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Detection of Microbial Contamination of Processed Beef Meat by Using API Strips and Automated Vitek 2 Compact System

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Authors' contributions

This work was carried out in collaboration between both authors. Author YAS designed the study, performed the statistical analysis, wrote the protocol, managed the analyses of the study, literature searches and supervised the laboratory work. Author AAA wrote the first draft of the manuscript, managed literature searches and carried the laboratory work. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: Food safety is a complex issue, where animal proteins such as meats, meat products, and fish and fishery products are generally regarded as high risk commodity with pathogenic microrganisms. The present study was conducted to evaluate microbial contamination of beef meat products; minced meat, sausage, beef burger and Shawerma in supermarkets and cafeterias in Khartoum towns.
Study Design: Fifteen samples of minced meat, 12 samples of beef burger, 12 samples of sausage and 12 samples of Shawerma were collected randomly at Khartoum State (Khartoum, Omdurman and Bari towns markets) during the period from October 2011-August 2012 and examined for bacterial contamination.
Methodology: Primary culturing of the samples were conducted onto blood agar plates and the

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purified isolates of the Gram-Negative bacteria were identified by rapid biochemical tests (Api 20 E, Api 20 NE) while Gram-Positive bacteria were identified by Api Staph and automated system (Vitek 2 compact) according to manufacturer's instructions. The *Bacillus* spp. was identified by conventional bacteriological methods.

Results: One hundred and two different bacteria were isolated and identified from 51 beef meat products. The most Gram- Positive isolates were *Lactobacillus* spp*.* 11(10.7%), and *Kocuria kristinae* 9(8.8%) while the most Gram-Negative isolates were *Proteus mirabilis* 7(6.8%) and *Klebsiella pneumoniae* 7(6.8%).

Conclusions: This study revealed that microbiological quality of beef meat products is strongly influenced by implementation of hygienic precautions during production and handling. The environment in butcher shops and areas of production can acts as important source of microbial contamination.

Keywords: API 20E; API 20NE; API Staph; Vitek 2 compact system; beef meat.

1. INTRODUCTION

The three towns of Khartoum State consume about 10-15 tons of sausage, 2.5- 4 tons of minced meat, 4-5 tons of burger and 1.0-1.5 tons of Shawerma per/day [1]. Minced beef meat is usually made from lean meat and used for different types of cooking and meals. In the Sudan it is widely used in local dishes, like Kofta and Molah Sharmoot and, may be sold in a readymade form in butcher shops. Meat has a high nutritional value and not only highly susceptible to spoilage, but frequently implicated in the spread of food-borne diseases [2]. In a study in Mafikeng, different bacteria isolates were detected in raw minced meat including, *Serratia odorifera, Escherichia coli (E. coli), Klebsiella oxytoca* and *Enterobacter aerogenes.* It was reported that different genera of bacteria are associated with contamination of meat and meat products including, *Escherichia*, *Salmonella*, *Shigella*, *Proteus*, and the mesophillic aerobic bacteria revealed high counts and is thought to be attributed to poor sanitary and hygienic condition [3,4]. Other bacteria isolated from fresh minced beef meat are detected in low percentage included, the genera *Enterobacter*, *Citrobacter*, *Bacillus*, *Serratia*, *Yersinia*, *Klebsiella*, *Proteus*, *Flavobacterium*, *Corynebacterium* and *Staphylococcus*. Previous studies showed that the coliform bacteria were observed in 48.8 % of burger samples and out of these 19% were *E*. *coli* [5,6]. *Listeria monocytogenes* and *Salmonella* spp. are also isolated from cured dried sausage samples of Italian dry fermented sausage and *lactobacillus* spp. is the least isolates [7,8]. More contamination of Shawerma can occur during slicing and initial post processing handling [9]. *Proteus* spp. is the predominant bacteria isolated from Shawerma and *Micrococcus* spp. are the least isolates [10]. Other bacteria detected in Shawerma including *Bacillus cereus*, *Streptococcus* (*Strep*) *faecalis* and *Staphylococcus* (*Staph*) *aureus* [11]. Sandwich from meat –based fast food found to be contaminated with *Salmonella paratyphi* serogroup A and Shiga toxin producing *E. coli* [12].

The aim of the present study was to evaluate the microbial contamination of beef meat products; minced meat, beef burger, sausage and Shawerma, collected at Khartoum supermarkets, by using Api 20E, Api 20 NE, Api Staph (Biomerieux, France) and automated Vitek 2 system (Biomerieux, Reference 276327600, version NA,2007, USA).

2. MATERIALS AND METHODS

The study area was Khartoum, capital of Sudan, is located in the central of the Sudan between the Latitudes 15° 33 06 N and longitudes 32°31 56 E. The area of Khartoum is 22,142 Km^2 and elevated above the sea 1250 feet and the population was estimated 7,055,148 millions [13].

A total of 51 samples, about 25 g each including 15 samples of minced meat,12 samples of beef burger, 12 samples of sausage and 12 samples of Shawerma were collected randomly from Khartoum State supermarkets and butchers while Shawerma samples were collected from cafeterias. Each sample was collected in sterile plastic bag and placed into thermo flasks containing ice bags and transported to the Veterinary Research Institute for microbiological examinations. About 10 g from each sample was placed into selenite broth medium, and incubated in a water bath at 42℃ for 24 hours. Then sub-cultured onto MacConkey agar (Himedia, India) and Xylose Lysine Deoxycholate agar medium (MAST group, UK), incubated at 37℃ for 24

hours. Five ml 0.85% Sodium Chloride was added to 5 g of the sample mixed gently to give a homogenous suspension. A loop full of the homogenous suspension was streaked onto blood agar plates and incubated at 37℃ for 24 hours. Purification was done by sub-culturing of typical and well isolated colonies onto nutrient agar plates. The purified isolates were sub-cultured onto blood agar slant, incubated at 37℃ for 24 hours then stored in a refrigerator (LG, Model GR M262YQ, Korea) for further characterization.

2.1 Rapid Biochemical Tests

2.1.1 API identification strips

Api Strips (Biomerieux, France) for Enterobacteriaceae, non enteric Gram-Negative rods and Gram- Positive cocci with catalase positive were used. Catalase test was conducted to differentiate between *Staphylococcus* and *Streptococcus* while oxidase test was carried out for differentiation between enteric and non enteric Gram-Negative isolates. For enteric Gram-Negative bacteria the Api 20 E Strips were used. The test was performed by removing 1-3 well isolated colonies of young culture from a solid medium and emulsified into 5ml normal saline, then adjusted to 0.5 McFarland's standard tube. The bacterial suspension was distributed into the tubules of the strip using 5ml disposable syringe. For citrate, Voges-Proskauer test and gelatin tests both the tubules and the cupules were filled, while for the other tests only the cupules were filled. The tests Arginine Dihydrolase (ADH), Lysine Decarboxylase (LDC), Ornithine Decarboylase, Hydrogen sulphite production (H_2S) and urease activity tests were covered by sterile paraffin oil. The strips were placed in the incubation box containing a little volume of water at the bottom to prevent dehydration, and then incubated at 37℃ for 18 hours. After incubation the reagents were added to some tests according to the instructions of the manufacturer and the results were read and recorded. The Api NE and API Staph were carried out according to manufacturer instructions. Seventy two isolates of Enterobacteriaceae were tested by API 20E kits, 19 isolates by API Staph and 3 isolates by Api 20 NE kits.

2.2 Identification of Gram Positive Isolates by Vitek-2 Compact

Twenty eight isolates of different Gram -Positive bacteria were tested by Gram –Positive (GP) cards of the Vitek 2 Compact device (Biomerieux, Reference 276327600, version NA, 2007, USA).

Gram stain was done to each tested isolate to insure the Gram reaction and purification, 2-3 colonies of young culture of the tested organism were picked and suspended into 3 ml of 0.45% saline solution (ready to use), mixed by vortex and adjusted to turbidity of 0.5 McFarland standard tubes according to the manufacturer's instructions. The prepared tubes and the identification cards were placed in the tube racks then loaded into the device. The results were obtained printable within 4-10 hours.

3. RESULTS

Of the 51 samples of meat products 102 different bacteria were isolated and identified *Proteus mirabilis* was isolated from all products of beef meat examined in this study which could be attributed to the use of contaminated water for washing and cleaning during processing (Table 1). Thirty two (32%) of the total isolates were recovered from 15 minced meat samples and *Lactobacillus* spp. and *Kocuria kristinae* were the most isolated organisms (Fig. 1). Identification isolates of beef burger samples revealed 25 bacteria and *Kocuria kristinae* was the most isolated bacteria 4(16%) followed by *Bacillus badius* and *Enterobacter gergoviae* 3(12%) each (Fig. 2). Similar number of isolates of beef burger samples was found in sausage sample and *Staph. capitis* was the predominant bacteria (Fig. 3). In Shawerma samples lesser bacteria were isolated compare to other meat products with highest isolation rate 6(24%) of *Bacillus licheniformis* which could be due to environmental contaminations.

4. DISCUSSION

The members of the family Enterobacteriaceae are usually associated with the contamination of meat products and the sources are the soil and intestinal tract of humans and animals, their incidence in meat was considered as a public health Previous studies have showed that meat and meat products were most frequently contaminated with *Salmonella* spp. [14-16]. This finding was in agreement with the results of this study which could be due to focally contaminated hands of infected food handler. *E. coli* was detected in 8% of burger samples, similar results were reported by other investigators [6,2]. This study revealed that the genera [17].

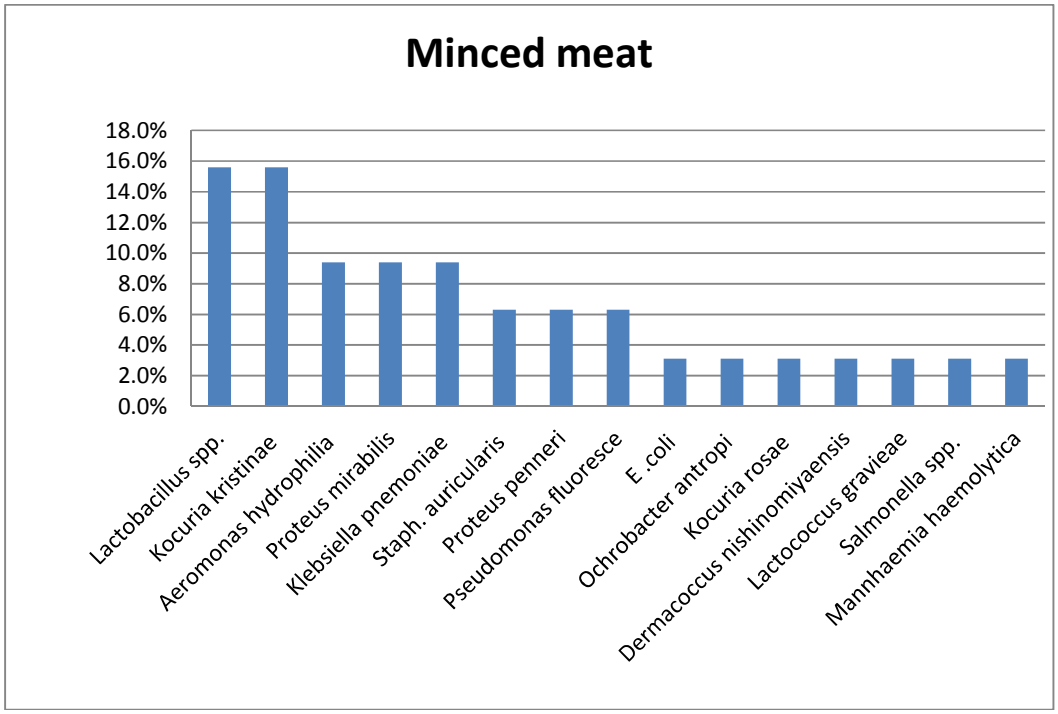

Fig. 1. Percentage of bacterial isolation from minced meat samples

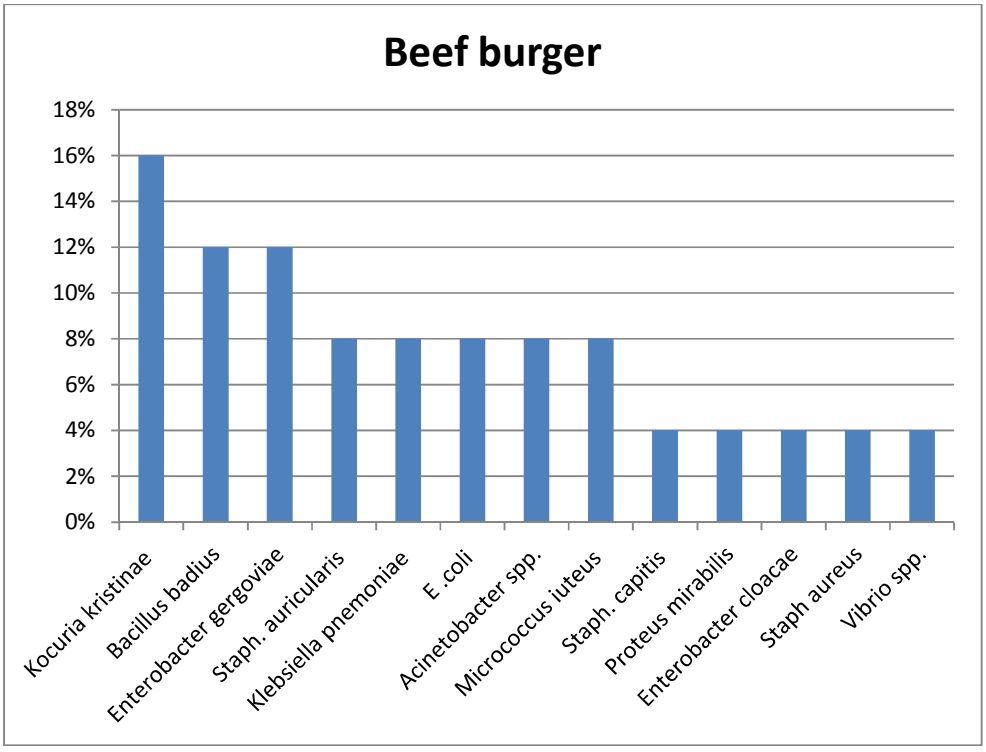

Fig. 2. Percentage of bacterial isolation from Burger samples

Staphylococcus, *Micrococcus*, *Bacillus*, *Escherichia* and *Proteus* were isolated from sausage samples, in other study similar genera were detected in sausage samples.

Whereas *Micrococcus* spp. was the only bacteria detected in refrigerated sausage [5]. Contamination of meat products with these organisms could be due to the use of herbs, spices and intestine of gelatinous casing or could be due to hand contamination during processing. Different types of bacteria were isolated from Shawerma samples including; *Bacillus* spp. and *Staphylococcus* spp. Similar finding was also reported by other researchers [9,11] which could be due to the contamination of Shawerma during slicing and initial post processing handling or could be due to the mixing of contaminated vegetables like tomato and carrot with the cooked Shawerma prior consumption or In addition, the high level of pathogenic microrganisms in Shawerma samples may be attributed to poor sanitary measures during the preparation.

In the present study, the use of API strips and the Vitek 2 Compact system for the identification of the isolates to the species level was found reliable and time consuming. Previous reports have showed that the Vitek 2 system provided a rapid and reliable identification of most species of *Staphylococcus* isolated from clinical cases as well as from environments [18]. It was also noted that the Vitek 2 system successfully identified 580 Gram-Positive and Gram-Negative bacteria isolated from minced meat; 87.5% to the Genus level and 94.4% to the species level [19]. In addition, extended β- lactamase (ESBL)

Table 1. Bacteria isolated from 51 samples beef meat products in Khartoum State

Isolates	Minced meat	Beef burger	Sausage	Shawerma	Total
Lactobacillus spp.	5(15.6%)	0(0%)	4(16%)	2(10%)	11 (10.7%)
Kocuria kristinae	5(15.6%)	4(16%)	0(0%)	0(0%)	9(8.8%)
Staph. auricularis	2(6.3%)	2(8%)	2(8%)	2(10%)	8 (7.8%)
Staph. capitis	0(0%)	1(4%)	6(24%)	1(5%)	8(7.8%)
Proteus mirabilis	3(9.4%)	1(4%)	2(8%)	1(5%)	7 (6.8%)
Klebsiella pneumoniae	3(9.4%)	2(8%)	2(8%)	0(0%)	7 (6.8%)
Bacillus licheniformis	0(0%)	0(0%)	0(0%)	6(30%)	6(5.8%)
Micrococcus variants	0(0%)	0(0%)	2(8%)	3(15%)	5 (4.9%)
Bacillus badius	0(0%)	3(12%)	1	0(0%)	4 (3.9%)
E. coli	1(3.1%)	2(8%)	1(4%)	0(0%)	4 (3.9%)
Pantoea spp.	0(0%)	0(0%)	1(4%)	3	4 (0.9%)
Aeromonas hydrophilia	3(9.4%)	0(0%)	0(0%)	0(0%)	3 (2.9%)
Enterobacter cloacae	0(0%)	1(4%)	1(4%)	1(5%)	3 (2.9%)
Enterobacter gergoviae	0(0%)	3(12%)	0(0%)	0(0%)	3(2.9%)
Proteus penneri	2(6.3%)	0(0%)	0(0%)	0(0%)	2 (1.9%)
Micrococcus iuteus	0(0%)	2(8%)	0(0%)	0(0%)	2 (1.9%)
Pseudomonas iuteola	0(0%)	0(0%)	1(4%)	1(5%)	2(1.9%)
Stomatococcus spp.	0(0%)	0(0%)	2(8%)	0(0%)	2 (1.9%)
Acinetobacter spp.	0(0%)	2(8%)	0(0%)	0(0%)	2 (1.9%)
Pseudomonas fluoresce	2(6.3%)	0(0%)	0(0%)	0(0%)	2 (1.9%)
Ochrobacter antropi	1(3.1%)	0(0%)	0(0%)	0(0%)	1 (.98%)
Kocuria rosae	1(3.1%)	0(0%)	0(0%)	0(0%)	1 (.98%)
Dermacoccus nishinomiyaensis/ Kytococcus sedentarius	1(3.1%)	0(0%)	0(0%)	0(0%)	1 (.98%)
Lactococcus gravieae	1(3.1%)	0(0%)	0(0%)	0(0%)	1 (.98%)
Staph aureus	0(0%)	1(4%)	0(0%)	0(0%)	1 (.98%)
Salmonella spp.	1(3.1%)	0(0%)	0(0%)	0(0%)	1 (.98%)
Mannhaemia haemolytica	1(3.1%)	0(0%)	0(0%)	0(0%)	1(.98%)
Vibrio spp.	0(0%)	1(4%)	0(0%)	0(0%)	1 (.98%)
Total isolates	**32(100%)**	**25(100%)**	**25(100%)**	**20(100%)**	**102(100%)**

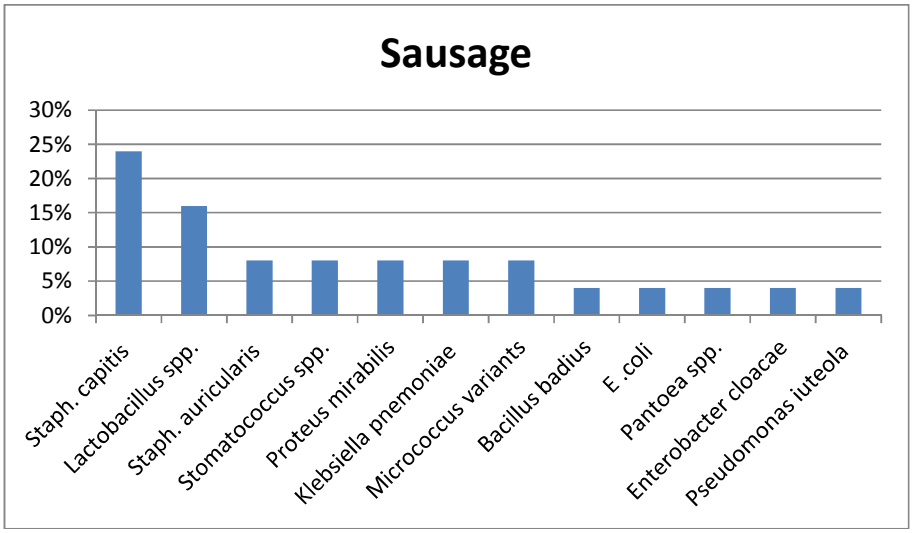

Fig. 3. Percentage of bacterial isolation from sausage samples

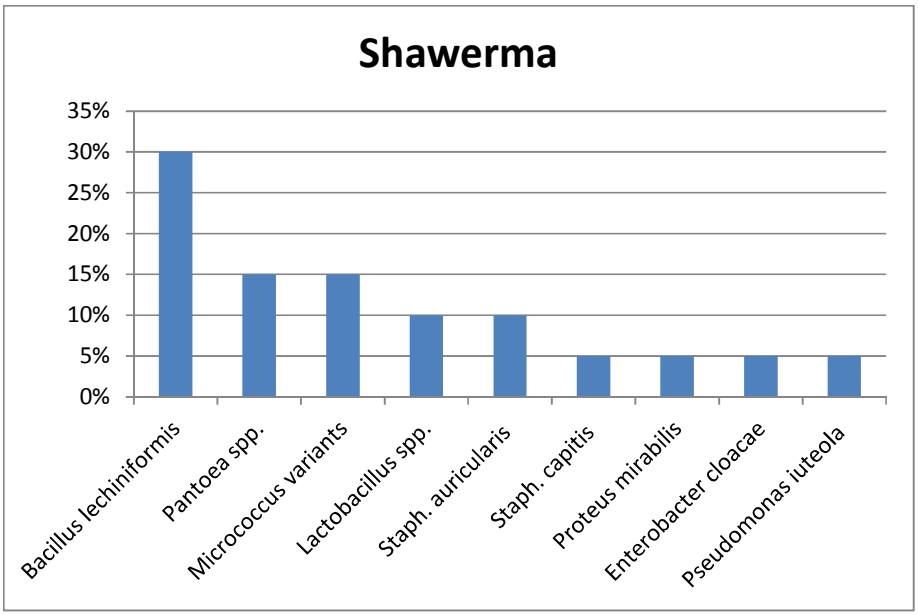

Fig. 4. Percentage of bacterial isolation from Shawerma samples

producing enterobacteria and Methicillin – Resistant *Staphylococcus aureus* were accurately identified by Vitek 2 system [20]. The API strips were extensively used for the identification of bacteria isolated from food samples [21,22].

5. CONCLUSION

In this study many Gram- Positive and Gram-Negative bacteria were isolated from meat product including some organisms of public health concern. The Viteck 2 Compact System facilitated the identification and differentiation between the closely related Gram - Positive bacteria contaminating meat products like *Kocuria kristinae, Ochrobacter anthropi*, *Dermacoccus nishinomiyaensis/ Kytcoccus sedentarius and Lactococcus gravieae*. Implementation of hygienic parameters during processing of meat including proper cleaning of hands, dressing of masks, regular cleaning and disinfection of the processing equipments beside proper handling and ideal preservation conditions

may greatly reduce the microbial load in the processed meat.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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