + | - | F | + | + | - | + | + | + | + | + | + | + | d | d | + | + | B. licheniformis |
| 9 | KhNSP | | 2 | + | + | + | - | + | + | - | + | - | F | + | d | - | + | - | + | + | d | - | - | - | - | + | - | B. cereus |
| 10 | OSP | 4 | | + | + | + | + | - | - | + | + | - | F | + | - | - | + | + | + | + | + | d | + | + | + | + | d | B. subtilis |
| 11 | OSP | | 1 | + | + | + | - | + | + | - | + | - | F | + | d | - | + | - | + | + | d | - | - | - | - | + | - | B. cereus |
| 12 | OSP | | 4 | + | + | + | - | + | + | - | + | - | F | + | d | - | + | - | + | + | d | - | - | - | - | + | - | B. cereus |
| 13 | OSP | 1 | | + | + | + | + | - | + | + | + | - | F | + | - | - | + | + | + | + | + | d | + | + | + | + | D | B. subtilis |

Legend: (d) Delayed reaction. 1----------10:Sugars tested
(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.
5-Sucrose. 10-Mannose

Table 7 (cont.). Specific identification of aerobic and facultative anaerobic spore-formers isolated from sausage samples from different sources

| Isolates No | Isolates code | Aerobic | Facultative | Gram staining | Endo-spore | Motility | Growth in air | Anaerobic | Growth at 50 | Growth in | Catalase | Oxidase | O/F | Citrate | Urease | Indole | VP | Nitrate | Starch | Acid from tested sugars 1 | 2 | 3 | 4 | 5 | 6 | 7 | Species |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 14 | LSP | | 1 | + | + | + | - | + | + | - | + | - | F | + | + | - | + | + | + | + | + | + | + | d | d | + | + | B. licheniformis |
| 15 | LSP | 2 | | + | + | + | + | - | + | + | + | - | F | + | + | + | + | + | - | + | - | - | d | - | - | - | - | B. firmus |
| 16 | LSP | 1 | | + | + | + | + | - | - | + | + | - | F | + | + | + | + | + | - | + | - | - | - | - | - | - | d | B. brevis |
| 17 | LSP | | 1 | + | + | + | - | + | + | - | + | - | F | + | d | - | + | - | + | + | d | - | - | - | - | + | - | B. cereus |
| 18 | LSP | | 3 | + | + | + | - | + | + | - | + | - | F | + | d | - | + | - | + | + | d | - | - | - | - | + | - | B.cereus |
| 19 | LSP | | 1 | + | + | + | - | + | + | - | + | - | F | + | + | - | + | + | + | + | + | + | + | d | d | + | + | B. licheniformis |
| 20 | LSP | | 1 | + | + | + | - | + | + | - | + | - | F | + | d | - | + | - | + | + | d | - | - | - | - | + | - | B. cereus |
| 21 | MSP | | 3 | + | + | + | - | + | + | - | + | - | F | + | d | - | + | - | + | + | d | - | - | - | - | + | - | B. cereus |
| 22 | MSP | 1 | | + | + | + | + | - | + | - | + | - | F | d | d | - | + | + | + | + | d | d | - | - | - | d | - | B. mycoids |

| | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 23 | MSP | 1 | | + | + | + | + | - | + | + | + | - | F | + | - | - | + | + | + | + | + | d | + | + | + | + | d | B. subtilis |
| 24 | MSP | | 3 | + | + | + | - | + | + | - | + | - | F | + | d | - | + | - | + | + | d | - | - | - | - | + | - | B. cereus |
| 25 | MSP | | 1 | + | + | + | - | + | + | - | + | - | F | + | + | - | + | + | + | + | + | + | + | d | d | + | + | B. lichenifo rmis |
| 26 | MSP | 1 | | + | + | + | + | - | + | + | + | - | F | + | - | - | + | + | + | + | + | d | + | + | + | + | d | B. subtilis |
| 27 | GSP | 2 | | + | + | + | + | - | + | + | + | - | F | + | + | + | + | + | - | + | - | - | d | - | - | - | - | B. firmus |
| 28 | GSP | | 1 | + | + | + | - | + | + | - | + | - | F | + | d | - | + | - | + | + | d | - | - | - | - | + | - | B. cereus |
| 29 | GSP | 2 | | + | + | + | + | - | - | + | + | - | F | + | + | + | + | + | - | + | - | - | - | - | - | - | d | B. brevis |

Table 7(cont.).Specific identification of aerobic and facultative anaerobic spore-formers isolated from sausage samples from different sources

| Isolates No | Isolates code | Aerobic | Facultative | Gram | Endo- | Motility | Growth in | Anaerobic | Growth at | Growth in | Catalase | Oxidase | O/F | Citrate | Urease | Indole | VP | Nitrate | Starch | Acid from tested sugars 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | Species |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 30 | GSP | 1 | | + | + | + | + | - | + | - | + | - | F | + | - | - | + | - | + | + | + | + | + | + | d | + | + | B. pumilus |
| 31 | GSP | 2 | | + | + | + | + | - | - | + | + | - | F | + | + | + | + | + | - | + | - | - | - | - | - | - | d | B. brevis |
| 32 | GSP | | 1 | + | + | + | - | + | + | - | + | - | F | + | + | - | + | + | + | + | + | + | + | d | d | + | + | B. lichenifo rmis |
| 33 | GSP | | 1 | + | + | + | - | + | d | - | - | + | F | d | - | - | - | d | + | + | + | + | + | + | | + | + | B. circulans |
| 34 | WSP | 1 | | + | + | + | + | - | + | - | + | - | F | d | d | - | + | + | + | + | d | d | - | - | - | d | - | B. mycoids |
| 35 | WSP | 3 | | + | + | + | + | - | + | + | + | - | F | + | - | - | + | + | + | + | + | d | + | + | + | + | d | B. subtilis |
| 36 | WSP | | 1 | + | + | + | - | + | + | - | + | - | F | + | d | - | + | - | + | + | d | - | - | - | - | + | - | B. cereus |
| 37 | WSP | | 3 | + | + | + | - | + | + | - | + | - | F | + | d | - | + | - | + | + | d | - | - | - | - | + | - | B. cereus |
| 38 | WSP | 1 | | + | + | + | + | - | + | + | + | - | F | + | - | - | + | + | + | + | + | d | + | + | + | + | d | B. subtilis |
| 39 | WSP | | 1 | + | + | + | - | + | + | - | + | - | F | + | - | - | + | + | + | + | d | - | - | - | - | + | - | B. coagulan s |
| 40 | HSP | | 1 | + | + | + | - | + | + | - | + | - | F | + | d | - | + | - | + | + | d | - | - | - | - | + | - | B. cereus |
| 41 | HSP | 4 | | + | + | + | + | - | + | + | + | - | F | + | - | - | + | + | + | + | + | d | + | + | + | + | d | B. subtilis |
| 42 | HSP | 4 | | + | + | + | + | - | + | + | + | - | F | + | - | - | + | + | + | + | + | d | + | + | + | + | d | B subtilis |
| 43 | HSP | | 1 | + | + | + | - | + | + | - | + | - | F | + | d | - | + | - | + | + | d | - | - | - | - | + | - | B. cereus |

Legend: (d) Delayed reaction. 1----------10:Sugars tested
(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.
5-Sucrose. 10-Mannose

Nigricans (87%) and Aspergillus niger (13%). Rhizopus nigricans has been isolated from different sources including cereals, vegetables, nuts, and meat. In Saudi Arabia, Easa (2010) isolated different types of moulds (including *Rhizopus nigricans)* from fast and traditional fast foods. Meat and soft cheeses (e.g. brie

cheese, cottage cheese) have more water content, allowing any bacteria, viruses or moulds present to multiply quickly (Bichai, 2008). This study revealed that the sausage hygiene in Khartoum State is questionable and needs intensive hygiene efforts and standard safety measures before, during, and after processing operations.

## REFERENCES


Abd Elaziz T. 1996. Screening of some food poisoning bacteria in sausage and hamburger meat. Journal of Egypt Public Health Assoc. (1-2): 47-61.

Abrahim A, Anna P, Nikoloas S, Loonnis A and Antonis A. 1998. Antibiotic resistance of *Samonella* sp. and *Listeria* sp. isolates from traditional meat fresh sausage in Greece. Journal of Food Protection. 61(10): pp 1378-1380(3).

Abugroun HA, Cousin MA and Judge MD. 1993. Extended shelf-life of un-refrigerated prerigor cooked meat. Journal of Meat Science. 2: 207-229.

Adams MR and Moss MO. 2008. *Food Microbiology*, 3rd edition. Royal Society of Chemistry.

Humodi HS and Hatim HH. 2010. Bacteriological and Parastological Assessment of Food Handlers in the Omdurman Area of Sudan. J. Microbiol Immuno lnfact. 43 (1): 70-73.

Al-Mutairi MF. 2011. The incidence of Enterobacteriaceae Causing Food Poisoning in Some Meat Products. Advance Journal of Food Science and Technology *3* (2): 116-121.

Andrews W. 1992. Manual of food quality control. 4. Rev. 1. Microbiological Analysis, FAO, Food and Nutrition Paper, 14/4. Rev.1. FAO, Rome, Italy.

Banerjee M and Sarkar PK. 2003. Microbiology quality of some retail spices in India. Food Res. Int. 36:469-474.

Barrow GISJ and Feltham RKA. 1993. *Manual for Identification of Medical Bacteria*, third edition. Cambridge University Press, England.

Bichai F, Payment P and Barbeau B. 2008. Protection of waterborne pathogens by higher organisms in drinking water, a review. Canadian Journal Microbiology. 54 (7): 509-524.

Chapman PA, Cerdan Malo AT, Ellin M, Ashton R and Harkin B. 2001. Escherichia coli o157 in cattle and sheep at slaughter, on beef and lamb products in South Yorkshire. UK. Int. J. Food Microbial. 64 (1-2): 139-150.

Codex Alimentarius 5. 2013. Food Safety Situation in Sudan. Prepred in Honour of 50th Anniversary of codex The Codex Alimentarius Commission.

Cohen N. 2008. Microbiological quality control of raw ground beef and fresh sausage in Casablanca (Morocco). *Journal of Environmental Health.* 71(4): 51-55.

Colak H, Bingol EB, Hampikyan H and Nalzi B. 2006. Determination of aflatoxin contamination in red and black pepper by ELSA and HPLC. *Journal of Food and Drug Analysis.* 14(3): 292-296. .

Dharmaveer SV, Rajkumar V and Mukesh KP. 2007. Quality and shelf life of smoked chevon sausages packed under vacuum and stored at 4ºC. Am. Journal of Food Technology. 2: 238-247.

Dowdell MJ and Board RG. 1968. A microbiological survey of British fresh susage. Journal of Applied Bacteriology. 31: 378-396.

Drosinos EH, Mataragas M, Xiraphi N, Moschonas G, Gaitis F and Metaxopoulos J. 2005. Characterization of the microbial flora from traditional Greek fermented sausage. Meat Science. 69: 307-317.

Elfaki AE and Abdalla SAE. 2011. Quality Evaluation of Two Sudanese Street Foods of Animal Origin. Advance Journal of Food Science and Technology. 3(3): 219-223.

Ellis MB. 1976. More Dematiaceous Hyphomycetes. Common Wealth Institute of Seed Pathology for Developing Countries, Denmark.

Easa SMH. 2010. Microorganisms found in fast and traditional fast food. Journal of American Science. 6 (10): 515-531.

FAO news release. 2005. Food-borne diseases are a serious threat in Africa.http://news.mongabay.com/2005/1003-fao.html.

FAO. 1992. Food and Agriculture Organization. *Manual of Food Quality Control*. 14/4. Rev. 1. microbiological analysis. Rome, ltaly.

FAO. 1990. Food and Agriculture Organization of United Nation. Manual on Simple Methods of Meat Preservation. Animal Production and Health Paper 79. Rome.

Gamal El-Deen MRE. 2010. Studies on contamination and quqlity of fresh fish meats during storage. Egypt. Acad. J. Biolog. Sci. 2 (2): 65-74.

Granum PE. 2005. *"Bacillus cereus" Food Borne Pathogenes: Microbiology and Molecular Biology.* Caister Academic Press. ISBN 9781-1-904455-00-4.

Hampikyan H, Bingol E, Colak H and Aydin A. 2009. The evaluation of microbiological profile of some spices used in Turkish meat industry. Journal of Food, Agriculture and Environment. 7 (3and 4): 111-115.

Harrigan WE. 1998. *Laboratory Methods in Food and Dairy Microbiology.* Academic press. USA.

Harrigan WE and MacCance ME. 1976. Laboratory Methods in Microbiology. Academic Press, London and New York.

Ibrahim SA, Asakir SF, Idris AA, Martinez-Utaza J and Hag Elsafi HE. 2013. Prevalence of *Salmonella* species amongasymptomatic food handlers in Khartoum State, Sudan. British Journal of Biomedical Science. 70 (2): 88-89.

Jamilah MB, Abbas KA and Abdoul RR. 2008. A Review on some Organic Acids Additives as Shelf -Life Extenders of Fresh Beef Cuts. *American. J.* Agric. Biol. Sci.3: 566-574.

Jay JM, Lossner MJ and Golden DA. 2005. Fresh meats and poultry, In Modern Food Microbiology. Eds. Jay. JM. Loessner, MJ. and Golden, DA. New York, Springer Science. 63-99.

Kulwant S, Jens CF, Uif I and Mathur BS. 1992. An illustrated manual on identification of seed-borne *Aspergillus*, *Fusarium*, *Penicilluim* and their mycotoxin, Danish Government Institute of Seed Pathology for developing Countries and Department of Boitechnology. The Technical University of Denmark. Denmark.

Lefoka, M. (2009). The survival of Microbial pathogens in Dairy products. Magister Scientiae. Faculty of Natural and Agricultural Sciences, Department of Microbial, Biochemical, and Food Biotechnology. University of the Free State, Bloemfontein. Lin, C.; Fernando, SY and Wei, C. (1996). Occurrence of *Listeria monocytogenes*, *Salmonella* sp., *Escherichia coli* and *E coli* 0157:H7 in vegetable salad. Food Control. 3: 135-140.

Lin C, Fernando SY and Wei C. 1996. Occurrence of *listeria monocytogenes*, *Salmonella* Spp. *Escherichia coli* and *E. coli* 0157: H7 in vegetable salads.Food Control 7: 135-140.

Mattick KL, Bailey RA, Jørgensen F and Humphrey T. 2002. The prevalence number of *Salmonella* in sausage and their destruction by frying, grilling or barbecuing. *Journal of Applied Microbiology*. 93: 541-547.

Mrema. N, Mpuchane S, Berhanu A and Gashe BA. 2006. Prevalence of *Salmonella* in raw minced meat, raw fresh sausages and raw burger patties from retail outlet in Gaborone, Botswana. Food Control. 17 Issue. 3: 207-212.

Musa WH. 2004. *Load and Types of Aerobic Bacteria in Fresh and Processed Beef*. M.Sc. thesis, Veterinary Medicine University of Khartoum, Sudan.

National Health Information Center. 2010. Annual Health Statistical Report. National Ministry of Health. Republic of Sudan.

Oluwafemi F and Simisaye MT. 2006. Extent of microbial contamination of sausages sold in two Nigerian cites. African Journal of Biomedical Research. 9 (2): 133-136.

Özbey G, KöK F and Muz A. 2007. Isolation of *Salmonella* spp. in Camel Sausages from Retail Markets in Aydin, Turkey and polymerase Chain Reaction (PCR) Confirmation Turk. J. Vet. Anim. Sci. 31(1): 67-71.

Pearson AM and Tauber FW. 1984. Spices and condiments. In: *Processed Meat*. 2nd edition, Pearson, AM. and Gillet, TA, Avi Publishing Company Inc. Westport, Connecticut. pp.256.

Pitt JI and Hoking AD. 1985. *Fungi and Food Spoilage*. CSIRO of Food Research. Academic Press. Sideny.

Podpecan B, Pengov A and Vadnjal S. 2007. The source of contamination of ground meat for production of meat products with bacteria *Staphylococcus aureus*. Vet. Res. 44:25-30.

Pringar TA, Cooke ME. 1985. *E. coli* retail processed food. J. Hrg. Comb., 95: 39.

Rebeechi A, Crivori S, Sarra PG and Cocconcelli PS. 1998. Physiological and molecular techniques for the study of bacterial community development in sausage fermentation. *J. Appl. Microbiol.* 84(6):1043-1049.

Saleh YE, El-Fuly MZ, Khalil MS and Abo-State MM. 1993. Incidence and characterization of *Bacillus* isolated from Egyptian foods. *Qatar Univ. Sci. J.* 13(1): 75-80.

Samappito W, Leenanon B and Robert EL. 2011. Microbiological characteristics of "Mhom", a Thi traditional meat sausage. The Open Food Science Journal. 5: 31-36.

Schmidl U, and Kaya M. 1990. Verhalten von *Listeria monocytogenes* in Vakuumverpaekten. *Brühwurstaufschnitt Fleischwirtschaft* 70: 236-240.

Selven P, Narendra Babu R, Sureshkumar and Venkataramanujam V. 2007. Microbial quality of retail meat products available in Chennal City. *Am. J. Food Technol.* 2: 55-59.

Sneath PHA, Mair NS, Sharp ME and Holt JG(eds). 1986. Berg's Mannual of Systematic Bacteriology. Vol. 2, (Baltimre: Williams and Wilkins).

Snyder P. 1997. *Antimicrobial effects of spices and herbs*. Hospitability Institute of Technology and Management, St. Paul. Minnesota, Retreived March1, 2002, from WWW. hitm.com/ documents/spices.html.

SSMO. 2001. Sudanese Standards and Metrology Organization. *Sudanese Microbiological Standards for Foods*. Khartoum, Sudan. The Belgian retail market. J Food Prot 62: 735-740.

The World Factbook. 2013. Sudan Mijor Diseases. FADS-Central Intelligence Agency. The World Factbook/ Library/ Production The World factbook 53k-2013-06-21.

Uyttendaele MDE, Troy P and Debevere J. 1999. Incidence of *Salmonella*, Campylobacter jejuni, Campylobacter coli and Listeria monocytogenes in poultry carcasses and different types of poultry products for sale on the Belgian retail market. Journal of Food Protection. 62: 735-740.

Zdeněck M. 1992. The State of Food Hygiene and Food-borne Diseases: Requirement for future. Ann. ISI, Super Santa. 28(4): 451.