PHENOTYPIC AND GENOTYPIC CHARACTERIZATION OF BACTERIAL CONTAMINANTS ISOLATED FROM EGYPTIAN PROCESSED MEATS

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ABSTRACT

A total of 60 Egyptian processed meat samples, categorized as 30 raw samples (10 every of frozen packaged ground beef, butchers' ground beef and frozen packaged beef sausage) besides 30 cooked samples (10 each of fried beef sausage, beef luncheon and fried hamburger) purchased from supermarkets, butchers' shops and restaurants in Mansoura city-Egypt, were subjected for both phenotypic and genotypic bacteriological analyses. The former analysis was done at the laboratory of Food Hygiene and Control Department, Faculty of Veterinary Medicine, Mansoura University, Egypt, while the latter works were conducted at the Department of Bacterial Infections, Research Institute for Microbial Diseases, Osaka University, Osaka, Japan.

Plates of plate count agar revealed the presence of aerobic mesophiles in the tissues of all surveyed raw and cooked samples. Whilst the Enterobacteriaceae organisms were detected in 30%-80% of raw processed meat samples, besides 30% every of beef luncheon and fried hamburger samples, meanwhile fried beef sausage samples, the violet red bile glucose agar plates could not detect such organisms in their tissues. Concerning the occurrence of coagulase-positive Staphylococcus aureus organisms, the plates of Baird-Parker agar combined with coagulase test (tube method) showed 30%, 80% and 60% incidence of this organism in frozen packaged ground beef, butchers' ground beef and frozen packaged beef sausage, in addition to 30%, 40% and 20% were obtained in fried beef sausage, beef luncheon and fried hamburger samples, respectively. Furthermore, the tissues of raw processed meats exhibited the presence of Bacillus cereus organisms by a prevalence of 60-90%, whereas, cooked processed ones possessed 50-90%, after their suspension being inoculated onto the dried surface of plates of mannitol egg-yolk polymyxin agar.

The aforementioned agar plates showed the bacterial counts per gram of surveyed Egyptian raw and cooked processed meats as 10^5 - $2.8x10^9$ *with a mean of* $1.3x10^8$ - $8.5x10^8$ *and* $3x10^4$ - $7.2x10^7$ *with an average of* $4.7x10^6$ - $2.6x10^7$ *aerobic mesophilic organisms;* 10^4 - $2.6x10^6$ *with a mean of* $1.3x10^4$ - $5.8x10^5$ *and* $5x10^2$ - $7x10^3$ *with an average of* $2.8x10^3$ - $4.1x10^3$ *Enterobacteriaceae organisms;* $1.6x10^2$ - $2x10^5$ *with a mean of* $8.5x10^2$ - $6x10^4$ *and* 10^2 - $1.5x10^4$ *with an average of* $2.4x10^2$ -

$6.8x10^3$ S. aureus "coagulase-positive" organisms; besides $6x10^2$ - $5.1x10^5$ with a mean of $1.2x10^4$ - 10^5 and 10^3 - 1.1_10^5 with an average of $1.2x10^4$ - $5.3x10^4$ B. cereus organisms, consecutively.

Microbiological risk assessment of tested processed meats, through comparing different bacterial populations contained in their tissues with the corresponding recommended limits resulted in 90-100% of cooked (ready-to-eat) processed meat samples, in addition to 40 - 70% of raw ones exceeded the recommended limits of aerobic mesophiles (10^4 - 10^5 organisms per gram for cooked and 10^7 organisms per gram for raw meats). None of the cooked meats was contaminated with Enterobacteriaceae organisms by levels more than the recommended limit (10^4 per gram). Additionally, out of the examined cooked processed meats, 40% beef luncheon and 10% fried hamburger besides none of fried beef sausage samples contained S. aureus "coagulase-positive" organisms by populations more than the recommended limit (10^3 per gram), on the other hand, the analyzed raw meats exhibited 30% frozen packaged beef sausage, 20% butchers' ground beef and none of frozen packaged ground beef samples harbored the same organisms by levels more than the recommended limit (10^4 per gram). Finally, 10% samples each of beef luncheon and fried hamburger were contaminated with B. cereus organisms by levels more than the recommended limit ($<10^5$ per gram), while none of fried beef sausage samples exceeded such limit.

A sum of 67 bacterial strains, isolated from both raw and cooked processed meat samples, distributed as 13 strains recovered from frozen packaged ground beef, 16 from butchers' ground beef and 25 from frozen packaged beef sausage, in addition to 2 from fried beef sausage, 8 from beef luncheon besides 3 strains from fried hamburger samples. Phenotypic (conventional) and genotypic (16S rRNA gene sequencing) analyses of these bacteria identified them as 13 Escherichia coli; 7 Enterobacter hormaechei; 8 strains each of Enterobacter cloacae and Pseudomonas aeruginosa; 5 Enterobacter sakazakii; 3 strains every of Enterobacter aerogenes, Enterococcus faecalis and Pseudomonas stutzeri; 2 strains each of Bacillus cereus, Bacillus licheniformis, Bacillus subtilis, Citrobacter freundii and Enterococcus faecium; besides one strain every of Enterobacter asburiae, Pantoea agglomerans, Proteus mirabilis, Serratia marcescens, Staphylococcus aureus, Staphylococcus cohnii and Staphylococcus xylosus.

INTRODUCTION

Processed meats are considered an excellent source of high quality proteins containing a good balance of essential amino acids and having a high biological value, a good source of most B-complex vitamins and also contribute significant levels of minerals including iron, copper, zinc, sodium, potassium and magnesium.

Risk assessment denotes the scientific evaluation of known or potential adverse health effects resulting from human exposure to foodborne hazards. Risk from microbiological hazards is of immediate and serious concern to human health. One of the difficulties associated with microbiological risk assessment is in determining the number of microorganisms in food at given time, i.e. estimating the exposure of an individual to the microorganism. The numbers of bacteria in food can be changed at all stages of food production and processing depending on the nature of the food and the way it is handled, stored and processed **(Walls and Scott, 1997)**.

Accurate identification of bacterial isolates is an essential task for microbiological laboratories. Traditional phenotypic identification is difficult and time-consuming, and when phenotypic methods are used to identify bacteria, interpretation of test results can involve a substantial amount of subjective judgement requires the recognition of differences in morphology, growth, enzymatic activity, and metabolism to define genera and species. Phenotypic variability among strains belonging to the same species also results in some bacterial isolates presenting characteristics that are atypical for identification. To get around the pitfalls of the conventional methods, identification techniques based on nucleic acid amplification may offer a good alternative. Full and partial 16S rRNA gene sequencing methods have emerged as useful tools for identifying phenotypically aberrant microorganisms as it is a more objective identification tool, unaffected by phenotypic variation or technologist bias, and has the potential to reduce laboratory errors **(Petti et al., 2005)**.

Therefore, the overall objectives of this work were intended to assess the microbiological risk in some popular Egyptian processed meats comprising ground beef, beef sausage, hamburger and beef luncheon through: (1) estimating the total bacterial counts of aerobic mesophiles, Enterobacteriaceae, *S. aureus* "coagulase-positives" and B. cereus per each gram of the examined processed meats and (2) accurate identification of isolated bacteria by using 16S rRNA gene sequencing.

MATERIALS AND METHODS

A total of 60 Egyptian processed meat samples, categorized as 30 raw samples (10 every of frozen packaged ground beef, butchers' ground beef and frozen packaged beef sausage) besides 30 cooked samples (10 each of fried beef sausage, beef luncheon and fried hamburger) purchased from supermarkets, butchers' shops and restaurants in Mansoura city, Egypt. Each sample was approximately represented by 100 grams. Each of all samples was aseptically packed into a polyethylene bag then marked and transferred -in icebox with a minimum of delay- to the laboratory of Food Hygiene and Control Department, Faculty of Veterinary Medicine, Mansoura University, wherein the preliminary bacteriological analyses were done.

[A] Preliminary bacteriological analyses (in Egypt):

Ten grams from each processed meat sample were homogenized with 90 ml of 0.1% sterile peptone water (Oxoid CM0009) for 1 min in a laboratory blender for obtaining an original dilution of 1:10, from which serial dilutions were prepared (AOAC, 1990), for the following analyses:

(1) Aerobic plate count (ICMSF, 1978):

A tenth ml from each prepared serial dilution was transferred and evenly spread over a dry surface of duplicated, previously prepared sterile plate count agar medium (Oxoid CM0325). The surface of inoculated plates was allowed to dry for 15 min before being placed inverted with control plates in the incubator adjusted at 30oC for 48 h. The bacterial colonies were enumerated and the aerobic plate count per gram of the examined sample was then calculated and recorded.

(2) Enterobacteriaceae count (ISO, 1993a):

Duplicated sets of sterile Petri dishes were inoculated with 1-ml amounts of the chosen range of prepared dilutions. A quantity of about 15ml of violet red bile glucose agar (Oxoid CM0485), melted and cooled to 45°C, was added to each inoculated Petri dish, then mixed well and allowed to set. Another 5 ml of the same agar/temperature was finally overlain every plate, which left to solidify, then incubated at 30°C for 24 h. Typical colonies of Enterobacteriaceae (red surrounded by precipitation of bile salts in the medium and having 0.5 mm or more in diameter) were enumerated and the Enterobacteriaceae count per gram of the examined sample was calculated and recorded.

(3) *Staphylococcus aureus* "coagulase-positive" count (AOAC, 1984):

From the previously prepared serial dilutions, 0.2 ml from selected dilutions were transferred and evenly spread onto dried surfaces of duplicate plates of Baird-Parker selective agar (Oxoid CM0275) with egg-yolk tellurite emulsion, then incubated at 37°C for 48 h. Colonies exhibiting typical morphology, grey-black to jet-black, circular, smooth, convex, 2-3 mm in diameter with a narrow white entire margin and may show an opaque zone surrounded by a zone of clearing extended 2-5 mm in the opaque medium, were considered a presumptive *S. aureus*. The top part of five suspected colonies was picked up and inoculated into test tubes containing 5 ml of sterile brain heart infusion broth (Oxoid CM0225) then incubated at 37°C for up to 24 h for biochemical confirmation and coagulase test then the coagulase-positive S. aureus count per gram of the examined sample was calculated and recorded.

(4) *Bacillus cereus* count (ISO, 1993b):

From each prepared serial dilutions, 0.1 ml was aseptically transferred and evenly spread onto dried surfaces of duplicate plates of sterile mannitol egg-yolk polymyxin agar (MYP, Oxoid CM0929) (Polymyxin B supplement, Oxoid SR0099E) then incubated at 30°C for 24-48 h. The typical colonies (dry, rough surface with a pink to purple base and surrounded by a ring of dense precipitate) were enumerated. The typical colonies were picked up and spread on nutrient agar slopes then incubated at 37°C for 24 h for confirmation then B. cereus count per gram of the examined samples was calculated and recorded.

[B] Confirmatory bacteriological analyses by genotyping "16s rRNA gene sequencing" (in Osaka/Japan):

These analyses were taken place at Department of Bacterial Infections, Research Institute for Microbial Diseases, Osaka University, Osaka, Japan, for accurate identification of the whole aforementioned bacterial strains - isolated from the surveyed Egyptian processed meats- by the aid of reference strains of *Cronobacter sakazakii* (RIMD0377001). A sum of 67 different bacterial strains, picked up from the different agar plates after being recovered from the tissues of surveyed samples of both raw and cooked processed meats, distributed as 13 strains recovered from frozen packaged ground beef, 16 from butchers' ground beef and 25 from frozen packaged beef sausage, in addition to 2 strains each of fried beef sausage, 8 from beef luncheon besides 3 strains from fried hamburger samples, were analyzed by partial 16S rRNA gene sequencing **(Hall et al., 2003)** using 16S-1 primer (5΄ - CAGGAAACAGCTATGACCGSITRAIRCA TGCAAGTCG-3΄) and 16S-2 primer (5΄-TATTACCGCRGCTGCTGG-3΄), by the aid of DNA thermal cycler [GeneAmp® PCR System 9700 (Applied Biosystems, USA)]. In a 200µl-PCR tube, a total of 25µl PCR mixture consisted of 14.75µl distilled water, 5µl 10x PCR buffer, 2.5µl deoxyribonucleotide triphosphate (dNTPs) mixture, 0.25µl each of, 2µl DNA template (extracted from each strain) and 0.25µl ExTaq DNA polymerase (Takara, Japan), were placed then subjected for a temperature program involved the initial denaturation of the DNA template at 94°C for 2 min, followed by 30 cycles; every of them comprised the denaturation at 94°C for 30 s, primer annealing at 55°C for 30 s and synthesis of complementary chain at 72°C for 45 s, then ended by an additional extension at 72°C for 5 min. The amplified DNA fragments or amplicons (about 770 base pairs) were subjected to electrophoresis in 1.5% Agarose gel, stained for 30 min in ethidium bromide solution (0.5µg/ml), viewed under a UV Transilluminator having a wave length of 302nm (BioDoc-It Systems) (Figure,1); then each DNA fragment, was excised with its agarose gel and subjected for purification using QIAquick Gel Extraction Kit (Qiagen, Germany) then subjected for nucleotide sequencing by the aid of ABI Prism 3100 DNA Sequencer (Applied Biosystems, USA) according to standard protocol for cycle sequencing. The resultant partial 16S rRNA gene sequences were compared with those available in the online GenBank database.The mean length of the sequences was 700± 60 nucleotides. Identification of 67 bacterial isolates into species level was defined as a 16S rRNA sequence similarity of ≥99% with that of the prototype strain sequence in GenBank, whilst genus-level identification was defined as a 16S rRNA sequence similarity of ≥97% with that of the prototype strain sequence in GenBank. A failure to identify was defined as a 16S rRNA sequence similarity score of lower than 97% with those deposited in GenBank **(Stackebrandt and Goebel, 1994)**.

The data obtained in this study were statistically analyzed according to methods described by **Snedecor (1971)**.

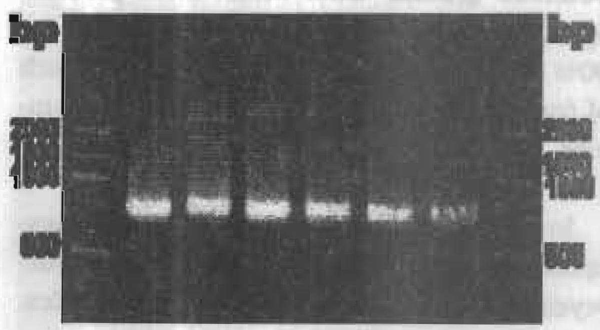

Figure (1): Agarose gel electrophoresis of polymerase chain reaction products obtained using DNA extracted from pure cultures identified using partial (770 bp) 16S rRNA gene sequencing. lane M: DNA 100 bp marker, lane 1: positive control (Cronobacter sakazakii reference strain RIMD 0377001), lane 2-6: Different bacterial strains obtained from processed meats.

RESULTS & DISCUSSION

Presence of different bacterial populations in tested both 3 types of raw and 3 types of cooked Egyptian processed meats (10 samples for each type) was described in Table (1) that denotes the contamination of all surveyed raw and cooked samples with aerobic mesophiles (100% each), whilst the occurrence of Enterobacteriaceae organisms were represented by all types of raw meats where found in 80% frozen packaged beef sausage, 60% butchers' ground beef and 30% frozen packaged ground beef samples, meanwhile such occurrence was only limited to 30% samples each of beef luncheon and fried hamburger among the surveyed types of cooked meats. Similarly, the coagulase-positive organisms of *S. aureus* were detected in raw meats by extremely higher prevalence than that found in cooked ones, as 80% butchers' ground beef, besides 60% and 30% in frozen packaged beef sausage and frozen packaged ground beef samples, respectively, while this bacterial contamination was restricted to 40% beef luncheon, 30% fried beef sausage and 20% fried hamburger samples. Additionally, the percentages of *B. cereus*-contaminated samples of raw meats were moderately higher than those of cooked ones, as represented for raw meats by 90% each of frozen packaged ground beef and frozen packaged beef sausage, besides 60% of butchers' ground beef, whilst these percentages for cooked meats were represented by 90% fried hamburger in addition to 50% each of fried beef sausage and beef luncheon samples.

Inevitable contamination with aerobic mesophilic bacteria, detected in all tissues of surveyed both raw and cooked meat samples, can be explained by the literatures of **Dickson and Anderson (1992)** who emphasized that carcass surfaces often become heavily contaminated during dressing, even with using a current slaughterhouse technology, in addition to the declaration of **Doyle et al. (2001)** which mentioned that the microorganisms in processed meats originate not only from the meat itself, but also from the non-meat ingredients like spices, fillers and salts. The numbers and percentages of both Enterobacteriaceae- and *S. aureus*- contaminated raw samples were almost equal as well as fewer than that of *B. cereus*-contaminated raw ones; these findings may be attributed to the multiple sources of processed meats contamination with the latter organisms as **Varnam and Evans (1991)** mentioned that the meat additives -like spices and fillers- contribute in the high incidence of processed meats contamination with *B. cereus* organisms. Viewing the Enterobacteriaceae and *S. aureus* contaminants in both types of surveyed ground beef samples, they were detected in more -manually prepared- butchers' ground beef samples than that determined in frozen packaged ground beef; these results agree with literature of **Dworkin et al. (2006)** which emphasized the food handlers -particularly those having infected cuts and sores- besides utensils, air, soil and water are considered among the major sources of meat contamination with these organisms during manufacturing, packaging and marketing. Furthermore, the occurrence of non-sporeforming Enterobacteriaceae and *S. aureus* organisms in cooked samples was clearly lower than that detected for sporeforming *B. cereus* organisms in the same samples; these results is expected owing to the high thermal resistance of bacterial spores against cooking temperatures **(Frazier and Westhoff, 1988)**.

Comparing the incidence of different bacterial populations -in raw processed meats- obtained in this work with those determined by other workers, higher incidence of Enterobacteriaceae organisms (100%) were detected in both ground beef and sausage samples by **Lotfi et al. (1986) and Oteiza et al. (2006)**, in addition to *S. aureus* "coagulase-positive" organisms were recovered from 24.7%–38.3% ground beef samples **(El-Gohary, 1993 and Kaldes et al., 1994)**, also **Abd El-Aziz (1987)** recognized such organisms in 75% of ground beef samples, nearly similar to that obtained in surveyed butchers' ground beef. Higher prevalence of *S. aureus* organisms in raw fresh and ground beef samples were reported by **Roushdy et al. (1983)** as 100% and in raw sausage samples by **El-Nawawy and Nouman (1981)** as 76%, whilst lower incidence of such contaminants were estimated by **Abd El-Monem (1998) and Malicki and Bruzewicz (2005)** in raw ground beef as 0.8%-20%, as well as by **El-Gohary (1993), Ouf (2001) and Hamouda (2005)** in raw sausage as 10-48%, besides, **Youssef et al. (1985) and Gergis (2005)** who could detect these organisms in 60% and 47% of raw ground beef samples, consecutively; both findings were higher than those obtained in frozen packaged ground beef but lower than found in butchers' ground beef samples, however **Oteiza et al. (2006)** could not isolate such contaminants from 100 Argentina raw sausage samples.

Concerning the percentages of *B. cereus*-contaminated samples among raw processed meats, detected by other researchers in relation to those calculated in the present study, almost equal percentages of contaminated ground beef samples -similar to that obtained in frozen packaged ground beef- (72% and 74%) were estimated by **Eldaly et al. (1988) and Lotfi et al. (1988)**, respectively as well as similar to that found in butchers' ground beef (52%-58%) were reported by **El-Sayed et al. (1999), Hassan (2001) and Hamouda (2005)** in addition to approximately identical *B. cereus*-contaminated samples of raw sausage (80% and 84%) were evaluated by **El-Ghamry (2004) and Hamouda (2005)**, whilst lower percentages of *B. cereus*-contaminated samples were recognized by **Hafez et al. (1990) and Hassan (1991)** as 5.75%-35% in both raw fresh and ground beef; by **Eldaly et al. (1988), Nortje et al. (1999), and El-Mossalami (2003)** as 9.8%-60% in raw sausage; however, **Nortje et al. (1999)** could not detect B. cereus organisms in 51 ground beef samples.

The prevalence of different bacterial populations in tested samples of cooked processed meats was at the top (100%) for aerobic mesophiles, followed by 50%-90% samples for *B. cereus*, then coagulase-positive organisms of S. aureus (0%-40% samples) and Enterobacteriaceae (20%-80% samples). These findings agreed with those reported in many literatures; as **ICMSF (1978) and Kiss (1984)** mentioned that the high incidence of bacterial contamination in processed meats indicate heavily contaminated raw materials and/or unsanitary processing besides improper time-temperature storage conditions. Furthermore, representation of Enterobacteriaceae contamination by 30% of examined samples of both beef luncheon and fried hamburger can be explained by the declaration of **Doyle et al. (2001)** who emphasized that some members of Enterobacteriaceae organisms can survive heat treatment of foods. Also, presence of *S. aureus* organisms in cooked meat samples indicates a poor sanitation of such meats because those organisms are highly vulnerable to destruction by heat treatment and approximately all sanitizers (FDA, 1998). S. aureus-contaminated samples in ground beef (55.6%) were estimated by **Tavakoli and Riazipour (2008)**, whereas lower contaminated samples (8%-15%) were evaluated in beef luncheon by **(Abd El-All, 1993; Ouf, 2001 and Hamouda, 2005)**, however, **Hemeida et al. (1986)** could not recover these S. aureus organisms from any sample of locally-manufactured beef luncheon. Furthermore, almost equal B. cereus-contaminated samples were obtained by **Lotfi et al. (1988)** as 48% of beef luncheon, meanwhile higher contaminated samples (70% and 80%) of beef luncheon were recognized by **El-Ghamry (2004) and Hamouda (2005)**, respectively besides lower contaminated samples (22% and 48%) of ready-to-eat hamburger were determined by **Shinagawa et al. (1985) and Ahmed (1991)**, although Ouf (2001) could not find *B. cereus* organisms in any sample of beef luncheon.

Intensities of four bacterial populations, estimated in tissues of examined both raw and cooked processed meat samples, were arranged in Table (2) and exhibit the range (minimum-maximum) with mean value ± standard error of aerobic plate counts (APC) in raw meats as $9x10^5$-$2x10^9$ and 10^5-$7x10^8$ with mean values of $2.7x10^8 \pm 1.9x10^8$ and $1.3x10^8 \pm 0.78x10^8$ organisms per gram in frozen packaged and butchers' ground beef, whilst these findings were $2x10^5$-$2.8x10^9$ with mean value of $8.5x10^8 \pm 4.4x10^8$ organisms per gram in frozen packaged beef sausage, respectively. These levels were also estimated in cooked meats as $3x10^4$-$6.7x10^7$ with a mean of $1.4x10^7 \pm 0.75x10^7$ organisms per gram in fried sausage, $2x10^5$-$1.6x10^7$ with a mean of $4.7x10^6 \pm 1.6x10^6$ organisms per gram in beef luncheon, besides $9x10^5$-$7.2x10^7$ with a mean of $2.6x10^7 \pm 0.79x10^7$ organisms per gram in fried hamburger samples, successively. Viewing the aforementioned mean counts reveals the aerobic plate counts were found in tissues of cooked (ready-to-eat) processed meats by lower levels than those detected in raw ones; these findings can be explained by the literature of **Pearson and Gillett (1997)** who emphasized that the cooking of processed meats causing destruction a lot of microorganisms in their tissues by a number depend upon the time and temperature relationship. By comparison, **Abd El-Aziz (1979 & 1987) and Ambrosiadis et al. (2004)** evaluated the mean values of aerobic plate counts in raw sausage samples by $1.2x10^8$-$3.4x10^8$ organisms per gram; almost similar to those found in this work, whilst lower APC mean values ($3.55x10^3$-$4x10^5$ organisms per gram) determined in raw ground beef by **Hamouda (2005) and Malicki and Bruzewicz (2005)** as well as in raw sausage (<102-10^8 organisms per gram) by **El-Nawawy and Nouman (1981), Rheinbaben and Hadlok (1984), El-Khateib (1997) and Oteiza et al. (2006)**. On the other hand, approximately similar APC mean values in beef luncheon (>106 and $1.7x10^6$ organisms per gram) were estimated by **Duitschaever (1977) and Gab-Allah (1990)**, respectively, whilst lower APC mean values were found in ready-to-eat hamburger ($2x10^2$-$1.8x10^3$ organisms per gram) by **Soliman et al. (2002)**, as well as in beef luncheon (<102–$9x10^5$ organisms per gram) by **Aiedia (1995)**, **Ouf (2001) and Hamouda (2005)**.

Plates of violet red bile glucose agar estimated the counts of Enterobacteriaceae organisms in tissues of tested raw meats as ranges of 10^4-$3x10^4$ and $3x10^4$-$2.6x10^6$ with mean values of $1.3x10^4 \pm 0.87x10^4$ and $5.8x10^5 \pm 4.1x10^5$ organisms per gram in frozen packaged and butchers' ground beef and 104-$2x10^6$ with mean of $3.7x10^5 \pm 2.3x10^5$ organisms per gram in frozen packaged beef sausage samples, successively, whereas the same plates could only detect these contaminants in both beef luncheon and fried hamburger samples, among the tested cooked meats, by ranges of $5x10^2$–6_103 and 10^3-$7x10^3$ with mean of $2.8x10^3 \pm 1.6x10^3$ and $4.1x10^3 \pm 1.7x10^3$ organisms per gram, respectively (Table, 2). Enterobacteriaceae organisms, enumerated in both examined raw and cooked meats, reflect the contamination of their raw materials -comprising fresh meat, fillers and spices- with the intestinal material **(Kiss, 1984 and Doyle et al., 2001)**. Also, detection and counting such enteric organisms in cooked meats like beef luncheon and fried hamburger denote inadequate cooking temperature and/or post-processing contamination. By comparison, **Lotfi et al. (1986) and Oluwafemi and Simisaye (2006)** estimated the mean value and ranges of Enterobacteriaceae counts in raw samples of both ground beef and sausage by levels of $9x10^4$ and $1.57x10^6$-$5.09x10^8$ organisms per gram, consecutively; higher than those recognized in present study, whereas lower intensities of Enterobacteriaceae populations in raw sausage samples of $9.1x10^4$ and $2x10^2$-$1.1x10^5$ organisms per gram were detected by **Lotfi et al. (1986) and Oteiza et al. (2006)**, successively.

Inspection of Table (2) reveal the contamination levels of *S. aureus* "coagulase-positive" organisms in both raw and cooked meat samples; these levels were represented in raw samples by ranges $1.6x10^2$-$1.4x10^3$ and $4x10^2$-$3.5x10^4$ with mean of $8.5x10^2 \pm 3.6x10^2$ and 9.9_$10^3 \pm 4.8x10^3$ organisms per gram in frozen packaged and butchers' ground beef and 10^3-$2x10^5$ with mean of 6_$10^4 \pm 3.1$_10^4 organisms per gram in frozen packaged sausage samples, successively, whilst these values in tested cooked meats were 10^2-$5x10^2$ and $2.3x10^3$-$1.5x10^4$ with mean of $2.4x10^2 \pm 1.3x10^2$ and 6.8x103$\pm 2.9x10^3$ organisms per gram in fried beef sausage and beef luncheon besides $5x10^2$-3_103 with mean of $1.8x10^3 \pm 1.2x10^3$ organisms per gram in fried hamburger samples, respectively. Several researchers could obtain coagulase-positive organisms of *S. aureus* in raw meats by counts nearly similar to those estimated in this work, where **Abd El-Aziz (1987) and Hamouda (2005)** evaluated the mean counts of such organisms in raw ground beef by $6x10^2$ and $4x10^3$ organisms per gram, consecutively, while this value was evaluated as $3.6x10^4$ organisms per gram in raw sausage by **Mousa et al. (1993)**, whilst higher intensities of those organisms were recovered from raw ground beef as a range of $1.5x10^3$-$1.2x10^5$/g by **Roushdy et al. (1983) and Kaldes et al. (1994)** as well as from raw sausage as a range of $1.8x10^5$-$2x10^7$/g by **Abd El-Aziz (1987) and Oluwafemi and Sim-**

isaye (2006), although lower populations of the same organisms were determined in raw ground beef as a range of 0.3-2.8x10/g by **Hassan (2001) and Minematsu et al. (2006)** besides from raw sausage as a range of <10-1.8x10^3/g by **Sumner et al. (1979), El-Mossalami (2003) and Hamouda (2005), however Oteiza et al. (2006)** could not isolate these organisms from 100 samples of Argentina raw sausage. Similarly, as well as in beef luncheon as a mean of 5.5x10^3 organisms per gram by **Gab-Allah (1990)**, although higher contamination levels of such organisms were evaluated as a range of 7.91x10^2-1.8x10^3/g in ready-to-eat sausage by **Soliman et al. (2002)**, meanwhile lower contamination as a mean of 2x10^2 organisms per gram were estimated by both **Tolba (1994) and Hamouda (2005)** in beef luncheon. The aforementioned processed and ready-to-eat meats that contaminated with coagulase-positive organisms of *S. aureus* represent a significant health hazard, because microbes that normally compete them have been eliminated. Improper storage temperature of such meats also allows staphylococci multiply soon after being introduced into the meats. The enterotoxins produced during cell growth generally do not affect the sensory characteristics of the contaminated meats and may therefore go unnoticed **(Jablonski and Bohach, 2001)**.

Plates of mannitol egg-yolk phenol red polymyxin agar showed the levels of *B. cereus* contamination in tested both raw and cooked processed meat samples as ranges of 6x10^2-5x10^5 and 5x10^3-3x10^4 with mean of 6.8x10^4± 5.4x10^4 and 1.2x10^4±0.41x10^4 organisms per gram in frozen packaged and butchers' ground beef besides 6x10^3-5.1x10^5 with mean of 10^5±0.52x10^5 organisms per gram in frozen packaged beef sausage samples, respectively, for raw meats (Table, 2). Comparing the obtained results with those determined by other workers, approximately identical counts of B. cereus organisms in raw ground beef (10^3-4x10^5 organisms per gram) were estimated by **Eldaly et al. (1988) and El-Ghamry (2004)** as well as in raw sausage (10^4-10^5 organisms per gram) by **Torky (1995)**, successively, whereas higher *B. cereus* counts in raw ground beef were detected by **Hafez et al. (1990)** as a mean of 1.8x10^5 organisms per gram, in raw sausage by **Lotfi et al. (1988) and El-Ghamry (2004)** as mean levels of 8.79x10^5-10^6 organisms per gram, consecutively, although lower *B. cereus* populations were recovered in raw ground beef by **Lotfi et al. (1988) and Hamouda (2005)** as mean counts of 2x10^2 -2x10^3 organisms per gram, in raw sausage by **Soliman (1988), Hassan (2001), and Hamouda (2005)** as mean counts of 1.5x10^3 -3.3x10^4 organisms per gram.

Using of the same aforementioned bacteriological analysis, for enumerating the *B. cereus* organisms in tested cooked meats, resulted in their detection by counts ranged from 103-3x10^4, 1.4x10^4 -10^5 and 3x10^3-1.1x10^5 with mean values of 1.2x104 ± 0.51x10^4, 5.3x10^4 ± 1.4x10^4 and 2.7x10^4±1.1x10^4 organisms per gram in cooked tissues of fried beef sausage, beef luncheon and fried hamburger, respectively (Table, 2). Detailed inspection of the obtained mean values of B. cereus counts in tested cooked (ready-to-eat) meats, exhibit that beef luncheon samples contained the highest populations of these organisms whilst the lowest intensities were found in the tissues of fried beef sausage, whereas fried hamburger samples harbored moderate intensity of *B. cereus* populations. Similarly, almost equal *B. cereus* contaminants were detected by **Nassif et al. (2002)** in grilled (ready-to-eat) sausage as a range of $4x10^3$-$3x10^4$ organisms per gram, also by **Ahmed (1991) and El-Sherif et al. (1991)** in cooked hamburger as mean levels of 8.3x104 and 3x104 organisms per gram, respectively, as well as by **Lotfi et al. (1988) and El-Ghamry (2004)** in beef luncheon as mean levels of $6x10^5$ and $6.23x10^5$ organisms per gram, consecutively, whereas lower contamination levels of B. cereus organisms were obtained in cooked sausage by **Soliman et al. (2002)** as a range of $5.6x10^2$-$1.3x10^3$, in ready-to-eat hamburger by **Nassif et al. (2002) and Soliman et al. (2002)** as ranges of $6x10^2$-10^4 and $3x10^2$-$3.2x10^2$ organisms per gram, respectively. General view on the intensities of the all four bacterial populations in the tissues of both raw and cooked meats, reveal the highest bacterial populations (represented by aerobic mesophiles) in both raw and cooked sample, followed by Enterobacteriaceae organisms in raw meats then *B. cereus* organisms in both meats succeeded by coagulase-positive organisms of S. aureus in raw meats.

Results in Table (3) assess the microbiological risk of the surveyed cooked samples of Egyptian processed meats, through comparing the obtained intensities of different bacterial contaminants in their tissues with those limits recommended by **Gilbert et al. (2000)** as 100% each of beef luncheon and fried hamburger samples were contaminated with aerobic mesophiles by APC levels more than the corresponding limit (10^4 organisms per gram) in addition 90% of fried beef sausage samples showed the same contaminants by counts exceeded the specified limit (10^5 organisms per gram), on the contrary, none of the tested cooked samples contained Enterobacteriaceae organisms by levels exceeded the related limit (10^4 organisms per gram), however, only 40% of beef luncheon besides 10% of fried hamburger samples were among the cooked meat samples that harbored the coagulase-positive organisms of *S. aureus* by more numbers than the corresponding limit (10^3 organisms per gram), finally, the *B. cereus*-contaminated samples that possessed higher levels of organisms than specified limit ($<10^5$ organisms per gram) were restricted to10% every of beef luncheon, fried hamburger and samples. Similar microbiological risk assessment for examined raw meats was also carried out, after comparing their different bacterial contamination levels with the corresponding limits stated by **ICMSF (1986)**, as 70% of frozen packaged ground beef, 60% of frozen packaged beef sausage besides 40% of butchers' ground beef samples were harbored the aerobic mesophiles by APC values exceeded the specified limit (10^7 organisms per gram), whereas 50% of frozen packaged hamburger, besides 30% and 20% of frozen packaged beef sausage and butchers' ground beef samples, consecutively were contaminated by more organisms of *S. aureus* "coagulase-positive" than the recommended limit (10^4 organisms per gram). Microbiological risk assessment of surveyed raw meats, in relation to the obtained counts of both Enterobacteriaceae and *B. cereus* organisms in their tissues, became impossible due to unavailability of the recommended limits specifying such organisms in raw processed meats (Table, 3).

Phenotypic (conventional) and genotypic characterization of a total of 67 bacterial strains; picked up from the agar plates after being recovered from the tissues of surveyed samples of both raw and cooked processed meats; identified them as 13 Escherichia coli; 7 Enterobacter hormaechei; 8 strains each of Enterobacter cloacae and Pseudomonas aeruginosa; 5 Enterobacter sakazakii; 3 strains every of Enterobacter aerogenes, Enterococcus faecalis and Pseudomonas stutzeri; 2 strains each of Bacillus cereus, Bacillus licheniformis, Bacillus subtilis, Citrobacter freundii and Enterococcus faecium; besides one strain every of Enterobacter asburiae, Pantoea agglomerans, Proteus mirabilis, Serratia marcescens, Staphylococcus aureus, Staphylococcus cohnii and Staphylococcus xylosus (Table, 4). Concerning the origin of the identified 67 bacterial strains, Bacillus cereus originates from butchers' ground beef and frozen packaged beef sausage; Bacillus licheniformis from beef luncheon and fried hamburger; Bacillus subtilis from fried beef sausage and fried hamburger; Citrobacter freundii from frozen packaged ground beef and beef luncheon; Enterobacter aerogenes from frozen packaged ground beef; Enterobacter asburiae from butchers' ground beef; Enterobacter cloacae from all types of raw meats; Enterobacter hormaechei from butchers' ground beef, frozen packaged beef sausage and beef luncheon; Enterobacter sakazakii from frozen packaged ground beef, butchers' ground beef and frozen packaged beef sausage; Enterococcus faecalis from butchers' ground beef and beef luncheon; Enterococcus faecium from frozen packaged beef sausage; Escherichia coli from all types of raw meats and beef luncheon; Pantoea agglomerans from beef luncheon; Proteus mirabilis from frozen packaged beef sausage; Pseudomonas aeruginosa from all types of raw meats; Pseudomonas stutzeri from frozen packaged beef sausage and fried hamburger; Serratia marcescens from frozen packaged ground beef; Staphylococcus aureus from frozen packaged ground beef; Staphylococcus cohnii from fried beef sausage; besides Staphylococcus xylosus from frozen packaged beef sausage samples (Table, 4). Several researchers could isolate most of the aforementioned bacterial strains from fresh and processed meats; as Enterobacter sakazakii -the new emerging pathogens- from fresh beef, ground beef and sausage **(Goullet and Picard, 1986; Watanabe and Esaki, 1994; Kimura et al., 1999 and Leclercq et al., 2002)**. Also, **Eldaly (1983)**, **Sallam (1993)**, **El-Daym (2005) and El-Shopary (2010)** could isolate Escherichia coli, Enterobacter aerogenes, Pantoea agglomerans, Enterobacter cloacae, Klebsiella pneumoniae, Citrobacter freundii, Proteus mirabilis, Streptococcus faecalis, Streptococcus faecium, Staphylococcus aureus, Staphylococcus epidermidis, Bacillus cereus, Shigella spp, Pseudomonas aeruginosa, Pseudomonas stutzeri, and Serratia marcescens organisms in fresh chilled and frozen meats as well as in raw and cooked processed meats like ground beef, sausage, hamburger and beef luncheon samples, similar to those recognized in the present work.

Table (1): Numbers and percentages of Egyptian raw and cooked processed meats, contaminated with different bacterial populations (n*=10 each).

Types of examined samples		*Aerobic mesophiles-contaminated samples*	*Enterobacteriaceae-contaminated samples*	*Staphylococcus aureus "coagulase positive"-contaminated samples*	*Bacillus cereus-contaminated samples*
Raw processed meats	Frozen packaged ground beef	10 (100%)	3 (30%)	3 (30%)	9 (90%)
	Butchers' ground beef	10 (100%)	6 (60%)	8 (80%)	6 (60%)
	Frozen packaged beef sausage	10 (100%)	8 (80%)	6 (60%)	9 (90%)
Cooked processed meats	Fried beef sausage	10 (100%)	0 (0%)	3 (30%)	5 (50%)
	Beef luncheon	10 (100%)	3 (30%)	4 (40%)	5 (50%)
	Fried hamburger	10 (100%)	3 (30%)	2 (20%)	9 (90%)

N * = number of examined samples.

Table (2): Bacterial populations per gram of Egyptian raw and cooked processed meats (n*=10 each).

Types of examined samples		*Aerobic plate counts*			*Enterobacteriaceae counts*			*Staphylococcus aureus "coagulase positive" counts*			*Bacillus cereus counts*		
		Min	Max	Mean ± SE	Min	Max	Mean ± SE	Min	Max	Mean ± SE	Min	Max	Mean ± SE
Raw processed meats	Frozen packaged ground beef	$9x10^5$	$2x10^9$	$2.7x10^8$ $\pm1.9x10^8$	10^4	$3x10^4$	$1.3x10^4$ $\pm0.87x10^4$	$1.6x10^2$	$1.4x10^3$	$8.5x10^2$ $\pm3.6x10^2$	$6x10^2$	$5x10^5$	$6.8x10^4$ $\pm5.4x10^4$
	Butchers' ground beef	10^5	$7x10^8$	$1.3x10^8$ $\pm0.78x10^8$	$3x10^4$	$2.6x10^6$	$5.8x10^5$ $\pm4.1x10^5$	$4x10^2$	$3.5x10^4$	$9.9x10^3$ $\pm4.8x10^3$	$5x10^3$	$3x10^4$	$1.2x10^4$ $\pm0.41x10^4$
	Frozen packaged beef sausage	$2x10^5$	$2.8x10^9$	$8.5x10^8$ $\pm4.4x10^8$	10^4	$2x10^6$	$3.7x10^5$ $\pm2.3x10^5$	10^3	$2x10^5$	$6x10^4$ $\pm3.1x10^4$	$6x10^3$	$5.1x10^5$	10^5 $\pm0.52x10^5$
Cooked processed meats	Fried beef sausage	$3x10^4$	$6.7x10^7$	$1.4x10^7$ $\pm0.75x10^7$	0	0	0	10^2	$5x10^2$	$2.4x10^2$ $\pm1.3x10^2$	10^3	$3x10^4$	$1.2x10^4$ $\pm0.51x10^4$
	Beef luncheon	$2x10^5$	$1.6x10^7$	$4.7x10^6$ $\pm1.6x10^6$	$5x10^2$	$6x10^3$	$2.8x10^3$ $\pm1.6x10^3$	$2.3x10^3$	$1.5x10^4$	$6.8x10^3$ $\pm2.9x10^3$	$1.4x10^4$	10^5	$5.3x10^4$ $\pm1.4x10^4$
	Fried hamburger	$9x10^5$	$7.2x10^7$	$2.6x10^7$ $\pm0.79x10^7$	10^3	$7x10^3$	$4.1x10^3$ $\pm1.7x10^3$	$5x10^2$	$3x10^3$	$1.8x10^3$ $\pm1.2x10^3$	$3x10^3$	$1.1x10^5$	$2.7x10^4$ $\pm1.1x10^4$

n*= number of examined samples. Min= minimum. Max= maximum. SE= standard error.

Table (3): Microbiological risk assessment of surveyed Egyptian processed meats (n*=10 each), through comparing different bacterial populations contained in their tissues with the recommended limits.

Types of examined samples		*Aerobic plate count*		*Enterobacteriaceae count*		*Staphylococcus aureus* "coagulase positive" count		*Bacillus cereus count*	
		Recommended limits**	No. (%) of samples exceeding the limits	Recommended limits**	No. (%) of samples exceeding the limits	Recommended limits**	No. (%) of samples exceeding the limits	Recommended limits**	No. (%) of samples exceeding the limits
Cooked processed meats	Fried beef sausage	10^5	9 (90%)	10^4	0 (0%)	10^3	0 (0%)	$< 10^5$	0 (0%)
	Beef luncheon	10^4	10 (100%)	10^4	0 (0%)	10^3	4 (40%)	$< 10^5$	1 (10%)
	Fried hamburger	10^4	10 (100%)	10^4	0 (0%)	10^3	1 (10%)	$< 10^5$	1 (10%)
Raw processed meats	Frozen packaged ground beef	10^7	7 (70%)	NA***		10^4	0 (0%)	NA	
	Butchers' ground beef	10^7	4 (40%)	NA		10^4	2 (20%)	NA	
	Frozen packaged beef sausage	10^7	6 (60%)	NA		10^4	3 (30%)	NA	

n*= number of examined samples. NA *** = not available.
** = different recommended limits were reported by Gilbert et al. (2000) for ready-to-eat meats whilst for raw meats were stated by ICMSF (1986).

Table (4) : Types and numbers of bacterial strains isolated from Egyptian raw and cooked processed meats, genotyping "16S rRNA gene sequencing" (n*=10 each).

Types and numbers of bacterial strains	Types of examined samples					
	Raw processed meats			Cooked processed meats		
	Frozen packaged ground beef	Butchers' ground beef	Frozen packaged beef sausage	Fried beef sausage	Beef luncheon	Fried hamburger
Bacillus cereus (2)	-	1	1	-	-	-
Bacillus licheniformis (2)	-	-	-	-	1	1
Bacillus subtilis (2)	-	-	-	1	-	1
Citrobacter freundii (2)	1	-	-	-	1	-
Enterobacter aerogenes (3)	3	-	-	-	-	-
Enterobacter asburiae (1)	-	1	-	-	-	-
Enterobacter cloacae (10)	1	2	5	-	-	-
Enterobacter hormaechei (7)	-	1	3	-	3	-
Enterobacter sakazakii **(5)	2	1	2	-	-	-
Enterococcus faecalis (3)	-	2	-	-	1	-
Enterococcus faecium (2)	-	-	2	-	-	-
Escherichia coli (13)	1	6	5	-	1	-
Pantoea agglomerans (1)	-	-	-	-	1	-
Proteus mirabilis (1)	-	-	1	-	-	-
Pseudomonas aeruginosa (8)	3	2	3	-	-	-
Pseudomonas stutzeri (3)	-	-	2	-	-	1
Serratia marcescens (1)	1	-	-	-	-	-
Staphylococcus aureus (3)	1	-	-	-	-	-
Staphylococcus cohnii (1)	-	-	-	1	-	-
Staphylococcus xylosus (1)	-	-	1	-	-	-
Total strains = (67) ***	(13)	(16)	(25)	(2)	(8)	(3)

n*= number of examined samples. **= the current name: *Cronobacter sakazakii*, according to Iversen et al. (2008).
*** = the obtained strains were picked up from the plates of examined processed meat samples.

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الملخص العربى

التوصيف المظهرى والوراثى للملوثات الجرثومية المعزولة من مصنعات اللحوم المصرية

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تناولت الدراسة الفحص الجرثومى لعدد ستين من عينات اللحوم المصنعة الأكثر إستهلاكاً فى مصر وهى ثلاثين عينة من المصنعات النيئة (عشر عينات من كل من مفروم اللحم البقرى المجمد المعبأ، مفروم اللحم البقرى المجهز فى محلات الجزارة من لحوم مجمدة مستوردة والسجق البقرى المجمد المعبأ) بالإضافة إلى ثلاثين عينة من اللحوم المصنعة المطهية الجاهزة للأكل (عشر عينات من كل من السجق البقرى المقلى، اللانشون البقرى والهامبورجر البقرى المقلى) تم شراؤها من محلات السوبر ماركت والجزارة والمطاعم المنتشرة فى مدينة المنصورة، وقد أجريت مراحل الدراسة الأولى التى شملت التجهيز والفحص الجرثومى على مستنبتات الأجار المختلفة ثم العد والتصنيف المبدئى لأنواع الجراثيم المختلفة بمعمل الرقابة الصحية على الأغذية – كلية الطب البيطرى – جامعة المنصورة – مصر، بينما أجريت التجارب الخاصة بالتصنيف النهائى للمعزولات الجرثومية باستخدام التقنيات الحديثة بقسم العدوى الجرثومية – معهد أبحاث الأمراض الميكروبية – جامعة أوساكا – اليابان.

وقد أظهرت أطباق العد الجرثومى تواجد الجراثيم الهوائية المحبة للحرارة المعتدلة aerobic mesophiles فى جميع عينات اللحوم المصنعة المختبرة (١٠٠٪) النيئة منها والجاهزة للأكل على حد سواء، بينما وجدت الجراثين المعوية Enterobacteriaceae فى ٣٠–٨٠٪ من عينات اللحوم المصنعة النيئة، بالإضافة إلى ٣٠٪ فى كل من اللانشون البقرى والهامبورجر البقرى المقلى بينما لم تظهر هذه الجراثيم على الأطباق الخاصة بالسجق البقرى المقلى، كما تواجدت جراثيم المكور العنقودى الذهبى "القادر على تخثر بلازما الدم" "coagulase-positives" *Staphylococcus aureus* فى ٨٠٪ و ٦٠٪ و ٣٠٪ من كل من عينات مفروم اللحم البقرى المجهز فى محلات الجزارة من لحوم مجمدة مستوردة، مفروم اللحم البقرى المجمد المعبأ والسجق البقرى المجمد المعبأ، على الترتيب، فيما كانت نسب تواجدها بين ٢٠٪ – ٤٠٪ فى عينات اللحوم المصنعة المطهية الجاهزة للأكل، بينما تواجدت جراثيم باسيلاس سيريس *Bacillus cereus* فى عينات اللحوم المصنعة النيئة وتلك الجاهزة للأكل بنسب تراوحت بين ٦٠٪ – ٩٠٪ و ٥٠٪ – ٩٠٪ على التوالى

وفيما يتعلق بالمتوسطات الأسية للأعداد الجرثومية لكل جرام من أنسجة عينات اللحوم المصنعة النيئة والمطهية، فقد تراوحت أعداد الجراثيم الهوائية المحبة للحرارة المعتدلة بين $١٠^{٥}$–٨ر٢×$١٠^{٩}$ بمتوسطات تراوحت بين ٣ر١×$١٠^{٨}$ – ٥ر٨×$١٠^{٨}$ و ٣×$١٠^{٤}$ – ٢ر٧×$١٠^{٧}$ بمتوسطات تراوحت بين ٧ر٤×$١٠^{٦}$ – ٦ر٢×$١٠^{٧}$ بينما كانت تلك الأعداد للجراثيم المعوية $١٠^{٤}$ – ٦ر٢×$١٠^{٦}$ بمتوسطات تراوحت بين ٣ر١ × $١٠^{٤}$ – ٨ر٥×$١٠^{٥}$ و ٥ × $١٠^{٢}$–٧ × $١٠^{٣}$ بمتوسطات تراوحت بين ٨ر٢ × $١٠^{٣}$ – ٤ر١ × $١٠^{٣}$، وللجراثيم المكور العنقودى الذهبى "القادر على تخثر بلازما الدم" ٦ر١ × $١٠^{٢}$–٢×$١٠^{٥}$ بمتوسطات تراوحت بين ٥ر٨ × $١٠^{٢}$ – ٦ × $١٠^{٤}$ و $١٠^{٢}$ – ٥ر١ × $١٠^{٤}$ بمتوسطات تراوحت

بين ٤ر٢ × $١٠^{٢}$ – ٨ر٦ × $١٠^{٣}$ وجراثيم باسيلاس سيرس ٦ × $١٠^{٢}$ – ٥ر١ × $١٠^{٥}$ بمتوسطات تراوحت بين ٢ر١ × $١٠^{٤}$ – ٦ز١ × $١٠^{٥}$ و $١٠^{٣}$ – ١ر١ × $١٠^{٥}$ بمتوسطات تراوحت بين ٢ر١ × $١٠^{٤}$ – $١٠^{٥}$ على الترتيب.

وفيما يتعلق بتقييم الخطر الميكروبيولوچى المحتمل لعينات اللحوم المصنعة المختبرة من خلال مقارنة التجمعات الجراثيمة الموجودة بأنسجتها مع تلك الحدود المسموح بها لكل نوع من هذه الجراثيم، فقد أسفر عن تجاوز ٩٠٪ – ١٠٠٪ من عينات اللحوم المصنعة المطهية عن الحد المسموح به ($١٠^{٤}$ – $١٠^{٥}$ لكل جرام)، و ٤٠٪ – ٧٠٪ من عينات اللحوم المصنعة النيئة للحد المسموح به ($١٠^{٧}$ لكل جرام) لتواجد الجراثيم الهوائية المحبة للحرارة المعتدلة، فى حين لم تزد أى من عينات اللحوم المصنعة المطهية عن الحد المسموح به ($١٠^{٤}$ لكل جرام) لتواجد الجراثيم المعوية، أما بالنسبة للحد المسموح به ($١٠^{٣}$ لكل جرام) لجراثيم المكور العنقودى الذهبى "القادر على تخثر بلازما الدم" فى اللحوم المصنعة المطهية فقد زادت ٤٠٪ من عينات اللانشون البقرى عن هذا الحد، بينما كانت ١٠٪ فقط من عينات الهامبورجر البقرى المقلى زائدة عن هذا الحد، فى حين لم تتجاوز أى من عينات السجق البقرى المقلى لهذا الحد، بينما فى عينات اللحوم المصنعة النيئة تجاوز ٣٠٪ من السجق البقرى المجمد المعبأ و ٢٠٪ من مفروم اللحم البقرى المجهز فى محلات الجزارة من لحوم مجمدة مستوردة الحد المسموح به ($١٠^{٤}$ لكل جرام) لتواجد هذه الجراثيم، فى حين لم تتخطى عينات مفروم اللحم البقرى المجمد المعبأ هذا الحد، وقد تجاوز ١٠٪ فقط من كل من عينات اللانشون البقرى والهامبورجر البقرى الحد المسموح به (أقل من $١٠^{٥}$ لكل جرام) لتواجد جراثيم باسيلاس سيريس.

وقد تم أخذ عدد سبع وستون من الجراثيم المعزولة من عينات اللحوم المصنعة النيئة (١٣ عترة من مفروم اللحم البقرى المجمد المعبأ، ١٦ عترة من مفروم اللحم البقرى المجهز فى محلات الجزارة من لحوم مجمدة مستوردة و٢٥ عترة من السجق البقرى المجمد المعبأ) وأيضاً من اللحوم المصنعة المطهية الجاهزة للأكل (عترتين من السجق البقرى المقلى، ٨ عترات من اللانشون البقرى، بالإضافة إلى ٣ عترات من الهامبورجر البقرى المقلى)، لتصنيفها باستخدام التصنيف الخاص بتتابع وحدات الحمض النووى للجين ١٦rRNA gene sequencing الذى أسفر عن تصنيف : ١٣ عترة أيشيريشيا كولاى، ٧ عترات انتيروباكتر هورماتشى، ٨ عترات من كل من انتيروباكتر كلواكى وسيدوموناس أيروجينوزا، ٥ عترات أنتيروباكتر ساكازاكى، ٣ عترات لكل من انتيروباكتر ايروجينز وانتيروكوكاس فيكاليس وسيدوموناس ستوتزارى، عترتين لكل من باسيلاس سيريس وباسيلاس ليشنيفورميس وباسيلاس ساتلاس وستروباكتر فروندى وانتيروكوكاس فيشيام، بالإضافة إلى عترة واحدة من كل من انتيروباكتر أسبارى وسراتيا مارسيسنز وبانتوا أجلوميرانس وبروتيس ميرابيلس والمكور العنقودى وستافيلوكوكاس كوهنى وستافيلوكوكاس زيلوساس.