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Detection of Foodborne Pathogenies and Nitrosamines Residues in Meat Products at Khartoum State, Sudan

OMER FADOUL
Ministry of Public Health, Qatar
MOHAMEDELFATIEH ISMAEL
College of Food Science and Engineering, Northwest A&F University, Yangling 712100, China
Sudanese Standard and Metrology Organization, Khartoum, 13573, Sudan
MUHAMMAD FAROOQ[1]
College of Food Science and Engineering, Northwest A&F University, Yangling 712100, China

Abstract

Strict application of the international standards requirements for food processing plants is deemed essential for public health and food trade. This is a cross-sectional analytic study that investigates the presence of pathogenies in raw beef (RB) from local slaughterhouses and in beef products (BP) from local meat processing plants (MPPs) in Khartoum state and their conformity to both requirements of national and international standards to ensure their safety. Additionally, determination of nitrite residual levels and nitrosamines content in processed meat products. A total of 140 raw and processed beef samples were examined from four MPPs. The BP samples are; beef burger, hotdog, mortadella, frankfurter, pasterma, sausage, and salami. The results showed frequencies bacterial isolates in RB were belonged to twenty-three genera as follows: Staphylococcus aureus (65%), Aerococcus spp (62.5%), Klebsiella pneumoniae (42.5%), Pasteurella multocida (42.5%), Micrococcus spp (40%), Salmonella spp (40%), E. coli (35%), Proteus vulgaris (32.5%), Acinetobacter spp (30%), Bordetella parapertucis (27.5%), Kurthia spp (22.5%), Streptococcus spp (22.5%), Corynebacterium ovis (17.5%), Listeria monocytogenes (17.5%.), Pseudomonas aeruginosa (15%), Bacillus cereus (12.5%), Sterptobacillus spp (12.5%), Haemophilus spp (7.5%), Rothia spp (7.5%), Nocardia asteroids (7.5%), Aeromonas spp (2.5%) Alcaligenes faecalis (2.5%), and Hafnia.alvei (2.5%). While, the BP samples revealed presence of S. aureus (82.5%), P. vulgaris (45%), E. coli (32.5%), B. cereus (32.5%), L. monocytogenes (7.5%), and P. aeruginosa (2.5%). The levels of residual nitrite ranged (0.00 – 0.99), (1.00 – 1.99), (2.00 – 2.99), (3.00 – 3.99), (4.00– 4.99), (5.00 – 5.99), (6.00 – 6.99), and (7.00->) mg/kg. in 49, 25, 13, 6, 2, 3, 1, and 1% of BP, respectively. Moreover, the detection of nine volatile N-nitrosamine compounds which are classified by IARC as probably carcinogenic to humans in PB samples exhibited concentration levels of N-nitrosodin-butylamine (0.41-91µgkg), N-nitrosodi-ethylamine(9-182µg/kg), N-nitrosodi-methylamine, (1.4-250µg/kg), N-nitrosodi-phenylamine (0.7-109µg/kg), N-nitrosodin-propylamine (5-250µg/kg), N-nitroso-methylethylamine (33-191µg/kg), N-nitroso-morpholine (2.4-305µg/kg), N-nitroso-piperidine (25.4-432µg/kg), N-nitroso-pyrrolidine (29-1033µg/kg). The findings revealed that RB has high pathogens load, a situation which necessitates improvement of hygiene in the slaughterhouses as well as, the nitrosamines contents in the BP were in compliance with the regulations of the European Union and FAO/WHO.

Keywords: Foodborne Pathogenies, Processed Beef, Nitrosamines, Residual Nitrite, HPLC

[1] Corresponding authors: Muhammad Farooq College of Food Science and Engineering, Northwest A&F University, Yangling 712100, China. Email: alfagold2008@windowslive.com / farooq.fst28@gmail.com

INTRODUCTION:

The muscle of a healthy animal is essentially sterile, but even under the most stringent conditions, muscles may become contaminated during the harvest process from the environment, hide, or from direct contact with the intestinal tract contents. Contamination ultimately can cause consumer illness if the product is not appropriately handled by the processor or the consumer him-self. Pathogens are of great concern for processors for both food safety and economic reasons. Processors should recall raw product if testing indicates the presence of pathogens [1]. In previous studies, organic acids and acidified sodium chlorite (ASC) have effectively reduced pathogen loads on beef carcasses or cuts [2]. However, Wolf, Miller [3] reported that ground beef from carcasses sprayed with 4% lactic acid had significantly reduced populations of *Salmonella* spp. and *E. coli* O157:H7. Moreover, Raftari, Jalilian [4] showed 2.5 and 1.5 log reductions by organic acid spray application for *E. coli* O157:H7 and Salmonella, respectively. It is highly unlikely that the coverage system as well as the dwell time observed in a laboratory setting would be followed in industry. [5] have found, in trims held for 24 hours after treatment application (organic acid sprays) before grinding, significant reductions of pathogens in both trims and ground beef inoculated with high inoculation doses. On the trim itself, *E. coli* O157:H7 and Salmonella typhimurium were reduced by 1.5 to 2.0 log cycles, with no differences among all treatments. In an attempt to replicate a commercial processing environment [2] used a belt system to transfer beef trim to a grinder, but they manually sprayed the trim on only one side. A limited amount of research has been done to determine the effectiveness of interventions under commercially simulated conditions on beef trim to reduce pathogens.

For at least 20 years, scientists have studied techniques to reduce the bacterial contamination on beef carcass and of beef variety meats as liver. These techniques are now used with Hazard Analysis and Critical Control Points system (HACCP) and have proven effective in reducing the level of contamination. However, potential microbiological decontamination techniques have been investigated for use on beef products in order to improve their microbiological status and so, quality and safety. Activities involved in the process of food animals slaughtering and carcass dressing result in contamination of the exposed cut surfaces of muscle tissue by both Gram-negative and Gram-positive bacteria as well as other microorganisms associated with meat. The origin of these microorganisms may be the gastrointestinal tract of animals and the environment with which the animal carcass or meat cuts had a contact with at some time before or during slaughter and carcass fabrication (cutting or breaking). Beef bacterial contaminant members are the genera's of Acinetobacter, *Aeromonas, Alcaligenes, Arcobacter, Bacillus, Brochothrix, Campylobacter, Carnobacterium, Caseobacter, Citrobacter, Clostridium, Corynebacterium, Enterobacter, Enterococcus, Erysipelothrix, Escherichia, Flavobacterium, Hafnia, Kocuria, Kurthia, Lactobacillus, Lactococcus, Leuconostoc, Listeria. Microbacterium, Micrococcus, Moraxella, Paenibacillus, Pantoea, Pediococcus, Proteus, Pseudomonas, Psychrobacter, Salmonella, Serratia, Shewanella, Staphylococcus, Vagococcus, Weissella*, and *Yersinia* have been reported to be associated with beef contamination [6, 7]. The spoilage microflora of fresh carcasses usually consists, almost exclusively, of Gram-negative rods (mainly Pseudomonads) besides micrococci and staphylococci. In addition, Gram-negative

bacteria such as members of *Acinetobacter, Alcaligenes*, and *Moraxella* genera and genera of enterobacteria. Gram-positive species including spore-forming bacteria, lactic acid-producing bacteria Brochothrix thermosphacta, yeasts and moulds, may be present in small numbers. However, conditions prevailing during storage, processing and handling are more important in determining the microbial association that dominates and spoils the product than the initial density of the various types of microorganisms present on meat carcass. In general, spoilage is caused by a small fraction of the total initial microflora that becomes dominant through selection under the conditions of product handling and storage. For example, it has been demonstrated that cold storage and the gaseous composition of meat packs exert strong selectivity on the microflora. In meat stored at cold temperatures under aerobic conditions, Pseudomonas spp. are considered to be the main spoilage organisms, while Gram-positive bacteria are responsible for spoilage under vacuum packaging and other modified-atmospheric packaging conditions.

Acetic acid and its related salts are widely used as acidulants and antimicrobials [8]. Several epidemiologic studies have indicated associations between consumption of raw red and processed meats and increased risk of colorectal, Stomach and Pancreatic cancers [9-11], cardiovascular diseases and other causes of death [12]. The association was stronger for high consumption of processed than red meat in these studies. In 2007, scientifically based evidence led the World Cancer Research Fund to recommend that consumption of processed meat should be avoided. Rohrmann, Overvad [12] estimated that daily consumption of more than 20 g of processed meat increased the mortality rate. Another possible alternative to nitrite in food preservation is nisin. It is a polycyclic antibacterial peptide produced by the *bacterium Lactococcus lactics* [13]. Nisin is used as a food preservative. It was used in foods for the first time in 1951 to prevent “blowing" of Swiss-type cheese caused by *Clostridium butyricum* [14].

Meat processed under sanitary and hygienic conditions should generally be contaminated with low levels of pathogens compared to the populations of saprophytic microflora. The most important pathogens associated with meat include, *Salmonellae, Staphylococcus aureus, verotoxigenic Escherichia coli, Clostridium perfringens, Campylobacter jejuni, Listeria monocytogenes, Yersinia enterocolitica* and *Aeromonas hydrophila. Salmonella* spp., pathogenic *E. coli* and *Campylobacter* spp are of enteric origin and considered to be the common food-borne pathogens in meat. In the case of *L. monocytogenes*, the plant environment represents the main source of contamination, but it is considered to be of more concern to human health as a post-processing contaminant of ready-to-eat products. The prevalence and levels of pathogenic bacteria on meat carcasses and cuts depend on a number of factors, including the origin of the animal, sanitation procedures and hygienic practices employed during handling and processing of the product, decontamination interventions and conditions of storage [15]. Consumers may now be accustomed to hearing about the health risks posed by meat and poultry. The pathogens commonly found in food animals could cause severe illness and death, especially in children [16]. The victims of that outbreak provided irrefutable evidence for consumers, industry, and policy makers that animal products carry inherent risks that are too dangerous to try to control exclusively in restaurant and home kitchens. Meat and poultry producers recognized that they needed to implement better pathogen controls throughout the food chain. Shortly thereafter, the government declared *E. coli* O157:H7 as an adulterant in food and mandated the use of HACCP

systems for all meat and poultry processors [17]. Working together, the meat and poultry industry and the United States Department of Agriculture (USDA) have made great strides towards decreasing the risk to consumers. But still the years, since the Jack in the Box outbreak, have seen many additional outbreaks and recalls linked to meat and poultry, and each is a reminder that a failure of control at any point in the food chain can be potentially deadly for consumers. The Center for Science in the Public Interest (CSPI) conclusions are based on 12 years of documented food-borne outbreaks from meat and poultry that occurred in the U.S. between 1998 and 2010 [18].

The objectives of this study were as follows: to isolate and identify the bacterial contaminant in raw beef meat (Slaughterhouse) and processed beef products obtained from meat processing plants in Khartoum as well as, to detect the level of residual nitrite and N-nitroso compounds (nitrosamines) in processed beef.

METHODS AND MATERIALS:

1. Samples collection and perpetration

A total of 140 RB and BP beef samples were collected. Raw beef samples were obtained from different slaughterhouses in Khartoum and the BP products samples such as beef burger, mortadella, frankfurter, pasterma, salami, hotdog and sausage were collected from four meat processing plants (MPPs; A, B, C and D). Samples were subjected to bacteriological investigation and to nitrite content determination using spectrophotometer. Moreover, high performance liquid chromatography (HPLC) to detect N-nitroso compounds (nitrosamines).

2. Microbial isolation

Methods of Yang, Pei [19], Ripolles-Avila, Hascoët [20] and Evans, Russell [21]. Quantification of the microbial groups was carried out according to the following standards: ISO (6887-2, 2003 and 15478 (2), 2004). Samples from PB products or RB were homogenized before aseptically taking, unless otherwise indicated, a mass of at least 10 g or a volume of 10 ml for the first dilution. The time lapse between preparation of the homogenate and inoculation of the counting media was worked out not to exceed 45 minutes. Samples were disinfected by flaming. Wrapped portions of meat were opened on trays by removing the packaging film starting beneath the tray. RB and BP samples were inoculated on MacConkey Agar and Blood Agar media. Plates were observed for appearance of colonies and discarded as negative if no growth was detected during specified incubation period. Subcultures were made for preparation of pure culture on Nutrient Agar or appropriate growth medium according to [22]. A typical and well isolated bacterial colony was partly picked up by a sterile wire loop and streaked onto the surface of a fresh plate of the same medium and incubated aerobically at 37°C for 24 hours. The process was repeated twice before the isolate was considered pure. Gram's stain was used to confirm purity.

3. Pathogens identification

3.1. Primary identification tests:

3.1.1. Gram's stain:

A culture smear was spread on a microscopic slide, fixed by flaming and put on a glass holder. The slide was then flooded with ammonium oxalate crystal violet stain for ½

minute, and washed with water. It was then covered by Lugol's iodine for ½ minute and the iodine was drained but not washed, decolorized with few drops of acetone, and immediately the slide was thoroughly washed with water. The slide was counterstained with diluted carbol fuchsin for ½ minute and washed with water again and allowed to dry. A drop of immersion oil was placed on each slide and examined under microscope. Gram-positive organism appeared purple in colour while Gram-negative ones appeared red [23].

3.1.2. Motility test:

The motility medium was inoculated with the organism under test by stabbing to a depth of 5mm. The medium was then incubated at/or below the optimum growth temperature (e.g. 37°C and 22°C) for 24 hours. Motile organism growth extended beyond the stab line while the that of non-motile organism was confined to the stab line [24].

3.1.3. Catalase test:

On a clean slide, a drop of 3% aqueous solution of hydrogen peroxide was placed. A colony of the test organism on the Nutrient Agar was picked up with a sterile glass rod and put on the drop of the hydrogen peroxide. Evolution of gas and appearance of bubbles indicated that the organism had produced catalase enzyme [25].

3.1.4. Oxidase test:

Drops of 1% tetramethyl-p-phenylenediamine dihdydrochloride were poured over wet strips of filter paper in a Petri dish and then sterilized in a hot air oven. Using sterile forceps, strips were laid on a clean slide. Growth on Nutrient Agar was picked up with sterile glass rod and rubbed onto small area on the filter paper. A purple color that developed within 10 seconds was considered a positive reaction [26].

3.1.5. Acid production from glucose:

Peptone water medium containing glucose was inoculated with the test organism and examined daily for seven days for acid and gas production [27].

3.1.6. OF test:

Duplicate tubes of Hugh and Leifson's medium were inoculated with the test organism using a straight wire loop, then a layer of melted soft paraffin, about 3cm, was added on the medium surface in one tube to seal it from air and the other was left without paraffin. The tubes were incubated at 37ºC and examined daily for up to 14 days. If the color was yellow in both tubes, fermentative reaction was indicated. If it was blue or green in the tube without paraffin and green in the sealed tube, this was an indication of alkali production. If it was yellow in the open tube only, oxidation of glucose was indicated. No change in both tubes means a negative reaction [28].

3.2. Secondary identification tests:

3.2.1. Methyl red (MR) test

The test organism was inoculated into Glucose Phosphate (MR) medium and incubated at 37°C for 2 days. Two drops of methyl red solution were added, shaken, and

examined. Positive MR reaction is shown by the appearance of a red color at the surface. An orange or yellow color should be regarded as negative [28].

3.2.2. Voges-Proskauer (VP) test:

The VP test was used to detect production of acetylmethylcarbinol by bacteria. Glucose Phosphate Medium was inoculated with the test organism and inoculated at 37°C for 48 hours. Then 0.6 ml of 5% alcoholic solution of a-naphthol and 0.2 ml of 40% KOH were added to culture which was then shaken and slopped. A positive reaction was indicated by development of bright pink color within 30 minutes [29].

3.2.3. Starch degradation:

The test isolates were cultured on starch agar medium. The plates were incubated at 37°C. After 3-5 days when good growth was noted, the plates were flooded with lugol's iodine solution. A positive starch reaction gave clear zones around the growth of each colony [28].

3.2.4. Coagulase tests:

The slide coagulase test was performed by placing a loopful of normal saline and two colonies of the test organism on a clean slide, mixed until homogenous suspension was obtained; then a drop of human plasma or rabbit plasma was added to the suspension; clumping occurrence within 5 seconds indicated a positive test. The slide test was confirmed by the tube test. For tube coagulase test a half ml of diluted, fresh human plasma in saline (1:10) was poured into small test tubes, then 0.1ml of an 18-24 hours broth culture was added to and incubated at 37°C. The tube was examined for coagulation after 6 hours then at regular intervals up to 24 hours. Definite clot formation indicated positive result [30].

3.2.5. Aesculin degradation (hydrolysis):

Aesculin broth medium was inoculated with each of test isolates then incubated at 37ºC and examined daily for up to 5 days. Positive aesculin degradation gave the medium black colour [28].

3.2.6. Sugar fermentation:

In this test nine sugars (xylose, cellobiose, raffinose, sucrose, maltose, D-mannose, D-mannitol, D-trehalose and lactose) were examined. The concentration of sugar and Andrade's indicator was 1% in peptone water. The peptone water sugar was inoculated with the organism under test, incubated at 37º C and examined daily for seven days. Acid production was indicated by appearance of reddish colour, while gas production was indicated by presence of empty space (air bubble) in the inverted Durham's tube. Negative cultures were examined at certain intervals for up to 30 days [31].

3.2.7 Gelatin liquefaction:

The test organism was stab-inoculated into nutrient gelatin using sterile long straight wire, incubated at 37°C for up to two weeks with daily examination, followed by putting in the refrigerator for two hours. A positive result was indicated by liquefaction of refrigerated gelatin [28].

3.2.8. Indole Production test:

A peptone water medium was inoculated with the test organism, incubated at 37°C for 48 hours, and then 1ml of Kovac's reagent was run down the side of the test tube. A pink ring layer within a minute indicated a positive reaction [25].

4. BACTERIAL SODIUM NITRATE SENSITIVITY TEST

This test was essentially carried out according to similar previously described test which was used for detection of antibacterial effect of silver nitrate [32]. Five species of bacteria isolated and identified in this study, were subjected to varying contents of nitrate in the form of sodium nitrate. The test was carried out as follows: a 0.1 g of sodium nitrate was accurately weighed and dissolved in 10 ml of distilled water, after that ten-fold serial dilutions, from 10-1 to 10-10 in ten test tubes, were made and then sterilized by autoclaving at 121ºC for 15 minutes. Then each tube content was poured into a Petri dish and the disks were impregnated with the revelant sodium nitrate concentration. *E. coli, Proteus vulgaris, Klebsiella pnuomniae, Staphylococcus aureus*, and *Pseudomonas aerurginosa* were each cultured onto Nutrient Agar plate. Each impregnated disk with a different $NaNO_3$ concentration was pasted on a Nutrient Agar culture of each of the test five bacterial species and incubated at 37ºC. The same method was repeated using 0.5 g, 1 g and 2g of sodium nitrate.

5. NITRITE DETERMINATION

This is of limited applicability to meat products containing reducing agents such as ascorbic acid. In the presence of the latter substance, the recovery of nitrite may be less than 50% according to ISO 6635, 1984. The test was conducted following the method of Wang, Yu [33] and ISO standards of in brief, a five zinc rods were added to cadmium sulphate solution in a beaker. The spongy cadmium deposit was removed from the zinc rods at about every hour by swirling them in the solution or rubbing them against each other. After about 7 hours the solution was decanted and the deposit was washed twice with a litre of distilled water, assuming that the cadmium is always covered with a layer of liquid. The cadmium deposit with approximately 01 M hydrochloric acid were blended by a mechanical mixer for 10 seconds and returned back to the beaker. Occasionally the deposit was stirred up with a glass rod, allowed to stand overnight under the 01 M HCl acid and stirred once more to remove all gas bubbles from the metal. The liquid was decanted and the cadmium slurry was washed twice with a liter of distilled water. A glass wool plug was fitted to the bottom of the glass column. Then the cadmium water suspension was transferred into the column with water until the height was about 17cm. The column was occasionally drained during filling, ensuring that the metal is always covered with a liquid. Inclusions of gas could be eliminated with a knitting needle. The liquid should had flowed out at a rate not exceeding 3 ml per min to avoid the possibility of incomplete reduction. The sample extract was prepared according to ISO 6635 (1984), the sample extraction was done by adding 100 ml hot water (80ºC), 5 ml borax solution and 0.5 g activated charcoal to 10 g comminuted beef into a 250 ml wide-necked conical flask. The mixture was heated, with repeat agitation, for 15 mm on a boiling water bath and allowed to cool for not less than an hour. Two ml of each of freshly prepared clearing reagents zinc ferrocyanide, zinc

acetate solution [21.9 g of crystallized zinc acetate (Zn $_{C2H3O2}$) H_2O_2], and 3ml of glacial acetic acid were added, diluted to 100 ml before adding of Potassium ferrocyanide solution (10.6% aqueous solution), accompanied by swirling after each addition. Five ml more of borax solution were added and the mixture was transferred to a 200 ml volumetric flask, allowed to stand for 30 min, made up to the mark with distilled water, mixed and filtered through a filter paper No. 4. A 10 ml volume of the filtrate sample (containing less than 100 mg) was pipetted into a 50 ml volumetric flask, diluted to approximately 40 ml and 5 ml sulphanilamide was added to. The absorbance of the solution was measured in a 1 cm cell using a spectrophotometer at a wave length of about 538 nm. A calibration curve was prepared by transferring 10ml water into each of 4 volumetric flasks, which each contained 10 ml of a standard sodium nitrite solution containing 0, 2.5, 5.0 or 10 µg of nitrite/ml; the colour developed and was measured.

-Nitrite content was calculated by the equation of $NaNO_2$= c x 2000/ M x V

Where; V = volume in ml of aliquot portion of filtrate taken for test; M =mass in g of sample taken; C = concentration of sodium nitrite in µg/ml which was read from the calibration curve that corresponded with the absorbance of the solution prepared from the sample.

6. EXTRACTION OF N-NITROSAMINES

Approximately 6 grams of meat sample was placed in the Pyrex tube into which 10 ml of 1N sodium hydroxide was poured. The tube was capped tightly and autoclaved at 121˚C for 10 min. After being allowed to stand at room temperature, the autoclaved solution was transferred to 50 ml separatory funnel. The tube was rinsed twice with 5 ml of ethanol and then 10 ml of dichloromethane. The rinsing solutions and10 ml of 10% aqueous sodium chloride was combined with the original extract in the separatory funnel. After being shaken, the dichloro-methane layer was collected, and the water layer was re-extracted with 10 ml of dichloromethane. The dichloromethane extracts were combined, dried over anhydrous sodium sulfate and concentrated to approximately 0.5 ml using KD concentrator under nitrogen gas flow. The concentrate was loaded onto a silica gel column (30cm × 1.5cm) and equilibrated with dichloromethane. The column was eluted with 10 ml of dichloromethane. After the addition of 100µl of octane (to prevent exsiccation of the solvent), the elute was concentrated to 1ml using KD concentrator and nitrogen gas, then extracting 3 ml methanol. This was repeated three times. The combined methanol extracts were concentrated to about 100 µl under a nitrogen stream [34].

7. N-NITROSAMINES DETERMINATION

Nitrosamines determination was carried out according to the methods described by Huang, Chen [35] and Komarova and Velikanov [36] by using high-performance liquid chromatography (HPLC). Nitrosamine standard mix was used [2000µg/mL of each of N-nitrosodi n-butylamine (NDnBA), N-nitrosodiethylamine (NDEA), N-nitrosodimethylamine (NDMA), N-nitrosodiphenylamine (NDPhA), N-nitrosodi n-propylamine (NDnPA), N-nitromethylethylamine (NMEA), N-nitromorpholine(NMOR), N-nitrosopi-peeridine (NPIP), and N-nitrosopyrrolidine (NPYR)] .It was purchased from ULTRA Science Corporations ,USA. Chemicals of sodium hydroxide, ethanol,

octane and dichloromethane (DCM) were purchased from Sigma-Aldrich corporation (St. Louis, Missouri, USA). All other chemicals (ascorbic acid, anhydrous sodium sulfate and sodium chloride) besides acetonitrile M300, Methanol, water (HPLC grade), and ammonium hydroxide were also obtained from Sigma-Aldrich. The 0.22 µm PVDF syringe filter was also used. Glass column (30 cm × 1.5 cm), Kuderna Danish (KD) concentrator (used for the concentration of organic solvents). Autoclave, and CAMAG UV Lamp dual wavelength, 254/366 nm, 2 × 8 watt in combination with KNAUER® HPLC system consisting of smart line manager, sample manager, and smart line UV detector 2500. System control, data collection, and data processing were accomplished using eurochrom™ chromatography data software. The chromatographic condition was optimized using the Knauer Symmetry C18, 5 µm (100 mm × 4.6 mm) column. A solvent A is 10 mM ammonium hydroxide and acetonitrile as solvent B. This was then filtered through a 0.22 µm PVDF membrane filter and degassed under vacuum prior to use. The separation of all components was achieved by gradient elution using solvent A and B. Solution A was used as diluent. The final selected and optimized conditions were as follows; injection volume 20 µl, gradient elution (solvent B from 0 to 90% during 10 min then maintained to the end of the run) at a flow rate of 1.0 ml/min at 80˚C (column oven temperature), detection wavelength of 231 nm, and sample temperature at 15˚C. Under these conditions, the backpressure in the system was about 2500 psi. A series of working standard solutions were prepared by appropriate dilution of the EPA 521 nitrosamine mix with solvent A and stored at –20˚C before use. From the primary stock solution 2000 µg/ml of each N-nitrosamine, a 1/50 dilution was done to get 40 µg/ml secondary stock solution of each N-nitrosamine. Sequentially, dilutions of the secondary stock solution were made to get titrating standards at 0.5, 1, 2.5, 5, 7.5, 10, and 15 µg/ml in 100 ml volumetric flasks; these solutions were kept in the absence of light. Nitrosamine detection samples were delivered to the Institute of Food Technology, Research Center, Algeza, Egypt. Twenty Samples were collected and sorted out as follows: four Mortadellas, four Sausages, four beef burgers three Frankfurters, two Hotdogs, two Pastermas and one Salami.

8. STATISTICAL ANALYSIS

The mean and standard errors were calculated for all measurements using statistical program (SPSS,18). ANOVA, descriptive statistical analysis and excel.

RESULTS:

1. Bacteriological analysis

1.1 The raw beef:

Tables 1-4 shows the isolated bacteria from raw beef samples and their biochemical reactions are shown in table S1. Forty raw beef samples were distributed according to the investigated locations, A.B.C and D, in ten samples each.

Table 1: Bacterial species isolated from RB from location A

Sample No.	Bacterial isolates – Location A																					
	Proteus vulgaris	*E. Coli*	*Staphylococcus aureus*	*Bacillus cereus*	*Pseudomonas aruginosa*	*Listeria monocytogenes*	*Salmonella spp*	*Aerococcus spp*	*Pasteurella multocida.*	*Micrococcus spp*	*Bordetella parapertussis*	*Acinetobacter spp.*	*Corynebacterium ovis.*	*Streptobacillus.spp*	*Klebsiella pneumoniae.*	*Karthia spp.*	*Streptococcus spp*	*Hafnia.alvei*	*Nocardia asteroides*	*Alcaligenes faecalis*	*Rothia spp.*	*Haemophilus spp.*
A1	×	×	√	×	×	√	√	√	√	√	√	√	×	×	×	×	×	×	×	×	×	×
A2	√	×	√	×	×	×	√	×	×	×	√	√	√	√	√	×	×	×	×	×	×	×
A3	×	×	×	×	√	×	×	√	×	×	×	×	×	×	×	√	√	×	×	×	×	×
A4	√	√	√	√	×	×	√	√	√	√	×	×	×	×	√	×	×	×	×	×	×	×
A5	×	√	×	√	×	×	√	×	×	×	×	×	√	×	×	×	×	×	×	×	×	×
A6	×	√	×	×	×	×	√	√	√	×	×	×	×	√	√	×	×	×	×	×	×	×
A7	×	√	×	×	×	×	√	×	×	×	×	×	×	×	√	×	×	√	×	×	×	×
A8	√	√	√	×	×	√	×	×	√	×	×	×	×	×	√	×	×	×	×	×	×	×
A9	×	×	√	×	×	×	√	×	√	√	√	√	×	×	×	×	×	×	×	×	×	×
A10	√	×	×	×	×	×	×	√	×	×	×	×	√	×	×	×	×	×	×	×	×	×

√= the bacterium isolated; ×= the bacterium not isolated.

Table 2: Bacterial species isolated from RB from location B

Sample No	Bacterial isolates – Location B																					
	Proteus vulgaris	*E. Coli*	*Staphylococcus aureus*	*Bacillus cereus*	*Pseudomonas aruginosa*	*Listeria monocytogenes*	*Salmonella spp*	*Aerococcus spp*	*Pasteurella multocida.*	*Micrococcus spp*	*Bordetella parapertussis*	*Acinetobacter spp.*	*Corynebacterium ovis.*	*Streptobacillus.spp*	*Klebsiella pneumoniae.*	*Karthia spp.*	*Streptococcus spp*	*Hafnia.alvei*	*Nocardia asteroides*	*Alcaligenes faecalis*	*Rothia spp.*	*Haemophilus spp.*
B1	√	√	√	×	×	×	×	√	×	×	×	×	√	×	√	√	×	×	√	×	×	×
B2	×	√	×	√	×	×	√	×	×	×	×	×	×	×	√	×	×	×	×	×	×	×
B3	√	√	×	×	×	×	√	×	√	×	×	×	×	×	√	×	×	×	×	×	×	×
B4	×	√	×	×	×	×	√	×	×	×	×	×	×	×	√	×	×	×	√	×	×	×
B5	√	√	×	×	×	×	×	×	√	×	×	×	×	×	√	×	×	×	×	×	×	×
B6	×	×	×	×	×	√	×	√	×	×	√	√	×	×	√	√	√	×	×	√	×	×
B7	×	×	√	×	√	×	×	√	×	√	√	√	×	×	×	×	√	×	×	×	×	×
B8	×	×	√	×	√	×	×	×	×	√	×	×	×	×	×	×	×	×	×	×	√	√
B9	√	×	√	×	×	×	×	√	×	×	×	×	×	√	√	√	×	×	×	×	×	×
B10	×	×	√	×	×	×	√	√	√	×	√	×	×	×	×	×	×	×	×	×	×	×

√= the bacterium isolated; ×= the bacterium not isolated.

Table 3: Bacterial species isolated from RB from location C

Sample No	Bacterial isolates – Location C																						
	Proteus vulgaris	*E. Coli*	*Staphylococcus aureus*	*Bacillus cereus*	*Pseudomonas aruginosa*	*Listeria monocytogenes*	*Salmonella spp*	*Aerococcus spp*	*Pasteurella multocida.*	*Micrococcus spp*	*Bordetella parapertussis*	*Acinetobacter spp.*	*Corynebacterium ovis.*	*Streptobacillus.spp*	*Klebsiella pneumoniae.*	*Kurthia spp.*	*Streptococcus spp*	*Hafnia.alvei*	*Nocardia asteroides*	*Alcaligenes faecalis*	*Rothia spp.*	*Haemophilus spp.*
C1	√	×	√	×	×	×	×	√	×	×	√	√	×	×	√	×	×	×	√	×	×	×
C2	×	×	√	×	×	√	√	√	√	√	×	×	×	×	√	×	×	×	×	×	×	×
C3	√	×	√	×	×	×	×	×	×	×	×	√	√	√	√	×	×	×	×	×	×	×
C4	×	×	√	×	×	×	√	√	√	×	√	×	×	×	×	√	√	×	×	×	×	×
C5	√	×	√	×	×	×	×	√	√	√	×	×	×	×	×	×	×	×	×	×	×	×
C6	×	√	×	√	×	×	√	×	×	×	×	×	√	×	√	×	×	×	×	×	×	×
C7	√	√	×	√	×	×	×	√	√	×	×	×	×	×	√	×	×	×	×	×	×	×
C8	×	√	×	×	×	×	√	×	×	×	×	×	×	×	√	×	×	√	×	×	×	×
C9	√	√	×	×	×	×	×	×	√	×	×	×	×	×	√	×	×	×	×	×	×	×
C10	×	×	√	×	×	√	×	√	√	√	×	×	×	×	√	×	√	×	×	×	×	×

√=the bacterium isolated; ×= the bacterium not isolated.

Table 4: Bacterial species isolated from RB from location D

Sample No	Bacterial isolates – Location D																						
	Proteus vulgaris	*E. Coli*	*Staphylococcus aureus*	*Bacillus cereus*	*Pseudomonas aruginosa*	*Listeria monocytogenes*	*Salmonella spp*	*Aerococcus spp*	*Pasteurella multocida.*	*Micrococcus spp*	*Bordetella parapertussis*	*Acinetobacter spp.*	*Corynebacterium ovis.*	*Streptobacillus.spp*	*Klebsiella pneumoniae.*	*Kurthia spp.*	*Streptococcus spp*	*Hafnia.alvei*	*Nocardia asteroides*	*Alcaligenes faecalis*	*Rothia spp.*	*Haemophilus spp.*	*Aeromonas spp.*
D1	×	×	√	×	×	×	×	√	×	×	√	√	×	×	×	√	√	×	×	×	×	×	√
D2	×	×	√	×	√	×	×	√	√	√	×	√	×	×	×	√	×	×	×	×	√	×	×
D3	×	×	√	×	×	×	×	√	×	√	√	√	×	×	×	×	×	×	×	×	×	×	×
D4	×	×	√	×	×	√	×	√	×	√	×	×	×	×	×	×	×	×	×	×	√	√	×
D5	×	×	√	×	√	×	×	√	√	√	×	×	×	×	×	√	√	×	×	×	×	×	×
D6	×	×	×	×	×	√	×	√	×	√	×	×	×	×	×	×	×	×	×	×	×	√	×
D7	×	×	√	×	×	×	×	√	×	√	√	√	×	×	×	√	√	×	×	×	×	×	×
D8	×	×	√	×	√	×	×	√	√	√	×	×	×	×	√	×	√	×	×	×	×	×	×
D9	×	×	√	×	×	×	×	×	×	×	×	√	√	√	×	√	×	×	×	×	×	×	×
D10	×	×	√	×	×	×	√	√	×	√	×	×	×	×	×	×	√	×	×	×	√	×	×

√=the bacterium isolated; ×= the bacterium not isolated.

1.2 Processed beef products:

Bacteria isolated from PB products are shown in Table 5. Seventy-nine isolates of six bacterial species were isolated from forty samples of PB products; they are also shown in table S2.

Table 5. Bacterial species isolated from PB products.

No	PB Products	Bacterial species					
		P. vulgaris	*E. coli*	*S. aureus*	*B. cereus*	*P. aeruginosa*	*L. monocytogenes*
1	Sausage	√	√	√	×	×	×
2	Burger	√	×	√	×	×	×
3	Salami	√	×	√	×	×	×
4	Mortadella	√	√	√	×	×	×
5	Hotdog	√	×	√	×	×	×
6	Mortadella	√	×	√	×	×	×
7	Burger	√	×	√	×	×	×
8	Hotdog	√	√	√	×	×	×
9	Pasterma	√	√	√	×	×	×
10	Sausage	√	×	√	×	×	×
11	Sausage	√	×	√	×	×	×
12	Burger	√	×	√	×	×	×
13	Salami	√	×	√	×	×	×
14	Mortadella	×	×	√	×	×	×
15	Hotdog	×	×	×	√	×	×
16	Mortadella	×	×	√	×	×	×
17	Burger	×	√	√	×	×	×
18	Hotdog	×	×	×	√	×	×
19	Pasterma	×	×	√	√	×	×
20	Sausage	×	√	×	×	×	×
21	Mortadella	×	√	×	√	×	×
22	Burger	√	√	×	×	√	×
23	Hotdog	×	×	√	×	×	×
24	Pasterma	×	√	×	√	×	×
25	Sausage	√	×	√	×	×	×
26	Mortadella	×	√	√	√	×	×
27	Burger	×	√	×	×	×	√
28	Hotdog	√	×	×	×	×	√
29	Pasterma	×	×	√	×	×	√
30	Sausage	√	×	√	×	×	×
31	Mortadella	×	×	√	√	×	×
32	Burger	×	×	√	√	×	×
33	Hotdog	×	×	√	√	×	×
34	Pasterma	×	×	√	√	×	×
35	Sausage	√	×	√	√	×	×
36	Mortadella	×	×	√	×	×	×
37	Burger	×	×	√	×	×	×
38	Hotdog	×	√	√	×	×	×
39	Salami	×	×	√	√	×	×
40	Sausage	×	√	√	×	×	×

√= bacterium was isolated; ×= bacterium not isolated; PB= Processed beef.

2. FREQUENCIES OF BACTERIAL ISOLATES ISOLATION PER LOCATION

2.1 The raw beef:

The highest frequency of isolations obtained from each location samples as shown in table S3. Location A was recorded for *Salmonella* spp (70%), followed by *Escherichia coli, Staphylococcus aureus, Aerococcus* spp., and *Pasteurella multocidea* (50% each) and *Proteus vulgaris* (40%). The low isolation frequencies were noticeable for *Micrococcus* spp., *Bordetella parapertussis, Acinetobacteria* spp., and *Corynebacterium ovis* (30% each), followed by those of *Bacillus cereus*, *Listeria monocytogenes*, and *Sterptobacillus* spp (20% each), and lastly those of *Pseudomonas aeruginosa* and *Klebsiella pneumonia* (10% each).

Location B, the highest frequency of isolation was recorded for *K. pneumoniae* (70%), followed by *E. coli, S. aureus,* and *Aerococcus* spp. (50% each) and *P. vulgaris* and *Salmonella* spp (40% each). The low frequencies of isolation were those of *P. multocida*, *B. parapertussis*, and *Kurthia* spp. (30% each), followed by those of *P. aeruginosa*, *Micrococcus* spp, *Acinetobacter* spp, *Streptococcs* spp and *N. asteroides* (20%

each), and lastly those of *B. cereus*, *L. monocytogenes* and *C. ovis*, *Streptobacillus* spp*, A. faecalis* and *Rothia* spp (10% each).

Location C the highest frequency of isolation of the bacteria isolated from its samples was that of *K. pneumoniae*. (80%), followed by those of *S. aureus. Aerococcus* spp *and P. multocide* (60% each*), P. vulgaris* (50%), *E. coli* and *Salmonella* spp (40% each). Low frequencies of isolation were manifested by *Micrococcus* spp (30%), followed by those of *B. cereus*, *L. monocytogenes*., *B. parapertussis*, *Acinetobacter* spp, *C. ovis*. and *Streptococcus* spp (20% each), and lastly those of *Sterptobacillus* spp, *Kurthia* spp., *H. alvei* and *N. astericoides* (10% each). Location D, the highest bacterial frequency of isolation was that of *S. aureus*. (100%), followed by *Aerococcus* spp (90%), *Micrococcus* spp (80%). *Acinetobacter* spp and *Streptococcus* spp (50% each). The low isolation frequencies are those of *P. aeruginosa*., *P. multocida and B. parapertussis* (30% each), followed by those of *L. monocytogenes and Rothia* spp (20% each) and lastly those of *Salmonella* spp*, C. ovis, Sterptobacillus* spp*, K. pneumoniae., and Aerom*onas spp (10% each).

2.2 Processed beef products:

The frequencies of isolation of bacterial contaminants of the whole PB products sample of the four MPPs investigated in Khartoum are shown in Table S4. The highest frequency of isolation was attained by *S. aureus* (65%) and the lowest by *Aeromonas* spp and *H. alvei* (2.5% each).

3. NITRITE ANALYSIS

Residual nitrite contents of PB products (mg/kg)

The concentrations of nitrite residue in one hundred samples of PB products were classified into eight group-ranges; they were 0.00 – 0.99, 1.00 –1.99, 2.00 – 2.99, 3.00 – 3.99, 4.00 – 4.99, 5.00 – 5.99, 6.00 – 6.99, and 7.00 mg/kg or more and were present in 49 %, 25%, 13%, 6%, 2%, 3%. 1%, and 1%, of the PB products tested, respectively (Table 6).

Table 6. Levels of Nitrites in different samples of PB (mg/ kg)

Serial No	Code of Meat processing plant	Name of product	Result of Nitrite content (mg/Kg)
1	A	Mortadella	3.3 mg/kg
2	A	burger	1.61mg/kg
3	A	Frankfurter	2.29mg/kg
4	A	pasterma	1.265mg/kg
5	A	sausage	1.61mg/kg
6	B	Mortadella	0.69mg/kg
7	B	Frankfurter	0.67m/kg
8	B	burger	0mg/kg
9	B	Hot dog	1.043mg/kg
10	B	sausage	0mg/kg
11	C	burger	0.524mg/kg
12	C	sausage	0.243mg/kg
13	C	Frankfurter	0.524mg/kg
14	C	selami	3.556mg/kg
15	C	Mortadella	6.763mg/kg
16	D	Mortadella	4.506mg/kg
17	D	burger	0.074mg/kg
18	D	Hot dog	3.355mg/kg
19	D	pasterma	0.205mg/kg
20	D	sausage	0.448mg/kg
21	A	Mortadella	2.88 mg/kg
22	A	burger	0.35mg/kg

23	A	Frankfurter	2.64mg/kg
24	A	pasterma	2.01mg/kg
25	A	sausage	0.48mg/kg
26	B	Mortadella	1.47mg/kg
27	B	burger	0.161mg/kg
28	B	Frankfurter	5.29mg/kg
29	B	pasterma	2.14mg/kg
30	B	sausage	0.39mg/kg
31	C	Mortadella	1.10mg/kg
32	C	burger	1.042mg/kg
33	C	Frankfurter	6.66mg/kg
34	C	slami	2.60mg/kg
35	C	sausage	0.41mg/kg
36	D	Mortadella	1.92mg/kg
37	D	burger	0.69mg/kg
38	D	Hot dog	0.96mg/kg
39	D	pasterma	0.904mg/kg
40	D	sausage	1.16mg/kg
41	A	Mortadella	0.87mg/kg
42	A	burger	1.06mg/kg
43	A	Frankfurter	3.35mg/kg
44	A	pasterma	5.71mg/kg
45	A	sausage	0.24mg/kg
46	B	Mortadella	14.8mg/kg
47	B	Frankfurter	0.5mg/kg
48	B	burger	0.8mg/kg
49	B	Hot dog	1.06mg/kg
50	B	sausage	0.82mg/kg
51	C	burger	0mg/kg
52	C	sausage	0mg/kg
53	C	Frankfurter	0.78mg/kg
54	C	selami	1.8mg/kg
55	C	Mortadella	0.4mg/kg
56	D	Mortadella	0.39mg/kg
57	D	burger	0.31mg/kg
58	D	Hot dog	0.54mg/kg
59	D	pasterma	0.19mg/kg
60	D	sausage	0.44mg/kg
61	A	Mortadella	0.24mg/kg
62	A	burger	0 mg/kg
63	A	Frankfurter	1.08mg/kg
64	A	pasterma	2.05mg/kg
65	A	sausage	0mg/kg
66	B	Mortadella	2.22mg/kg
67	B	Frankfurter	2.24mg/kg
68	B	burger	1.94mg/kg
69	B	Hot dog	2.72mg/kg
70	B	sausage	2.14mg/kg
71	C	burger	0mg/kg
72	C	sausage	0mg/kg
73	C	Frankfurter	0.33mg/kg
74	C	selami	1.5mg/kg
75	C	Mortadella	0.61mg\kg
76	D	Mortadella	0.97mg/kg
77	D	burger	0mg/kg
78	D	Hot dog	0.82mg/kg
79	D	pasterma	0.195mg/kg
80	D	sausage	2.39mg/kg
81	A	Mortadella	0.22mg/kg
82	A	burger	0mg/kg
83	A	Frankfurter	1.3mg/kg
84	A	pasterma	1.4mg/kg
85	A	sausage	0,57mg/kg
86	B	Mortadella	0.63mg/kg
87	B	Frankfurter	1.04mg/kg
88	B	burger	1.33mg/kg
89	B	Hot dog	1.51mg/kg
90	B	sausage	1.04mg/kg
91	C	burger	1.1mg/kg
92	C	sausage	1.57mg/kg
93	C	Frankfurter	4.00mg/kg

94	C	selami	3.76mg/kg
95	C	Mortadella	2.2mg/kg
96	D	Mortadella	1.54mg/kg
97	D	burger	0.97mg/kg
98	D	Hot dog	1.58mg/kg
99	D	pasterma	3.01mg/kg
100	D	sausage	0.93mg/kg

4. **N-nitrosamines detection in processed beef**

Nine volatile nitrosamines were detected in each of the twenty PB products samples examined [burger (4), Mortedlla (4), Sausage (4) frankfurter (3), hot dog (2), pasterma (2), and salami (1)] collected from four meat processing plants in Khartoum. They were N-nitrosodi n-butylamine , NDnBA (present as 0.4-91µg/kg), N-nitrosodiethylamine, NDEA(9-182µg/kg), N-nitrosodi -methylamine, NDMA (1.9-208 µg/kg), N-nitrosodiphenylamine, NDPhA(0.7-109 µg/kg), N-nitrosodin-propylamine, NDnPA (5-250 µg/kg), N-nitrometylethylamine, NMEA (3.3-191µg/kg), N-nitrosomorpholine, NMOR (2.4-305µg/kg), N-nitrsopiperidine, NPIP (25.4-432 µg/kg), N-nitrosopyrrolidine, NPYR (29-1033 µg/kg). The mean NA content of PB products samples are shown in Table 7 and figure 1.

Table 7: Mean N-nitrosamines content of processed beef products (µg/100g).

Meat proceeding plant	N-nitrosamines values (Mean ± SD)								
	NDnBA[1]	NDEA[2]	NDMA[3]	NDPA[4]	NDnPA[5]	NMEA[6]	NMOR[7]	NPIP[8]	NPYR[9]
A	0.932a ± 0.492	0.312a ± 0.117	1.166b ± 0.445	0.624 a ± 0.301	13.048a ± 3.529	1.902b ± 6.189	4.176a ± 2.506	7.662a ± 1.768	19.12a ± 8.217
B	0.200a ± 0.108	0.604a ± 0.436	1.738b a ± 1.037	2.062 a ± 1.689	9.844a ± 6.189	1.546b ± 2.506	5.966a ± 3.395	12.126a ± 6.33	33.86a ± 17.828
C	0.652a ± 0.509	0.628a ± 0.213	1.776b a ± 0.854	0.478a ± 0.224	7.460 a ± 3.395	1.790b ± 3.395	7.308a ± 6.189	13.232a ± 6.079	21.64a ± 10.048
D	3.120a ± 1.787	4.702a ± 3.401	7.616a ± 3.574	3.300a ± 1.921	13.048a ± 3.529	8.546a ± 3.529	10.404a ± 3.529	18.084a ± 6.352	46.08a ± 13.227

1: N-nitrosodin-butylamine; 2: N-nitrosodiethylamine; 3: N-nitrosodimethylamine;4: N-nitrosodiphenylamine; 5: N-nitrosodi n –propylamine; 6: N-nitrometylethylamine; 7: N-nitrosomorpholine; 8: N-nitrospiperidine; 9: N-nitrosopyrrolidine; Means followed by the same superscript letters on the same column are not significantly different.

The lowest NA content (0.4µg/kg) was detected for NDnBA in sausage and frankfurter (MPPs:B and C) , whereas the highest NA content (1033µg/kg) was detected for NPYR in a hotdog sample (MPP:B) .The NA content of PB samples were classified into group-ranges contnt as follows:0.4-20µg/kg (46.6%), 20.1-50µg/kg (17.2%), 50.1-100µg/kg (13.8%), 100.1-500µg/kg(20%), 500.1-1000µg/kg (1.6%) and 1000.1-1500µg/kg (0.5%).

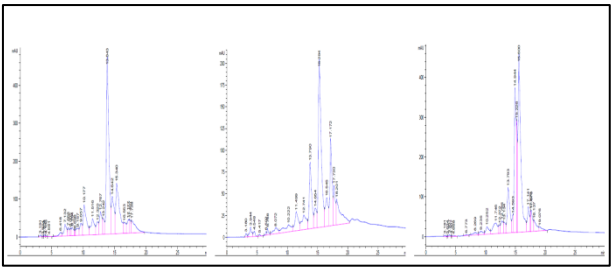

Figure 1. Area Percent of N-nitrosamines content

DISCUSSIONS:

The present study was designed to isolate the possible bacterial contaminants of RB and PB products, measure the residual nitrite content of PB products, and detect presence of nitrosamines.in PB products collected from four MPPs.

The significant rise of bacterial load in local slaughterhouses samples is the owners of slaughterhouses uncommitment to standard specifications and the poor slaughter, skinning and dressing methods, poor hygienic practice, improper and unhygienic handling of meat and meat products and bad sanitary operations and their failure to view that meat can be easily contaminated by aerial spores or bacterial spores carried in the air and several other insects, such as flies, which are uncountable at such sites, so also does dust particles from heavily contaminated atmospheres around market places and motor parks [6]. Also, one of the major sources of contamination arises from the handlers during preparation and display of meats for sale [37].

Meat surface is usually heavily contaminated with a wide range of microorganisms due to its high nutritional value (water, proteins, peptides, amino acids, nucleotides, sugars, minerals and vitamins). It is a suitable medium for the development of most bacteria [16].

The present study shows that members of twenty-three bacterial genera are associated with contaminated raw beef (Tables 1-4), while James M. Jay [38] mentioned that members of forty genera of bacteria were found to be associated with contamination of beef. In this study *Escherichia coli* was isolated from raw beef. This is similar to the finding of Abdissa, Haile [39] and Kitanov and Willms [40] who isolated the same bacterium from raw meat. *E. coli* have certain strains which are known to be pathogenic and some of them produce an enterotoxin that results in symptoms of abdominal pain and diarrhoea [21].

The isolation of *S. aureus, B. cereus, E. coli, K. pneumoniae*, and *P. aeriginosa*, is in agreement with [41] who had isolated the same organisms from raw meat.

Bradeeba and Sivakumaar [42] isolated *P. aeriginosa, P. vulgaris, B. cererus. Salmonella* spp*, K. pneumoniae, S. aureus* and *Acinetobacter* spp from beef, mutton and pork. Similar results are reported in this study.

Buncic and Panin [43] isolated *Aeromnas hydrophila* from meat products. This would have substantiated our result if identification of members of the *Aeromnas* genus was carried out the species levels. Similarly, isolation of the geneus Aeromnas bacteria was successfully done from various location of beef slaughtering process throughout [44].

Hussein [45] reported that the aerobic organisms were isolated from fresh meat such as members of the genera *Streptococcus, Micrococcus, Bacillus, Staphylococcus*, and *Lactobacillus*, besides *Diphtheriodes*. Similar results are reported in this study but disagree with his in the bacteria that belong to the genus *Lactobacillus* and the *diphtheriodes* (Brohothrix). Our results are also in line with Abdel-Mageed [46] who had isolated *Micrococcus* spp from Omdurman slaughterhouses. Moreover, they agree with Hamad [47] in the isolation of *E. coli*, and *S. aureus* from processed meat as well as substantiate the isolation of *E. coli* from frankfurter(turkey), beef roast and pasterma meat.

Hamad [47] examined 75 samples collected from three types of meat products from which 77 bacterial isolates were obtained. Fifty three percent of these isolates was S. aureus followed by *E. coli* (28%). Our findings agree with the latter study in six types of beef products; seventy-nine isolates were obtained from 40 PB products samples of which 82% were *S. aureus* and 32.5% *E. coli*.

This study doesn't agree with that of Tawfeek [48] who had isolated bacteria of *Salmonella-Shigella* group from cured meat (pasterma, beef Mortadella, smoked beef loaf and sausage) obtained from Jeddah market. In this study, neither Salmonella nor shigella could be isolated from all possessed beef products samples examined (burger, hotdog, mortedlla, pasterma, salami and sausage). However, it agrees with the findings of Hussein [45] and Amanie [49].

Sofos [50] isolated *E. coli* O157:H7 and *L. monocytogenes* from beef carcasses; this is in part similar to our finding sine identification of *E. coli* was not carried out the seerotyple level. The high bacterial loads present in local slaughterhouses could be due to improper sanitary procedures before and after slaughtering such as cleaning and sanitation, special personal hygiene of meat handlers and also checking of potable water quality and cleaning procedures for meat process line and meat contact surfaces.

Beef bacterial contamination is also attributed to improper storage which can increase the multiplication of bacteria of BP products, particularly sausages which are highly perishable products; a fact that will lead to fast microbial spoilage and oxidative rancidity. To avoid this problem, it must be stored immediately under freezing at -18 ºC [1].

This study investigated the levels of the residual sodium nitrite content of BP produced by four meat processing plants. Most of the meat processing plants in Khartoum are located in poor infrastructure industrial areas where sources of contamination such as dust, stagnant water, and traffic congestion are prevailing. Nevertheless, nitrite contents of one hundred samples of locally produced PB products are found to range from zero to 14.8 mg/kg. Accordingly, there is no safety concern about the nitrite content of these products as generally it showed not exceed 150 mg/kg in products [51, 52]. The nitrite lethal oral dose for human beings is established as 33-250 mg / kg live body weight. Nitrite residue content should be in compliance with FAO/WHO and the European Union recommendations. This is in agreement with Cockburn, Brambilla [53].

The Permissible limit for residual nitrites in meat products have been established worldwide. According to the processing condition of the meat product, it ranged from 40-100ppm (ESS/3597, 2005; ESS/3598, 2005; ISO 6635, 1984) [54, 55].

There is no convincing evidence that the residual amount of nitrite contributes to the microbiological safety of meat products. For example, in meat

products containing ascorbate (or isoascorbate/erythorbate) the residual nitrite content is very low and sometimes below the level of detection [56]. Furthermore, in the present study, 96.5% of the bacteria isolated from PB products were resistant to sodium nitrate, while 3.5% were sensitive. This leads to the conclusion that, in such a situation, sodium nitrate has no effect on preserving processed meat quality but it does contribute to its colour and flavor.

Consumption of red meat and processed meat has been associated with increased risk of stomach cancer [10], Pancreatic cancer [11] and colorectal cancer [9] besides an increase risk cardiovascular disease and other causes of death [12]. The latter authors have estimated that consumption of more than 20g of processed meat per day increases the mortality rate. The IARC has evaluated that NDMA, NDEA belong to the of probably carcinogenic to human and NDnBA, NPIP, and NPYR belong to the group pf possibly carcinogenic to human [57]. All thes live NA are detected in all PM products investigated in this study, Moreover, NPIP and NPYR were precent in high level. Level of less than 5µg/kg are considered low [58].

In a study conducted by M. Al-Kaseem [59] a low level of volatile NA could be detected in cured meat and canned meat. This is in agreement with our study because NA could also be detected in all samples collected from products produced by the four MPPs.

Herrmann [60] has found on her study that the formation and mitigation of NA and especially non-volatile (NVNA) NA in meat products is scarce and the present study is therefore a relevant contribution NA content of beef products she found high levels of NPIP, NDMA and NPYR in cooked pork sausages. Similar findings are reported on this study in all PB products samples for NPIP, NDMA and NPYR. It has been suggested that NA can be only found in over cooked or fried processed meat products which were previously cured with nitrite. Therefore , fresh meat for cooking and fresh burgers and sausages for frying , which don't contain nitrite but salt only , do not constitute a risk of NA formation in them [52].The current clearly demonstrate that NA formation can take place in uncooked ,unfried but cured sodium nitrate added to products and level of NA formation is assumed [60]. However, in addition to nitrites, the formation of NA which may be increased or reduced by some commonly used food additives, ingredients, heat treatment of fat content [60]. Calcium carbonate may also be used to migrate NA formation [61].

CONCLUSION:

Some of the isolated bacteria, from RB and PB products, in this study, are of public health importance; thus, their potential risk to humans as pathogenic bacteria and major causes of gastro-intestinal disorders, food poisoning and food-borne diseases should be considered.

In the light of the above-mentioned criterion,100% of the beef test samples collected from Khartoum are not in conformity to the bacteriological requirement according to International and Sudanese standard (SDS 4139/2010). On the other hand, 100% of the samples collected from the four plants revealed levels of residual nitrite matching with relevant laws and regulations of the European Union legislations which recommend that sodium nitrite (E.250) content should not exceed 150 mg/kg. They are also in conformity and identical to WHO/ FAO, (2007) requirement which state an

average of 50-100 ppm residual nitrite in processed meat products. In any case, the amount of residual nitrite in the finished product should not exceed 125 ppm. The maximum ingoing amount for processed meat products is normally up to 200 mg/kg of product. This value is conformable to ESS/3597.2005, ESS/3598.2005. Nevertheless; there is a risk in the addition of nitrites. This would result in the formation of the N-nitrosamines the main problem that appeared from this study and the control of most risk factors has not been commenced due to lack of oversight of food control authorities. Therefore, emergent problems need full commitment and participation by the state food control authorities because of presence of carcinogenic N-nitrosamines. The Maximum permissible level of sodium nitrite added to PB as preservative should be specified by the regulatory / legislative body of food control in Sudan.

Reference to the data generated from this study we recommended that, it is vitally important to recommend the following: (1) Establishment of a National Food legislative Authority, independent of the Ministry of Health and Ministry of Animal Resources, Fisheries and Rangelands, to monitor the hygienic state of slaughterhouses and meat processing plants and ensure their compliance with the international and national standards and specifications to reduce the levels of microbial contamination chemicals, drugs and insecticides residues in meats and meat products. (2)Enhancement of consumers awareness about risk of buying meat and meat product from butcheries or meat processing plants that are not complying with the above-mentioned international and national requirements. (3) Carrying out more studies on meat analysis (Chemical and bacteriological), in order to produce high quality food products and to decrease the possibility of acquiring food-borne diseases. (4) Usage of an up-to-date, high quality and advanced Methodology. (5) Carrying out further studies on N-nitrosamines in meat and meat products to provide essential and necessary data about their presence type's concentration, effects, other epidemiological aspects and control measures. (6) The concentration of nitrite should be included in the product label.

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Table S1. The biochemical reactions of isolated bacteria from raw beef samples.

Sample No	label	Gram stain	shape	motility	Growth in air	catalase	oxidase	glucose	*Carbohyd-rate	VP	Caogulase	Asculin hydrolysis	Growth at 15 c
1	a	+	Rod	-	+	+	-	+	F	ND	ND	+	+
	b	+	cocci	-	+	+	-	+	F	-	+	ND	ND
	c	+	cocci	-	+	+	-	+	F	-	+	ND	ND
	d	-	Rod	-	+	+	-	+	F	ND	ND	ND	ND
	e	+	cocci	-	+	+	-	+	F	+	ND	ND	ND
	f	+	cocci	-	+	+	-	+	F	-	+	ND	ND
	g	+	cocci	-	+	+	-	+	F	-	+	ND	ND
	h	-	Rod	-	+	+	+	+	F	-	ND	ND	ND
	i	+	cocci	-	+	+	+	-	o	-	ND	ND	ND
2	a	+	Rod	-	+	+	-	-	-	ND	ND	ND	ND
	b	-	Rod	-	+	+	-	+	F	ND	ND	ND	ND
	c	-	S\R	-	+	+	-	+	F	-	+	ND	ND
	d	+	Rod	-	+	+	-	-	-	ND	ND	ND	ND
	e	-	Rod	-	+	+	-	+	F	+	ND	ND	ND
	f	+	cocci	-	+	+	-	+	F	-	+	ND	ND
	g	-	rod	-	+	+	-	+	F	-	ND	ND	ND
	h	+	cocci	-	+	+	-	+	F	-	+	ND	ND
	i	-	rod	-	+	+	-	+	F	-			
	j	+	cocci	-	+	+	-	+	F	-			
	k	+	cocci	-	+	+	-	+	F	-			
	l	+	cocci	-	+	+	-	+	F	-			
3	a	+	rod	-	+	+	-	-	-	-			
	b	+	cocci	-	+	+	-	+	F	+			
	c	+	cocci	-	+	+	-	+	F	-			
	d	+	cocci	-	+	+	-	+	F	+			
	e	+	rod	-	+	+	-	-	-	-			
	f	+	cocci	-	+	+	-	+	F	+			
	g	+	cocci	-	+	+	-	+	F	-			
	h	+	cocci	-	+	+	-	+	F	+			
	i	-	rod	-	+	+	+	+	F	-			
	j	+	cocci	-	+	+	-	+	F	-			
	k	+	cocci	-	+	-	+	-	F	-			
4	a	-	rod	+	+	+	-	+	F				
	b	+	cocci	-	+	+	-	+	F	-			
	c	-	rod	-	+	+	+	+	F	-			
	d	+	cocci	-	+	+	-	+	F	+			
	e	+	cocci	-	+	+	+	-	o	-			
	f	+	cocci	-	+	+	-	+	F	-			
	g	-	rod	+	+	+	-	+	F				
	h	+	cocci	-	+	+	-	+	F	-			
	i	+	rod	-	+	+	-	+	o	-			
	j	+	cocci	-	+	+	-	+	F	-			
5	a	+	rod	-	+	+	-	-	-				
	b	-	rod	-	+	+	-	+	F	-			
	c	-	rod	-	+	+	-	+	F	-			
	d	-	rod	-	+	+	-	+	F	-			
	e	+	rod	-	+	+	+	-	o	-			
	f	+	rod	-	+	+	+	-	o	-			
	g	-	rod	+	+	+	+	+	F				
	h	-	rod	-	+	+	-	+	F	-			
6	a	-	rod	-	+	+	-	+	F				
	b	-	rod	-	+	+	-	+	F	-			
	c	-	rod	-	+	+	-	+	F	-			
	d	-	rod	-	+	+	-	+	F	-			
	e	-	rod	+	+	+	-	+	F				
	f	-	rod	-	+	+	-	+	F	-			
	g	-	rod	-	+	+	-	+	F	-			
	h	-	rod	-	+	+	+	+	F	-			
	i	-	rod	-	+	+	-	+	F				
	j	-	rod	-	+	+	-	+	F	-			
	k	+	cocci	-	+	+	-	+	F	+			
	l	-	rod	-	+	+	-	+	F	-			
7	a	-	rod	-	+	+	-	+	F	-			
	b	-	rod	-	+	+	-	+	F	-			
	c	-	rod	-	+	+	-	+	F	-			
	d	-	rod	-	+	+	-	+	F	-			
	e	-	rod	-	+	+w	-	+	F				
	f	-	rod	-	+	+	-	+	F	-			
	g	-	rod	-	+	+	-	+	F				
	h	-	rod	-	+	+	-	+	F	-			
8	a	-	rod	-	+	+	+	+	F	-			
	b	-	rod	-	+	+	+	+	F	-			
	c	-	rod	+	+	+	-	+	F				
	d	-	rod	+	+	+	-	+	F				
	e	-	rod	+	+	+	-	+	F				
	f	-	rod	-	+	+	-	+	F	-			
	g	-	rod	+	+	+	-	+	F				
	h	-	rod	+	+	+	-	+	F				
	i	+	rod	-	+	+	-	+	F			+	+
	j	+	cocci	-	+	+	-	+	F	-			
	k	+	cocci	-	+	+	-	+	F	-			
	l	+	cocci	-	+	+	-	+	F	-			
9	a	-	rod	-	+	+	-	+	F				

	b	+	cocci	-	+	+	-	+	F	-			
	c	+	cocci	-	+	+	-	+	F	-			
	d	-	rod	+	+	+	-	+	F				
	e	-	rod	+	+	+	-	+	F				
	f	-	rod	-	+	+	-	+	F	-			
	g	-	rod	+	+	+	-	+	F				
	h	-	rod	+	+	+	-	+	F				
	i	+	rod	-	+	+	-	+	F			+	+
	j	+	cocci	-	+	+	-	+	F	-			
	k	+	cocci	-	+	+	-	+	F	-			
	l	+	cocci	-	+	+	-	+	F	-			
9	a	-	rod	-	+	+	-	+	F				
	b	+	cocci	-	+	+	-	+	F	-			
	c	+	cocci	-	+	+	-	+	F	-			
	d	-	rod	-	+	+	+	+	F	-			
	e	+	cocci	-	+	+	+	-	o	-			
	f	+	rod	-	+	+	-	-	-				
	g	-	rod	-	+	+	-	+	F				
	h	-	S\R	-	+	+	-	+	F	-	+		
10	a	+	rod	-	+	+	-	-	-				
	b	-	rod	-	+	+	-	+	F	+			
	c	+	cocci	-	+	+	-	+	F	-			
	d	-	rod	-	+	+	-	+	F	-			
	e	+	cocci	-	+	+	-	+	F	-			
	f	+	cocci	-	+	+	-	+	F	-			
	g	+	cocci	-	+	+	-	+	F	-			
	h	+	cocci	-	+	+	-	+	F	-			
	i	-	rod	+	+	+	-	+	F				
	j	+	rod	-	+	+	-	-	-	-			
	k	+	cocci	-	+	+	-	+	F	+			
	l	+	cocci	-	+	+	-	+	F	-			

+=postive ; negative ; ND = Not done ; * Carbohyd-rate break down

Table S2. Bacterial species isolated from 40 PB products.

Meat Plants	Time/ week	No of Sample	Sample	Bacterial isolates
A	Week 1	1	Sausage	*Proteus vulgaris, E. coli, Staphylococcus aureus*
		2	Burger	*Proteus vulgaris, Staphylococcus aureus*
		3	Salami	*Proteus vulgaris, Staphylococcus aureus*
		4	Mortadella	*Proteus vulgaris, E. coli, Staphylococcus aureus*
		5	Hotdog	*Proteus vulgaris, Staphylococcus aureus*
		6	Mortadella	*Proteus vulgaris, Staphylococcus aureus*
		7	Burger	*Proteus vulgaris, Staphylococcus aureus*
		8	Hotdog	*Proteus vulgaris, E. coli, Staphylococcus aureus*
		9	Pasterma	*Proteus vulgaris, E. coli, Staphylococcus aureus*
		10	Sausage	*Proteus vulgaris, Staphylococcus aureus*
B	Week 2	11	Sausage	*Proteus vulgaris, Staphylococcus aureus*
		12	Burger	*Proteus vulgaris, Staphylococcus aureus*
		13	Salami	*Proteus vulgaris, Staphylococcus aureus*
		14	Mortadella	*Staphylococcus aureus*
		15	Hotdog	*Bacillus cerues*
		16	Mortadella	*Staphylococcus aureus*
		17	Burger	*E. coli, Staphylococcus aureus*
		18	Hotdog	Bacillus *cerues*
		19	Pasterma	*Staphylococcus aureus, Bacillus cerues*
		20	Sausage	*E. coli*
C	Week 3	21	Mortadella	*Staphylococcus aureus, Bacillus cerues*
		22	Burger	*Staphylococcus aureus, Psudomones aeuroginosa, Proteus vulgaris*
		23	Hotdog	*Staphylococcus aureus*
		24	Pasterma	*Staphylococcus aureus, Bacillus cerues*
		25	Sausage	*Staphylococcus aureus, Proteus vulgaris*
		26	Mortadella	*E. coli-Staphylococcus aureus, Bacillus cerues*
		27	Burger	*E. coli, Listeria moncytogenes*
		28	Hotdog	*Proteus vulgaris, Listeria moncytogenes*
		29	Pasterma	*Staphylococcus aureus, Listeria moncytogenes*
		30	Sausage	*Staphylococcus aureus, Proteus vulgaris*
D	Week 4	31	Mortadella	*Staphylococcus aureus, Bacillus cerues*
		32	Burger	*Staphylococcus aureus, Proteus vulgaris*
		33	Hotdog	*Staphylococcus aureus, Bacillus cerues*
		34	Pasterma	*Staphylococcus aureus, Bacillus cerues*
		35	Sausage	*Staphylococcus aureus - Proteus vulgaris*
		36	Mortadella	*Staphylococcus aureus*
		37	Burger	*E. coli, Staphylococcus aureus*
		38	Hotdog	*Staphylococcus aureus, Bacillus cerues*
		39	Salami	*Staphylococcus aureus, Listeria moncytogenus*
		40	Sausage	*E. coli, Staphylococcus aureus*
		Total		70 isolates

Table S3: Frequencies of isolation of bacterial isolates per location

Location	Percentages of Bacterial Isolates per Location																			
	Proteus vulgaris	*E. Coli*	*Staphylococcus aureus*	*Bacillus cereus*	*Pseudomonas aruginosa*	*Listeria monocytogenes*	*Salmonella spp*	*Aerococcus spp*	*Pasteurella multocida.*	*Micrococcus spp*	*Bordetella parapertussis*	*Acinetobacter spp.*	*Corynebacterium ovis.*	*Streptobacillus.spp*	*Klebsiella pneumoniae.*	*Kurthia spp.*	*Streptococcus* spp	*Hafnia.alvei*	*Nocardia asteroides*	*Alcaligenes faecalis*	*Rothia spp.*	*Haemophilus spp.*	*Aeromonas* spp.
A	40%	50%	50%	20%	10%	20%	70%	50%	50%	30%	30%	30%	30%	20%	10%	0%	0%	0%	0%	0%	0%	0%	0%
B	40%	50%	50%	10%	20%	10%	40%	50%	30%	20%	30%	20%	10%	10%	70%	30%	20%	0%	20%	10%	10%	10%	0%
C	50%	40%	60%	20%	0%	20%	40%	60%	60%	30%	20%	20%	20%	10%	80%	10%	20%	10%	10%	0%	0%	0%	0%
D	0%	0%	100%	0%	30%	20%	10%	90%	30%	80%	30%	50%	10%	10%	10%	50%	50%	0%	0%	0%	20%	30%	10%

Table S4. The frequencies of isolation of bacterial isolates from all RB samples examined

Location	**Frequency of isolation**																						
	Proteus vulgaris	*E. Coli*	*Staphylococcus aureus*	*Bacillus cereus*	*Pseudomonas aruginosa*	*Listeria monocytogenes*	*Salmonella* spp	*Aerococcus* spp	*Pasteurella multocida.*	*Micrococcus* spp	*Bordetella parapertussis*	*Acinetobacter spp.*	*Corynebacterium ovis.*	*Streptobacillus.spp*	*Klebsiella pneumonia.*	*Kurthia spp.*	*Streptococcus spp*	*Hafnia.alvei*	*Nocardia asteroides*	*Alcaligenes faecalis*	*Rothia spp.*	*Haemophilus* spp.	Aeromonas spp.
All slaughter-houses sampled	32.5%	35%	65%	12.5%	15%	17.5%	40%	62.5%	42.5%	40%	27.5%	30%	17.5%	12.5%	42.5%	22.5%	22.5%	2.5%	7.5%	2.5%	7.5%	7.5%	2.5%