

## Microbiological Studies On Some Meat Products At Sharkia Governorate Markets

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### ABSTRACT

A total of 120 samples (30 from each canned beef, beef burger, luncheon and sausage) were collected from different shops and supermarkets at Sharkia Governorate, Egypt. The samples were examined for the bacteriological evaluation. The results revealed the detection of 9 different types of aerobic spore forming bacteria, in addition to un-typed, the incidence of the total aerobic spore formers were 20%, 40%, 36.6% and 46.6% in canned beef, beef burger, luncheon and sausage respectively and their mean values of total count were  $2 \times 10^4$ ,  $5.1 \times 10^4$ ,  $1.5 \times 10^5$ , and  $9.6 \times 10^4$  per gm respectively in the same mentioned meat products. *Bacillus cereus* was distributed more than the other bacillus species among the examined samples followed by *B.coagulans*.

Concerning some food poisoning microorganisms, the incidence of *Salmonella typhimurium* were 3.3%, 6.6%, 6.6% and 13.3 % in canned beef, beef burger, luncheon and sausage respectively, while the incidence of *Staph aureus* were 6.6%, 10%, 13.3% and 16.6% respectively in these products, the average *Staph.aureus* count were  $1.9 \times 10^3$ ,  $3.1 \times 10^3$ ,  $4.6 \times 10^3$  and  $3.6 \times 10^3$  per gm in the same mentioned products respectively. On the other hand, the incidence of *Escherichia coli* were 6.6%, 10%, 10% and 6.6% in the previous mentioned products respectively. Biochemical identification detected three sero-types of *E.coli*, these sero-types were O157:H7, O111:K58 (B4) and O125:K70 (B15) among the examined meat products. The mean colony counts of the total coliform per gram in the previous four mentioned products were  $3 \times 10^4$ ,  $1.7 \times 10^5$ ,  $2.0 \times 10^5$  and  $1.2 \times 10^6$  respectively with the same incidence of *E.coli* among the different products.

The incidence of *Listeria monocytogenes* were 3.3%, 3.3%, 3.3% and 6.6% in canned beef, beef burger, luncheon and sausage respectively, meanwhile the incidence of *Yersinia enterocolitica* in these products were 3.3%, 10%, 6.6% and 13.3% respectively.

On the other aspect, the incidence of alpha hemolytic streptococci were 10%, 6.6%, 6.6% and 3.3% in canned beef, beef burger, luncheon and sausage respectively, while the incidence of beta strain were 6.6%, 3.3%, 0% and 3.3% respectively. Gamma strain was detected only in one (3.3%) sausage sample.

The levels of *S. aureus*, coliforms, *S. typhimurium*, and *E coli* count were exceeded the permissible limits in the all positive samples.

### INTRODUCTION

During the last few decades, the consumption of the meat products was highly developed in Egypt, these products characterized by the relatively low cost and easy preparation to eat or ready to eat without preparation; thus, it saves the time and effort, but in some instances, meat products cause a serious health problem for consumer due to the microbial contamination. These products contained major ingredients from two or more commercially categories. Therefore, the consumer not only exposed to the portable original microbial hazards of each ingredient,

but also the possible of magnified or additional microbial hazard due to further handling, processing or modification of the environment (1). Aerobic spore formers microorganisms are known to be associated food poisoning. At least one member of this group (*Bacillus cereus*) is a well known as a food poisoning microorganism, that cause illness due to the fact that it produces either heat stable emetic toxin or heat sensitive diarrheal entero-toxin which most frequently found in meat products (2).

Some food poisoning bacteria as *Sal.typhimurium*, *Staph.aureus*, *E.coli*, *Listeria*

*monocytogenes* and *Yersinia enterocolitica* exhibit a serious effect on the consumer health. Salmonella is one of the most common causes of food poisoning (3), the patient usually complains headache, vomiting, abdominal pain and fever. *Staph.aureus* produces one or more than heat stable extracellular toxins which resist many proteolytic enzymes and boiling (4). Symptoms of *Staph.aureus* food poisoning characterized by nausea, vomiting, diarrhea, weakness and general malasia (4,5).

On the other hand, *E.coli* was recognized as a food born pathogen, colonizes in the large intestine and produce shiga like toxin (*Stx*) which responsible for severe hemorrhagic colitis in human (6). *Listeria monocytogenes* is one of the etiological agents of the food born illness. This microorganism is associated with severe symptoms as abortion, stillbirth, meningitis, encephalitis and septicemia with high mortality rates (7). Moreover, *Yersinia enterocolitica* is a food born pathogen and it contaminates refrigerator foods due to its psychrotrophic nature and frequently associated with ground meat (8). While, the clinical manifestation typically includes abdominal pain, fever, diarrhea, nausea and sometimes fatal septicemia (9). *Hemolytic streptococci* may lead to pharyngitis in children school (10). On the other aspect, high incidence of *hemolytic streptococci* in throat swap was detected after consumption of contaminated foods from animal origin (11).

Hence, the current study was planned to detect some microorganisms in some meat products (canned beef, beef burger, luncheon and sausage) collected from Sharkia Governorate. Also, count the more risk types and compare these values with the permissible limits and also, discuss its public health significance.

## MATERIALS AND METHODS

### Collection of samples

A total of 120 random samples of meat products thirty from each of canned beef,

frozen beef burger, luncheon and frozen sausage were purchased from May to August 2005 from supermarkets at Sharkia Governorate. The samples (100 gm for each) were individually packaged in aseptic polyethylene bags and maintained in ice box during transport to the laboratory with a minimum of delay. Canned beef transported as a close can at room temperature to the laboratory.

Identification of aerobic spore formers microorganisms

### A - Direct enumeration and identification

Preparation of samples: ten gm of each sample were aseptically taken and mixed with 99 ml of 0.1% sterile peptone water in a sterile homogenizer flask to get a dilution of 1:10. One ml was transferred to a separate sterile test tube containing 9 ml of sterile saline from which serial dilutions up on  $10^8$  were prepared. One ml of each dilution was transferred to sterile Petri-dish, then the media (tryptone dextrose agar) was poured to plate, after solidification, plates were incubated in inverted position at 37°C for 48 – 72 hours as well as 0.1 ml of each dilution was transferred and spread over a dry surface of *Bacillus cereus* agar and egg yolk agar using surface plate technique after 24 hours at 37°C colonies appear on surface and sub-surface, these colonies were counted.

### B- Indirect enumeration and isolation

All ten fold dilution test tubes were put in a water bath at 80°C for 30 m., then the same steps as mentioned before in direct method were done. After incubation the suspected colonies were counted and picked up on semisolid agar.

### C- Identification of an aerobic spore former microorganism

Staining by gram's stain for detection of rod shaped gram positive bacilli.

Biochemical identification (12) catalase activity, sugar fermentation, hydrolysis of

gelatin, casein and starch, nitrate reduction, urea and citrate utilization, oxidation fermentation (O.F) and indole test were performed.

#### Detection of some food poisoning microorganisms

The samples were bacteriologically examined according to the recommended methods using of Cefusulin-Irgasan-Novobiocin (CIN) agar, nutrient agar and MacConkey agar (13). Gram's stain films were prepared from each bacterial growth.

Fermentation of salicin and sucrose, aesculin hydrolysis and urease test were used as a biochemical test, also motility at 22°C was tested (14).

Sero-typing of isolated microorganisms was carried out according to the recommended methods (15).

#### Salmonella detection method

After incubation of original sample at 37°C for 20 hours, one tenth of each dilution was transferred to tetrathionate broth and incubated at 43°C for 24 hours and 48 hours. These cultures were streaked on plates of brilliant green phenol red agar after incubation. The plates were incubated at 37°C for 24 hours. Then suspected colonies were examined biochemically. (16,17).

#### Staphylococcus aureus count

Over a dry surface Baird- Parker (B-P) agar plates, 0.1 ml amount from each of prepared dilutions (ten fold dilutions) of samples under investigation was transferred and evenly spread using surface plating technique (18).

#### Isolation of *E.coli*

Twenty gm from each sample were homogenized in sterile mixer flask with 180 ml of modified *E.coli* broth containing Novobiocin (20 mg/liter) (mEC+n; Sigma chemical Co., St. Louis) (19) after 16 – 20 hours of incubation at 37°C, a loopful from

enriched broth was streaked plated on to sorbitol MacConkey agar (AMAC, Oxoid Unipath, Ltd, Hampshire, UK) supplemented with cefixime (0.5 mg/l) and Potassium telurite (2.5 mg/l) (CT-SMAC, Dynalbiotech UK) (20) after incubation at 37° C for 18 – 20 hours, non sorbitol fermenting colonies (colourless, pale with brown centers) were selected and screened for lactose fermentation on Levine's eosin methylene blue (L-EMB) agar (flat with dark center) (21) and for absence of glucourindase on SMAC containing 4-methylum belliferyl-B-D glucouronide.

#### Confirmation of *E.coli*

Typical colonies taken from incubated plates were tested for agglutination with commercial latex antibody test for *E.coli* antigen (Oxoid). The colonies were streaked onto plate count agar and confirmed to be *E.coli* colonies with gram staining oxidase and catalase tests, and other biochemical tests (22). Determination of coliform count, the most probable number (MPN/gm) (23).

*Listeria monocytogenes* was isolated and identified (24,25). *Yersinia enterocolitica* was isolated and identified (26).

#### Diagnosis of Streptococcus Species

Isolation and identification were carried out after preparation of samples (23).

All samples streaked directly into sheep blood agar for detection of different types of hemolytic streptococcus species. Morphological identification, cultural characters and biochemical tests were done for different isolates.

## RESULTS AND DISCUSSION

The results achieved from Table 1. revealed the detection of 9 different types of aerobic spore former microorganisms (*Bacillus sp.*) in addition to un-typed spore former bacteria. *B. cereus* was highly distributed than the other detected bacillus species among the

examined products; it detected in 6.6%, 13.3%, 6.6% and 10% in canned beef, beef burger, luncheon and sausage respectively followed by *B. coagulans* which found in 6.6%, 10%, 6.6% and 3.3% in the mentioned products respectively.

Table 2. detected that the mean values of total aerobic spore formers microorganisms were  $2.0 \times 10^4$ ,  $5.1 \times 10^4$ ,  $1.5 \times 10^5$  and  $9.6 \times 10^4$  in canned beef, beef burger, luncheon and sausage samples respectively; also, the incidence of the total aerobic spore formers were 20%, 40%, 36.6% and 46.6% respectively in the same samples. These results coincide with those reported (31), while higher incidence of *B. cereus* was found in chicken meat products (32). On contrary, lower aerobic spore former bacteria than those in the present study were recorded in the meat products in Egypt (33) and in Italy (34).

On the other aspect, the risk of food born disease due to bacillus other than *B. cereus* was considered low (34). In spite of, some studies recorded that *B. pumilus* has been associated with some of clinical conditions and incidence of food born gastroenteritis (35). The result reporting in the present investigation is reassuring with regard to the potential risk due to the presences of Bacillus strains in the examined meat products. In any

case, some considerations need to be made. It should be noted that bacillus strains are usually counted after preparation of the product. In this way, only spores are counted, and therefore, the total amount of bacilli cell is unknown and their potential risk could be underestimated. Some information is available about the ability of bacillus spore to germinate during ripening of beef burger or sausage (36,37). Therefore, further studies are needed to clarify which processing parameters can stimulate or repress spore germination and cell replication.

As regarded the food poisoning microorganism, Table 3. showed that the incidence of *Salmonella typhimurium* in canned beef, beef burger, luncheon and sausage were 3.3%, 6.6%, 6.6% and 13.3% respectively. These results are consistent with those reported in beef burger (38), in chicken meat products (3,39); while, salmonellae could not be found in both examined beef burger and sausage (40). The detected *Salmonella typhimurium* in meat products in the current study may be carried by cattle or contaminated beef during the production processes (41). On the other aspect, the attachment of *Sal. Sp.* to beef muscle varied according to PH, temperature, compounds of the medium and the nature of the meat (42).

**Table 1. Incidence of isolated aerobic spore formers micro organisms in the examined meat products (n = 30 for each product).**

Type of bacteria	Canned beef		Beef burger		Luncheon		Sausage	
	No.	%	No.	%	No.	%	No.	%
<i>B. cereus</i>	2	6.6	4	13.3	2	6.6	3	10
<i>B. megaterium</i>	0	0	1	3.3	1	3.3	2	6.6
<i>B. coagulans</i>	2	6.6	3	10	2	6.6	1	3.3
<i>B. bervis</i>	1	3.3	1	3.3	0	0	2	6.6
<i>B. globisporous</i>	0	0	2	6.6	2	6.6	1	3.3
<i>B. circulans</i>	1	3.3	0	0	0	0	1	3.3
<i>B. mycoides</i>	0	0	2	6.6	1	3.3	2	6.6
<i>B. fermus</i>	1	3.3	1	3.3	3	10	2	6.6
<i>B. pumilus</i>	0	0	2	6.6	2	6.6	2	6.6
<i>B. un-typed</i>	1	3.3	1	3.3	2	6.6	4	13.3

**Table 2. Bacterial count per gm of some microorganisms detected in the examined meat products (n= 30 for each product).**

Meat products		Canned beef	Beef burger	Luncheon	Sausage
<i>Total aerobic spore formers</i>	%of +ve	20%	40%	36.6%	46.6%
	Minimum	$2.5 \times 10^3$	$3.7 \times 10^3$	$5.4 \times 10^3$	$6.3 \times 10^3$
	Maximum	$7.2 \times 10^4$	$2.2 \times 10^5$	$6.2 \times 10^5$	$4.4 \times 10^5$
	Mean	$2 \times 10^4$	$5.1 \times 10^4$	$1.5 \times 10^5$	$9.6 \times 10^4$
<i>Staph. aureus</i>	%of +ve	6.6%	10%	13.3%	16.6%
	Minimum	$7.1 \times 10^2$	$4.0 \times 10^2$	$8.7 \times 10^2$	$6.8 \times 10^2$
	Maximum	$3.2 \times 10^3$	$5.4 \times 10^3$	$6.2 \times 10^3$	$6.0 \times 10^3$
	Mean	$1.9 \times 10^3$	$3.1 \times 10^3$	$4.6 \times 10^3$	$3.6 \times 10^3$
<i>coliforms</i>	%of +ve	6.6%	10%	10%	6.6%
	Minimum	$2.2 \times 10^4$	$1.8 \times 10^4$	$8.1 \times 10^5$	$1.9 \times 10^5$
	Maximum	$3.7 \times 10^4$	$4.2 \times 10^5$	$3.6 \times 10^6$	$2.2 \times 10^6$
	Mean	$3.0 \times 10^4$	$1.7 \times 10^5$	$2.0 \times 10^6$	$1.2 \times 10^6$

**Table 3. Incidence of some food poisoning microorganisms in the examined meat products (n = 30 for each product).**

samples	<i>Salmonella typhimurium</i>		<i>Staph. aureus</i>		<i>E. Coli</i>		<i>Listeria monocytogenes</i>		<i>Yerisinia enterocolitica</i>	
	+ve No.	%	+ve No.	%	+ve No.	%	+ve No.	%	+ve No.	%
Canned beef	1	3.3	2	6.6	2	6.6	1	3.3	1	3.3
Beef burger	2	6.6	3	10	3	10	1	3.3	3	10
Luncheon	2	6.6	4	13.3	3	10	1	3.3	2	6.6
Sausage	4	13.3	5	16.6	2	6.6	2	6.6	4	13.3

**Table 4. Serological identification of isolated *E coli* strains using the biochemical testes (n=30 for each product).**

Samples	O157: H7		O111: K58 (B4)		O125: K70 (B15)	
	No.	%	No.	%	No.	%
Caned beef	0	0	1	3.3	1	3.3
Beef burger	1	3.3	2	6.6	0	0
Luncheon	1	3.3	1	3.3	1	3.3
Sausage	0	0	1	3.3	1	3.3

The incidences of *Staph. aureus* in canned beef, beef burger, luncheon and sausage were 6.6%, 10%, 13.3% and 16.6% respectively with the average count  $1.9 \times 10^3$ ,  $3.1 \times 10^3$ ,  $4.6 \times 10^3$  and  $3.6 \times 10^3$  per gram respectively Table 2. These results are similar with those recorded in chicken luncheon (3) and in chicken burger and luncheon (39). On

contrary, lower *S. aureus* count than our findings were reported in beef burger (43), beef burger and sausage (40), beef burger (38) and in ostrich burger (33). Lower *S. aureus* count with higher incidence rather than our findings were reported in Egypt (39). Staphylococcal enterotoxin A and D were inactivated completely by autoclaving and roasting; while, boiling had no effect on the

stability of enterotoxins, so that *S.aureus* growth and production of enterotoxins must be prevented in food because thermal processing can not relied upon to inactivate the toxins (44).

Table 3. showed that the incidence of *E coli* in the examined canned beef, beef burger, luncheon and sausage were 6.6%, 10%, 10% and 6.6% respectively. Meanwhile, the isolated *E coli* could be classified into three strains (Table 4). The incidence of O157: H7 strain was 0%, 3.3%, 3.3%, and 0% in canned beef, beef burger, luncheon, and sausage respectively; while, O111: K58 (B4) strain was detected in 6.6% of beef burger samples and in 3.3% of each other three products. O125: K70 (B15) not detected in beef burger, but it identified in 3.3% of each other three products. The obtained results are nearly similar to those found in rural area in Egypt (40); while, the same study could not detect *E coli* in meat products in urban area. On the other side, higher incidence of *E coli* in meat products rather than our figures were detected (38, 39). It is important to notice that the presence of *E coli* in any food product is an indicative of faulty methods of production and handling. On the other hand, table (2) revealed that the mean values of coliform count were  $3 \times 10^4$ ,  $1.7 \times 10^5$ ,  $2 \times 10^6$  and  $1.2 \times 10^6$  in canned beef, beef burger, luncheon and sausage respectively. Lower levels of total coliform in meat products than those in the current study were reported (38). The above results revealed

that the coliform count in canned beef is slightly lower than those in other products, this difference may be due to tight canning of this product, which protect it against microorganisms.

Table 3. revealed that the incidence of *Listeria Monocytogene* was 3.3% in each of canned beef, beef burger and luncheon, while it was 6.6% in sausage. This result was lower than those recorded in meat products in France and Russia (45, 46). On contrary, *L. Monocytogene* could not be detected in the examined sausage samples in Turkey (47). Human listeriosis is generally cause by consumption of ready to eat food that stored in refrigerator temperature and that permit the growth of this microorganism (48). The incidence of *Yersinia enterocolitica* in canned beef, beef burger, luncheon, and sausage were 3.3%, 10%, 6.6%, and 13.3% respectively. Nearly similar findings were reported in pork meat products (49). Higher incidence of *Y. Enterocolitica* than those detected in the current study were recorded in the meat products (50) and in fresh meat (51). On contrary *Yersinia sp.* could not be found in beef burger and sausage in an Egyptian study (40). The obtained results exhibited a relatively higher incidence of *Y. Enterocolitica* in frozen beef burger and frozen sausage samples than those in the other two products, this result may be explained by the psychotropic nature of this microorganism and its association with the ground meat (8).

**Table 5. Incidence of hemolytic streptococci in the examined samples (n= 30 for each product).**

Samples	<i>Hemolytic streptococci</i>					
	Alpha		Beta		Gamma	
	No. of +ve	%	No. of +ve	%	No. of +ve	%
Canned beef	3	10	2	6.6	0	0
Beef burger	2	6.6	1	3.3	0	0
Luncheon	2	6.6	0	0	0	0
Sausage	1	3.3	2	6.6	1	3.3

**Table 6. Incidence of the examined samples exceeded the permissible limits\* of some bacterial count per gm (n= 30 for each product).**

Meat products Microo- rganism	Canned beef			Beef burger			Luncheon			Sausage		
	P.L.**	No	%	P.L.	No	%	P.L.	No	%	P.L.	No.	%
<i>Staph aureus</i>	Free	2	6.6	$\leq 10^2$	3	10	Free	4	13.3	Free	5	16.6
<i>Total coliforms</i>	Free	2	6.6	$\leq 10^2$	3	10	$\leq 10^2$	3	10	$\leq 10^2$	2	6.6
<i>S. typhimurium</i>	Free	1	3.3	Free	2	6.6	Free	2	6.6	Free	4	13.3
<i>E. coli</i>	Free	2	6.6	Not found	-	-	Free	3	10	Free	2	6.6

\*: The recommended permissible limits (27- 30)

\*\*P.L.: permissible limits.

N.B.: The permissible limit of E coli in the beef burger was not mentioned in the standard.

Regarding the *hemolytic streptococci*, Table 5. illustrates that the incidence of alpha *hemolytic streptococci* were 10%, 6.6%, 6.6%, and 3.3% in canned beef, beef burger, luncheon and sausage respectively; while, the incidence of beta *hemolytic streptococci* were 6.6%, 3.3%, 0% and 6.6% respectively in the same samples. On contrary, gamma *hemolytic streptococci* were not detected in the examined samples except in one (3.3%) sausage sample. Higher incidences of alpha and beta *hemolytic streptococci* than our figures were recorded in meat products in Egypt (11), but the incidence of gamma strain in previously mentioned study was nearly similar to those in the present study. Higher incidences of human pharengitis (63.8%) were recorded following the consumption of luncheon (52). Furthermore, alpha hemolytic streptococci were isolated from 25.8% from peritonsillar abscess (53).

The obtained results indicate that the levels of *Staph. aureus*, total *coliforms*, *S. typhimurium* and *E coli* count exceeded the permissible limits recommended by Egyptian Organization for Standardization and Quality Control in the all positive samples Tables, 2,3 and 6. Both *S.typhimurium* and *E coli* were not counted in this study because their permissible limits were zero in the examined products.

### CONCLUSION AND RECOMMENDATIONS

Information given by the obtained results showed a relatively low incidence of the detected microorganisms in the different examined meat products, in spite of the bacterial count in this study exceeded the permissible limits intended by Egyptian Organization for Standardization and Quality Control. This result indicates a great fluctuation of the microbial pollutions within the examined samples. This was attributed to the nature of microbial load of the used meat, contamination levels of the other additive and microbial contaminations during manufacture, storage and transportation.

The following recommendations should be applied to throw some light on scientific solutions of this problem in order to minimize the microbial pollutions of the meat products:

- 1-The meat and additives used in the different products must be free from any dirties.
- 2- Different machines in the plants must be thoroughly washed periodically.
- 3- Maintaining clean water supply and air filtration system should be used to avoid water and air contamination.
- 4- Various steps of production must be under laboratory control.

- 5- Sanitary disposal of water and other effluents.
- 6- Continuous training and education programs were recommended for workers.
- 7- Transportation, storage and displaying of meat products should be under hygienic procedures.
- 8- Continuous monitoring of the hygienic state of different meat products is highly recommended.

### REFERENCES

- 1- *National Academy of Science (NAS) (1985):* An Evaluation of the role of microbiological criteria for foods and food ingredients. National Academy Press. Washington D.C.
- 2- *Granum P.E. and Lund T. (1997):* *Bacillus cereus* and its food poisoning toxins. FEMS Microbiol. Lett. 157:223-228.
- 3- *Essa H.H.; Makar N.H. and Sohair, Hussein, Z. (2004):* Bacteriological evaluation of chicken luncheon in Assiut city. Assiut Vet. Med. J. 50 (102):64- 72.
- 4- *Eley A.R. (1992):* Microbial food poisoning. 1<sup>st</sup> Ed. Charman. Hall, London, Glasgow and New York, USA.
- 5- *Ward D., Bernard D., Collette R., Kramer D., Hart K., Pricer R. and Otwell S. (1997):* Hazard found in seafood's Appendix III. In HACCP: Hazard Analysis And Critical Control Point, Training Curriculum 2<sup>nd</sup> ed. 173UNC- SG-96-02. North Carolina Sea Grant, Raleigh, NC, USA.
- 6- *Naim F., Serge M., Linda S. and Piette G.: (2004):* Post processing in vitro digestion challenge to evaluate survival of *Escherichia coli* O157:H7 in fermented dry sausages. Appl. Environ. Microbiol. 70 (11): 6637- 6642.
- 7- *Evans M.R., Swaminathan B., Graves L.M., Altermann E.A., Klaenhammer T.R., Fink R.C., Kernodle S. and Kathariou S. (2004):* Genetic Marker Unique to *Listeria monocytogene*. Serotype 4b differential epidemic clone (hot dog outbreak strain) from other lineages. Appl. Environ. Microbiol. April, 70 (4): 2383-2390.
- 8- *Vishnubhatla A, Fung D.Y.C., Obrat, RD, Hays M.P., Nagaraja T.G. and Flood S.J.A. (2000):* Rapid 5' Nuclease (Toq Man) Assay for detection of virulent strain of *Yersinia enterocolitica*. Appl. Environ. Microbiol. September 66 (9): 4131-4135.
- 9- *Bottone E. (1997):* *Yersinia enterocolitica* the charisma continues. Clin. Microbiol. Rev. 10: 257-276.
- 10- *Noel T.P., Zabriskie J, Macpherson C.N., Perratte G. (2005):* Beta hemolytic streptococci in school children 5-15 years of age with an emphasis on rheumatic fever in the tri- island of Grenada. West Indian Med. J. 54 (1): 22-27.
- 11- *Moawad A. M.A., YA Abd- El- Mawla and A.A. Moawad (2003):* The impact of streptococci isolation from some foods of animal origin on public health. Egypt J. Agric. Res., 81(2): 821-827, special Issue: 2<sup>nd</sup> Scientific Congress for Provincial Lab. 7-10 Sep.2003.
- 12- *Cowan S.T. and Steel K.J. (1974):* Manual for identification of Medical Bacteria 2<sup>nd</sup> ed. Cambridge University Press, UK.
- 13- *Brewer R.A. and Corbel M.J. (1985):* *Yersinia enterocolitica* and related species. In isolation and identification of microorganisms of medical and veterinary importance (ed C.H. Collins & J.M. Grange) society for applied bacteriology technical series. No.21, Academic Press, London, pp:83-104.
- 14- *Macfadin J. (1980):* Biochemical tests for identification of medical bacteria 2<sup>nd</sup> ed. Williams and Wilkins Co. USA.
- 15- *Scubert S.; Bockemuhl J.; Brendler, U. and Hessem J. (2003):* First isolation of



- virulent *Yersinia enterocolitica* O:8, biotype 1b in Germany Eur. J Clin. Microbiol. Infec. Dis.,22 (1): 66-68.
- 16-Mulder R.W.A.W.(1977): Inactivation of salmonellae on chilled and deep frozen broiler carcasses by irradiation. J. App. Bact., 42: 179-185.
- 17-Cheesbrough Monica (1993): Medical laboratory Manual for tropical countries. Vol.2: Microbiology, ELBS Edition reprinted 1993, Cambridge , England pp227-233.
- 18-Thatcher F.S. and Clark M.E. (1975): Microorganisms in foods. International Committee on microbiological specifications for foods. Univ. of Toronto Press, Toronto and Buffalo, Canada
- 19-Okrend A.T.; Rose LE and Bennett B. (1990): A screening method for isolation of *Escherichia coli* O157:H7 from ground beef. J. Food Prod. 53: 249-252.
- 20-Zadik, P.M.; Chapman P.A. and Siddons C.A. (1993): Use of tellurite for the selection of verocytotoxigenic *Echerichia coli* O157. J. Med. Microbiol. 39:155-158.
- 21-Food and Drug administration (FDA) (1998): Bacteriological Analytical manual. 8<sup>th</sup> Ed. Revision. A published and distributed by AOAC International, USA.
- 22-Avery S.M.; Small A.; Reid C.A. and Boncic S. (2002): Pulsed field gel electrophoresis characterization of shiga toxin producing *Echerichia coli* O157 from hides of cattle at slaughter. J of Food Port. 65 (7); 1172-1176.
- 23-International Commission on Microbiology Specifications for food "ICMSF" (1978): Micro- organisms in foods I. Univ. of Toronto, Press Toronto and Buffalo, Canada.
- 24-Mclauchin J. (1987): *Listeria monocytogenes*, recent advances in the taxonomy and epidemiology of listeriosis in human. J App. Bacteriol. 63: 1-9.
- 25-Mc. Clain D.F. and Lee W.H. (1988): Development of USDA. F sis method for isolation of *listeria monocytogenes* from raw meat and poultry. A.O.A.C. 71:660.
- 26-American Public Health Association "APHA" (1992): Compendium of methods for microbiological examination of foods. 2<sup>nd</sup> ED. APHA technical committee on microbiological methods of foods. Washington, D.C., USA.
- 27-Egyptian Organization for standardization and Quality Control (2000): The permissible limits for canned beef 3491/2000.
- 28-Egyptian Organization for standardization and Quality Control (2005): The permissible limits for frozen beef burger 1688/2005.
- 29-Egyptian Organization for standardization and Quality Control (2005): The permissible limits for luncheon 1114/2005.
- 30-Egyptian Organization for standardization and Quality Control (2005): The permissible limits for sausage 3492/2005.
- 31-Youssef M.S.H., Saleh O.A. and Bkeet A.A. (2003): Incidence of aerobic spore forming bacteria in some processed poultry meat products in Damanhour city. Egypt J. Agric. Res., 81(2): 833-840, special Issue: 2<sup>nd</sup> Scientific Congress for Provincial Lab. 7-10 Sep. 2003.
- 32-Smith, D.P., Berrang M.E., Feldner P.W., Meinersmann R.G. (2004): Detection of *Bacillus cereus* on selected retail chicken products. J. Food Prot. Aug.,67 (8): 1770-3.
- 33-Zaki E.M.S., Osman E.A. and Omima M.D. (2004): Incidence of *bacillus cereus*, *staphylococcus aureus* and their toxins in ostrich burger. J. Egypt Vet. Med. Ass. 64<sup>th</sup> (1): 285-293.

- 34-Matarante A., Baruzzi F., Sandra P., Cocconcelli and Mora M. (2004): Genotyping and toxigenic potential of *Bacillus subtilis* and *Bacillus pumilus* strain occurring in industrial and artisanal cured sausage. *Appl. Environ. Microbiol.* 70 (9): 5168-5176.
- 35-Turnbull P. (1997): The role of *Bacillus* group in infection culture 18: 5-5 (cited after Matarante et al, 2004).
- 36-Houben J.H. and Krol B. (1989): Effect of citric acid, citrate and slight decreases on bacteriological stability of Hogue liver sausage. *Meat Sci.* 24: 163-176.
- 37-Grohs B.M. and Kunz (1999): Antimicrobial effect of spices on sausage-spoiling microorganism using a medium for sausage type frankfurter. *Adv. Food Sci.* 21: 128-135.
- 38-Essa H.H. and Makar N.H. (2003): Bacteriological of beef burger in Assiut City. *Assiut Vet. Med. J.* 49 (99):81-89.
- 39-Khalifa E.M.I. and Abd El-Shaheed (2005): Bacteriological evaluation of chicken meat and some chicken meat products sold in Kafr El- Sheikh Governorate. 4<sup>th</sup> Int. Conf. Mansora 5-6 April, 2005.
- 40- Soliman M.R., Abd El- Mones K.H. and Saad S.M. (2002): Microbiological Quality of ready to eat meat products and fishes in urban and rural areas. *J. Egypt Vet. Med. Ass.* 62 (6a): 39-51.
- 41-Gallagher G.A., Berry E.D., Betancourt R.M., Arthur T.M. and Koocharaie M. (2002): Development of methods for the recovery of *Escherichia coli* O157:H7 and *Salmonellae* from beef carcass sponge samples and bovine fecal and hide samples. *J. Food Prot.* 65 (10): 1527-1534.
- 42-Bouttier S., Linxe C., Ntsama C., Morgant G., Bellon-Fontaine N.M. and Fournial J. (1997): Attachment of *Salmonella choleraesuis* of beef muscle and adipose tissues. *J. Food Prot.* 60 (1): 16-22.
- 43-Ebraheem G.M.M. (2001): Ready to eat meat sandiwiches as a source of potential pathogens in Assuit City. M.V.Sc. Thesis, Assiut Univ. (cited after Essa and Makar,2003).
- 44-Zaki S. Enam (1998): *Staphylococcus aureus* as a potential food poisoning in broiler processing plants. Ph.D. Thesis Fac. of Vet. Med. Moshtohor, Zag. Univ.
- 45-Thevenot D., Delignette-Muller M.L., Christianans S., Vernozy- Rozand C. (2005): Fate of *Listiria monocytogene* in experimentally contaminated French sausage. *Int. J. Food Microbiol.* May 25; 101 (2): 189-200.
- 46-Efimochkina N.R., Sheveleva S.A., Nitiaga I.M., Tortakovkii, Ermolaeva S.A., Karpova T.I. (2005): A new complex approaches for the identification of *Listiria* isolated during production of fermented sausages. *Vopr Pitan;* 74 (2): 39-45.
- 47- Akpolot N.O., Elci S., Atmaca S. and Gul K. (2004): *Listiria monocytogenes* in products of animal origin in Turkey. *Vet. Res. Commun.* Oct. 25 (7): 561-567.
- 48- Zhou X., Jiao X., Wiedmann M. (2005): *Listiria monocytogenes* in the Chinese food system: Strain characterization through partial act A sequencing and tissue- culture pathogenicity assay. *J. Med. Microbiol.* Mar., 54 (pt3): 217-24.
- 49- Lambertz T.S., and Danielesson- Tham M.L. (2005): Identification and characterization of pathogenic *Yersinia enterocolitica* isolated by PCR and pulsed – field gel electrophoresis. *Appl. Environ. Microbiol.* Jul. 71(7): 3674-81.
- 50- Ramiraez EI, Vazquez- Salinas C, Rodas- Suarez O.R. and Pedroche F.F. (2000): Isolation of *Yersinia* from raw (pork and chicken) and precooked meat (porcine tongues and sausage) collected from

- commercial establishment in Mexico city. J. food Port. Apr. 63(4): 542-4.
- 51- *Fridriksson- Ahomaa M., Koch U., Klemm C., Bucher M. and Stalle A. (2004):* Different genotype of *Yersinia enterocolitica* 4/0:3: strain widely distributed in butcher shop in Munich area. Int. J. Food Microbiol. Aug. 15; 95(1):89-94.
- 52- *Ultan F., Kurter K., Senol E. and Sultan (1989):* A food -born outbreak of group a streptococcal pharyngitis. Mikrobiyol. Bul., 23:302-311.
- 53- *Fujiyoshi T., Inaba T., Udaka T., Tanabe T., Yoshida M. and Makishima K. (2001):* Clinical significance of the *Streptococcus milleri* group in pertonsillar abscesses. Nippon Jibiinkoka Gakkai Kaiho., 104:866-871.

### الملخص العربي

#### دراسات ميكروبيولوجية لبعض منتجات اللحوم بأسواق محافظة الشرقية

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أجريت هذه الدراسة لتقييم بعض منتجات اللحوم في أسواق محافظة الشرقية من الناحية الميكروبيولوجية . وقد تم تجميع ١٢٠ عينة من منتجات اللحوم (٣٠ عينة من كل من اللحم البقري المعلب؛ البيف بيرجر؛ للنشون والسجق المجمد) من أسواق محافظة الشرقية ، وقد أسفرت الدراسة عن النتائج التالية.

تم التعرف علي ٩ أنواع مختلفة من البكتيريا الهوائية المتحوصلة في منتجات اللحوم التي تمت دراستها وذلك عدا ما لم يتم تصنيفه وكانت النسب المنوية لوجودها هي ٢٠%، ٤٠%، ٣٦,٦%، ٤٦,٦% في عينات اللحم البقري المعلب، البيف بيرجر، للنشون والسجق علي التوالي وكان متوسط العدد الكلي للبكتيريا الهوائية المتحوصلة  $10 \times 2$  ،  $10 \times 5,1$  ،  $10 \times 1,5$  ،  $10 \times 9,6$  لكل جرام في نفس العينات المذكورة علي التوالي، وقد كانت أكثر الأنواع وجودا هي باسيلس سيرس يليها باسيلس كواجيولانز.

أما فيما يتعلق ببعض أنواع بكتيريا التسمم الغذائي فقد وجد أن النسب المنوية لتواجد السالمونيلا تيفيموريوم هي ٣,٣%، ٦,٦%، ٦,٦%، ١٣,٣% في عينات اللحم المعلب، البيف بيرجر، للنشون والسجق علي التوالي، في حين كانت النسب المنوية لتواجد بكتيريا المكور العنقودي الذهبي هي ٦,٦%، ١٠%، ١٣,٣%، ١٦,٦% علي التوالي في نفس المنتجات وكان متوسط أعداد هذا النوع  $10 \times 1,9$  ،  $10 \times 3,1$  ،  $10 \times 4,6$  ،  $10 \times 3,6$  لكل جرام علي التوالي في نفس العينات المذكورة. ومن ناحية أخرى كان معدل تواجد الإشيريشيا كولاي ٦,٦%، ١٠%، ١٠% في نفس العينات سالفة الذكر علي التوالي. ومن ناحية أخرى أسفر تصنيف الإشيريشيا كولاي عن تواجد العترات التالية O157:H7 (B4) ، O111: K58 (B4) ، O125: K70 (B15) في العينات المختبرة. وكان متوسط مجموع بكتيريا القولون في العينات  $10 \times 3$  ،  $10 \times 1,7$  ،  $10 \times 2$  ،  $10 \times 1,2$  لكل جرام علي التوالي في عينات اللحم البقري المعلب، البيف بيرجر، للنشون والسجق و كانت نسب تواجد بكتيريا القولون هي نفس نسب تواجد الإشيريشيا كولاي. ومن ناحية أخرى كانت النسب المنوية لتواجد اللستيريا مونوسيتوجين هي ٣,٣%، ٣,٣%، ٦,٦%، ٦,٦% علي التوالي في عينات اللحم المعلب، البيف بيرجر، للنشون والسجق. أما اليرسينيا أنتركوليتيكا فقد كانت نسب تواجدها في نفس المنتجات هي ٣,٣%، ١٠%، ٦,٦%، ١٣,٣% علي التوالي .

من جهة أخرى كانت نسب تواجد نوع ألفا من البكتيريا السبحية ١٠%، ٦,٦%، ٦,٦%، ٣,٣% في اللحم المعلب، البيف بيرجر، للنشون، والسجق علي التوالي في حين كان تواجد نوع بيتا من البكتيريا السبحية هي ٦,٦%، ٣,٣%، ٦,٦%، ٦,٦% علي التوالي في نفس المنتجات المذكورة ومن جهة أخرى لم يتواجد نوع جاما من البكتيريا السبحية إلا في عينة سجق واحدة فقط (٣,٣%).

كان تواجد بكتيريا المكور العنقودي الذهبي، السالمونيلا تيفيموريوم، الإشيريشيا كولاي، و بكتيريا القولون أعلي من الحدود المسموح بها في كل العينات الموجبة.