International Journal of Microbiological Research 2 (3): 196-203, 2011
ISSN 2079-2093

SIncidence of *Clostridium perfringens* in Meat Products at Some Egyptian Governorates

[1]Elham I. Atwa and [2]Nahla A. Abou EI-Roos

[1]Department of Bacteriology, Animal Health Research Institute, Shebin El- Kom-Branch, Egypt
[2]Department of Food Hygiene, Animal Health Research Institute, Shebin El- Kom-Branch, Egypt

Abstract: Two hundreds of meat product samples were collected from large supermarket, grocery stores and retail meat shops at Menoufiea and Gharbia Governorates including 125 collected from ready to cook meat products;, beef burger, meat kofta, minced meat, beef sausage and kobeba, 25 samples of each and 75 collected from ready to eat products;basterma, canned beef and luncheon, 25 sample of each. *C. perfringens* was isolated from ready to cook and ready to eat meat products with incidence of (48.8 and 21.3%), respectively. Typing of *C. perfringens* revealed that the incidences of toxigenic and non toxigenic strains were 89.6 and 10.4%, respectively. Typing of toxigenic strains of *C. perfringens* revealed that type (A) was the most predominant one (46.8%) compared to type (D) and mixed types with the incidence of 19.5 and 23.3%. respectively. The incidence of type "A" and "D" from beef burger, meat kofta, minced meat, beef sausage, kobeba, basterma, canned beef and luncheon were 46.7, 46.1, 42.9, 58.8, 33.3, 50, 42.9 & 40% respectively and 26.7, 23.1, 14.3, 5.9, 33.3, 25, 14.3 & 20%, respectively. Meanwhile, the incidences of mixed types from the same products were 13.3, 23.1, 28.5, 23.5, 22.2, 25, 28.5 and 40%, respectively. The obtained result revealed that the total count of *C. perfringens* from the afore mentioned products, were $3.2x10^3$, $4.5x\ 10^4$, $1.2x\ 10^3$, $1.2x\ 10^3$, $2.3x\ 10^3$, 4.5x 10, $2.8x\ 10^2$ and 2.1x 10 organisms /g, respectively. While, the percentage of *C. perfringens* type "A" producing enterotoxin isolated from beef burger, meat kofta, minced meat, beef sausage, kobeba, basterma, canned beef and luncheon, by using the suckling mouse bioassay, were 28.5, 16.7, 0, 30, 33.3, 0%, 33.3 and 0%, respectively. The amplification of 1167bp and 233bp fragments from the extracted DNA of *C. perfringens* isolated from the tested meat product samples confirmed the presence of both *C. perfringens* alpha toxin (cpa) and enterotoxin (cpe) genes.

Key words: *Clostridium perfringens* · Alpha toxin · Enterotoxin · PCR meat products

INTRODUCTION

Meat is a good source of animal protein and minerals especially iron. The progress of food technology and the utilization of meat in any available form of meat product render the processor to convert the various types of meat into identified products [1,2]. Meat products such as kofta and sausage are gaining popularity in Egypt because they represent quick easily prepared meals and solve the problems of shortage in fresh meat [3]. All the preparation steps of meat from the time of slaughtering till it is ready to cook should be placed under ideal conditions of hygiene [4-6].

The curing of meat inhibits the growth of microorganism and improves the sensory characteristics of final products as well as the cooking increased their acceptability for consumers. Moreover, the cooking process gets ready to eat meat products, which become safe for all people [7].

The microbiological quality and safety of commercially processed meat and poultry products are major areas of concern for producers, consumers and public health officials worldwide [8]. Products excessively contaminated with microorganisms are undesirable from the stand point of public health, storage, quality and general aesthetics [9, 10]. Processed meat products constitute a good media for bacterial growth and multiplication, depending on many factors such as pH, temperature biosafety measures and personal hygiene, which may lead to food intoxication and affect on the public health [11-13].

Corresponding Author: Elham I. Atwa, Department of Bacteriology,
Animal Health Research Institute, Shebin El- Kom-Branch Egypt.

The quality of meat product depends on the quality of the used meat, additives, sanitary condition of the equipments and the processing procedures [14, 15]. Fresh sausage is one of the most popular furthered processed meat products. It is formulated from a combination of raw ingredients which yield a final product of acceptable quality and competitive price. Moreover, the high microbiological quality is necessary to improve the processed meat products [16].

Anaerobic bacteria constitute an important group of microorganisms which are responsible for many public health hazards as well as spoilage due to lack of oxygen. Clostridia are the most anaerobic organisms which contaminate food, due to production of their resistant spores [17].

C. perfringens is more widely spread than other pathogenic bacteria; its principal habitats are in the soil and the intestinal contents of man and animals [18]. As well as, it has a great effect on the human health causing food poisoning; also *C. perfringens* causes a number of human diseases ranging from necrotic enteritis to wound infection and life threatening gas gangrene. This pathogenicity is associated with lethal extra cellular toxins which have been defined as enzyme activity as collagenase, hyaluronidase and deoxyribonuclease [19]. All *C. perfringens* food poisoning outbreaks have been caused by strains type (A) in which meat is an excellent medium for the bacterial growth Meat products may also be contaminated by different types of such microbes.

Food outbreaks caused by *C. perfringens* are usually those presenting with high counts in the meat dishes or meat products which have been exposed to insufficient cooking. Contamination of meat and meat products with *C. perfringens* may be through different sources; mainly internally from animal after slaughtering as postmortem invasion or externally from contaminated hands, skin of animals, soil, water and processing equipments [20].

So, the aim of the present study was to determine the incidence of *C. perfringens* in manufactured meat products collected from large supermarket, grocery stores and retail meat shops at Menoufiea and Gharbia Governorates and type *C. perfringens* alpha toxin and enterotoxin (cpa & cpe) directly from the meat product samples using PCR.

MATERIALS AND METHODS

Samples: A total of two hundredsmeat product samples were collected from large supermarket, grocery stores and retail meat shops at Menoufiea and Gharbia Governorates. Samples were collected as follow; 125 from ready to cook meat products as beef burger, meat kofta, minced meat, beef sausage and kobeba, 25 samples each and 75 from the ready to eat products as basterma, canned beef and luncheon, 25 samples each.

Samples were transferred directly to the laboratory in separate clean sterile plastic bags in an ice box and subjected to the required investigations without delay.

Bacteriological Examination:

Isolation and Identification of *C. perfringens:* Ten gram portions of samples were diluted in 90 ml of sterile 0.1% peptone water and homogenized in a blender at 2000 r.p.m for 1-2 minutes. 1ml of each homogenized food suspension was added to each of two tubes containing 10 ml of sterile cooked meat broth (CMB). To enrich for any *C. perfringens* spores present in the food sample, one of those two tubes was heat shocked at 72°C for 20 min before anaerobic incubation at 37°C for 24 hrs. The other tube was directly incubated anaerobically at 37°C for 24 hrs to enrich primarily for *C. perfringens* vegetative cells present in the food sample. Each CMB enrichment culture showing growth was streaked onto one plate of nutrient agar containing 10% sheep blood and 40ug/ml neomycin and incubated for 24 hrs at 37°C in an anaerobic jar. The plates were examined for the characteristic colonies of *C. perfringens*. Subcultures from the suspected colonies were identified morphologically and biochemically according to Smith and Holdman [21], Koneman *et al.* [22] and Collee *et al.* [23].

Typing of *C. perfringens:* Positive strains of *C. perfringens* isolates were typed by intra dermal inoculation test in Albino Guinea pigs and mouse intravenous neutralization test according to Stern and Batty [24].

Biological Assay for Detection of Enterotoxigenic *C. perfringens:* Enterotoxigenic *C. perfringens* isolates were detected according to Giannella [25]. CMB culture of *C. perfringens* isolates were inoculated onto Duncan and Strong (DS) sporulation medium [26] anaerobically at 37°C for 8hrs. Infant albino mice (1-2 gm, 1-4 days old) were subjected to intra gastric inoculation with 0.1ml of crude culture filtrate. After 4 hours, there were necropsies of the small intestine and distention. The gut weight to the remaining body weight was calculated. A ratio of less than 0.083 is considered negative.

Total Anaerobic Count of *C. perfringens* in Meat Product: It was carried out according to the technique adapted by Cruickshank *et al.* [27] and APHA [28]. The selective media plates of *C. perfringens* (TSC Agar, Oxoid) were streaked with 0.1 ml of the first and second dilution prepared from the collected samples diluted in sterile peptone water, incubated anaerobically at 37°C for 18-24 hrs in the Gas Pack anaerobic jarand the average counts were calculated.

PCR Assay to Determine the *C. perfringens* Alpha Toxin (cpa) Andenterotoxin (cpe) Genes:

Extraction of *C. perfringens* DNA from Samples: To prepare template DNA for the PCR assay, a loopful of a pure CMB culture from each isolate confirmed as *C. perfringens* was pelleted by centrifugation and used as source of chromosomal DNA.

To isolate DNA from (6) meat product samples positive for isolation of *C. Perfringens* alpha toxin gene *(cpa)* and enterotoxin gene (*cpe*), approximately 0.5mg of each sample was minced and resuspended in 3 ml of phosphate buffered saline (PBS) and DNA was extracted by the conventional method [29]. DNA was eluted in 40ul of TE buffer (10mM Tris-HCL, 1mM EDTA, pH 8).

Primers Used in PCR: 1-The sequences of the primers for *C. perfringens* alpha toxin gene *(cpa)* were selected from the sequences published by Saint-Joanis *et al.* [30], a forward primer (5`-AAGATTTGTAAGGCGCTT-3`) and a reverse primer (5`-ATTTCCTGAAATCCACTC- 3`), with amplified fragment: 1167bp were used.

The sequences of the primers for *C. perfringens* enterotoxin (*cpe*) were selected from the sequences published by Meer and Songer [31], a forward primer (5'-GGA GAT GGT TGG ATA TTA GG-3`) and a reverse primer (5'-GGA CCA GCA GTT GTA GAT A-3`), with amplified fragment: 233bp were used.

PCR Amplification: The PCR was performed according to Fach and Guillou [32], in a touch-down thermocycler (Hybaid) in a total reaction volume of 50 µl containing 5µl of 10× PCR buffer (10ml M tris- HCL, pH 9, 50 mM KCL, 0.005% tween, 0.1% 2 triton ×100), 5µl of 25mM MgCl, 250mM each deoxynucleotide triphosphate, 2U of tag DNA polymerase, 1µM of each primer. Amplification was obtained with 35 cycles following an initial denaturating step at 94°C for 30 sec. each cycle involved denaturation at 94°C for 1 min, annealing at 56°C for 1 min for cpa gene and 55°C for 2 min for cpe gene and extention at 72°C for 2 min. The results were determined by electrophoresis of 20µl of PCR products in a 1% agarose gel for 30 min at 80V and staining with ethidium bromide. The 1167 and 233 bp PCR products of cpa and cpe, respectively, were observed. PCR markers (Biotechnology Department, BioBasic Inc. USA) consisting of nine DNA fragments ranging from 0.5 to 10.0 kilobase (KB) pairs were used as the standards. Amplified bands were visualized by UV illumination and photographed on high-density thermal paper film.

RESULTS

Table 1 shows that the prevalence of *C. perfringens* from ready to cook meat products was 48.8%with the incidence of 60, 52, 28, 68 and 36% from beef burger, meat kofta, minced meat, beef sausage and kobeba, respectively. Table 2 shows that the prevalence of *C. perfringens* from ready to eat meat products was 21.3% with the incidences of (16, 28 and 2o%) from basterma, canned beef and luncheon, respectively.

Results in Table 3 show the typing of *C. perfringens* by intradermal injection of Guinea pig which revealed that the incidence of toxigenic and non toxigenic strains were 89.6 and 10.4%, respectively. Typing total toxigenic strains of *C. perfringens* revealed that type A was the most predominant one (46.8%), while type D and mixed types displayed the incidence of 19.5 and 23.3%, respectively.

Table 4 shows that the mean *C. perfringens* counts of beef burger, meat kofta, minced meat, beef sausage, kobeba, basterma, canned beef and luncheon were $3.2x10^3$, $4.5x\ 10^4$, $1.2x\ 10^3$, $1.2x\ 10^3$, $2.3x\ 10^3$, 4.5x 10, $2.8x\ 10^2$ and 2.1x 10 CFU /g, respectively.

Also, results in Table 4 displays that the mean number of enterotoxigenic *C. perfringens* type "A" isolated from beef burger, meat kofta, minced meat, beef sausage, kobeba, basterma, canned beef and luncheon by using the suckling mouse bioassay were 28.5, 16.7, 0, 30, 33.3, 0, 33.3 and 0%, respectively.

Fig. 1 shows six samples of meat products representative for positive *C. perfringens* alpha toxin gene (*cpa*) (1167bp) and *C. perfringens* enterotoxigenic (*cpe*) (233bp) isolates.

Table 1: Incidence of *C. perfringens* in the examined ready to cook meat products samples

Samples	No. of examined samples	No. of positive samples	%*
Beef Burger	25	15	60
Meat Kofta	25	13	52
Minced Meat	25	7	28
Beef Sausage	25	17	68
Kobeba	25	9	36
Total	125	61	48.8

*% Calculated according to the number of tested samples

Table 2: Incidence of *C. perfringens* in the examined ready to eat meat products samples.

Samples	No. of examined samples	No. of positive samples	%*
Basterma	25	4	16
Canned Beef	25	7	28
Luncheon	25	5	20
Total	75	16	21.3

*% Calculated according to the number of tested samples

Table 3: Typing of *C. perfringens* isolated from the tested samples using guinea pig assay

				Type of toxigenic isolates of *C. perfringens*							
Samples	No. of *C. perfringens* isolated	No. of non toxigenic isolates	%*	A	%*	D	%*	Mixed	%*	Total**	%*
Beef Burger	15	2	13.3	7	46.7	4	26.7	2	13.3	13	86.7
Meat Kofta	13	1	7.7	6	46.1	3	23.1	3	23.1	12	92.3
Minced Meat	7	1	14.3	3	42.9	1	14.3	2	28.5	6	85.7
Beef Sausage	17	2	11.8	10	58.8	1	5.9	4	23.5	15	88.2
Kobeba	9	1	11.1	3	33.3	3	33.3	2	22.2	8	88.8
Basterma	4	0	0.0	2	50.0	1	25.0	1	25.0	4	100.0
Canned Beef	7	1	14.3	3	42.9	1	14.3	2	28.5	6	85.7
Luncheon	5	0	0.0	2	40.0	1	20.0	2	40.0	5	100.0
Total	77	8	10.4	36	46.8	15	19.5	18	23.3	69	89.6

*% Calculated according to the total number of isolated *C. perfringens*

** Total number of toxigenic *C. perfringens*

Table 4: Total viable count of *C. perfringens* isolated from the tested samples and number of type" A" enterotoxin producing *C. perfringens* (suckling mouse bioassy)

Samples	Total count of *C. perfringens* CFU/g	No. of *C. perfringens* type "A"	No. of *C. perfringens* type "A" enterotoxin	%*
Beef Burger	$3.2x10^3$	7	2	28.5
Meat Kofta	$4.5x10^4$	6	1	16.7
Minced Meat	$1.2x10^3$	3	0	0.0
Beef Sausage	$1.2x10^3$	10	3	30.0
Kobeba	$2.3x10^3$	3	1	33.3
Basterma	4.5x10	2	0	0.0
Canned Beef	$2.8x10^2$	3	1	33.3
Luncheon	2.1x10	2	0	0.0
Total	-	36	8	22.2

*% Calculated according to the total number of isolated *C. perfringens*

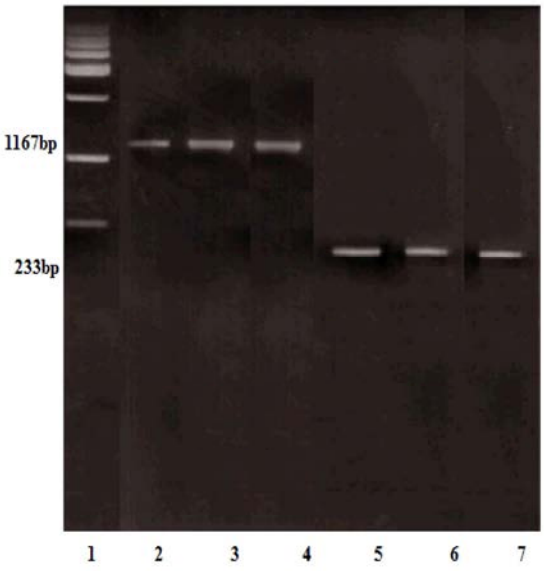

Fig. 1: Electrophoretic analysis of PCR products of amplified *C. perfringens* alpha toxin and enterotoxin genes (Please, put Fig. head below photo)
Lane (1):Standard molecular weight marker (100 bp)
Lanes (2, 3 and 4): Amplified *C. perfringens* alpha genes (cpa) at 1167bp
Lanes (5, 6 and 7): Amplified *C. perfringens* enterotoxin genes (cpe) at 233bp

DISCUSSION

Food illness caused by *C. perfringens* is among the common illnesses resulting from the consumption of contaminated food, the vehicles of infection are typically meat and poultry products. It has been firmly established that an enterotoxin produced in the intestine following sporulation of ingested vegetative cells is responsible for the illness [33]. In recent decades many surveys have been conducted on the incidence of *C. perfringens* in raw and processed meat and poultry. These reports indicate widespread occurrence of the organism in meat and poultry [34-35].

In the present study, the prevalence of *C. perfringens* isolated from ready to cook meat products was 48.8% from the total examined samples (125), with the incidence of (60, 52, 28, 68 and 36%)from beef burger, meat kofta, minced meat, beef sausage and kobeba, respectively. Similar results were obtained by El-Lawendy mention author (s) [36] who recorded that the incidences of *C. perfringens* in beef burger and meat kofta were (60.3% and 50.85%) respectively. Lower results were reported by (Torky mention author (s) [37] who recorded that the incidences of *C. perfringens* in meat kofta and minced meat were (45% and 35%), respectively} Put this sentence between red brackets at XX}. El-Lawendy Mention author (s) [36] and Sharma author (s) [38] found that the incidence of *C. perfringens* in beef sausage was 62%. Our results are also in agreement with Wen and McClane [39] who detected *C. perfringens* a contamination rate ranging between 20 to 40% in all meats and sea food.

In the current work, the prevalence of *C. perfringens* from ready to eat meat products was 21.3% from the total examined samples (75), with the incidences of (16, 28 and 20%) in basterma, canned beef and luncheon, respectively. These results agree with El-Lawendy [36] who recorded the incidence of *C. perfringens* in basterma and luncheon to be (16% and 25.53%). Also, Khater [40] isolated *C. perfringens* from basterma and luncheon at rates of (20%) and (5- 20%) and Torky [37] recorded *C. perfringens* isolation rate from luncheon of 25%.

Typing of *C. perfringens* revealed that the incidence of toxigenic and non toxigenic strains were (89.6 and 10.4%) respectivelyand type A was the most predominant typeHigher results were obtained by El-Lawendy [36] who reported incidence of 37.93% of type A & 31.03% of mixed types in kofta and 25% of type A and type D in basterma. Also, Khater [40] isolated *C. perfringens* type A and D from basterma with incidences of 66.6 & 33.3%, respectively and 25 & 75% from luncheon. Khairy [41] which isolated 60% of type A, 35% of type D and 20% of mixed types from beef burger. Shalaby and Elmahrouk [42] collected 169 camel meat samples from a butcher's shop and they identified *C. perfringens* type A with an incidence of 33.7%.

In the present investigayion, the mean *C. perfringens* counts of beef burger, meat kofta, minced meat, beef sausage, kobeba, basterma, canned beef and luncheon were $3.2x10^3$, $4.5x\ 10^4$, $1.2x\ 10^3$, $1.2x\ 10^3$, $2.3x\ 10^3$, 4.5x 10, $2.8x\ 10^2$ and 2.1x 10 CFU /g, respectively. On the other hand, lower result was obtained by Hassan [43] who recorded that the mean value of *C. perfringens* count in kofta was about 2.1×10^4 organisms /g. and Higher results were recorded by Torky [37] who reported mean count of $9.3x10^4$ organisms /g in kofta and ??Eleiwa [44] recorded mean count value in beef burger about $7.25x\ 10^3$ /g. Torky [37] and El-Mossalami [45] recorded counts of *C. perfringens* in beef burger of (1.2×10^4 and $6.7x10^4$ CFU/g.), respectively. While, Taman [46] failed to detect *C. perfringens* in canned beef and luncheon.

Results of the present studyshowed that enterotoxigenic strains of *C. perfringens* were isolated from beef burger, meat kofta, minced meat, beef sausage, kobeba, basterma, canned beef and luncheon at rates of 28.5, 16.7, 0, 30, 33.3, 0, 33.3 and 0%, respectively. Miwa *et al.* [47] found that enterotoxigenic strains of *C. perfringens* were present in an average of 37% of meat and poultry samples. Singh *et al.* [48] reported incidences of enterotoxigenic *C. perfringens* of 9.3, 32.4 and 15.5% in buffalo, goat and poultry meat, respectively. Meanwhile Shalaby and Elmahrouk [42] detected enterotoxin in camel meat samples with an incidence of 5.9% of *C. perfringens* isolates. On the other hand, Satio [20] reported incidence of enterotoxgenic *C. perfringens* strains from meat and fish of only 2%.

In the present work, the use of PCR revealed specificity of the oligonucleotide primers which was confirmed by the positive amplification of 1167bp fragments for *C. perfringens* alpha genes (cpa) and 233bp fragments for *C. perfringens* enterotoxin genes (cpe) from DNAs extracted from the collected meat products samples. These results agree with Singh, *et al.* [48] and Zheng *et al.* [49] who reported that the Immunomagnetic Separation Polymerase Chain Reaction (IMS-PCR) results gave a good agreement with the results obtained by conventional culture methods. In comparison to conventional culture methods, the IMS-PCR is a rapid and specific method and has potential use as a screening tool for enterotoxigenic *C. perfringens* in food samples. It can be concluded that the anaerobic counts of the examined samples were within the permissible limits requested by the Egyptian standard specification (Please mention these limits) and not enough to induce food poisoning in human, since millions of viable *C. perfringens* are required to induce food poisoning in human (10^6 microorganisms / g.). PCR assay is suitable for detection of *C. perfringens* alpha gene and enterotoxin gene directly from meat product samples.

REFERENCES

1. Hammed, M.I., 2001. Chemical evalution of non meat ingredients in some meat product. Ph. D. Thesis, Faculity of Veterinary Medicine, Cairo Univ., Egypt.
2. Basyoni, S.R., 2003. Studies on incidence of yeasts in some meat products. Egyptian Journal of Veterinary Medical Association, 63: 205- 211.
3. Hassan, M.A. and J.R. Daoud, 1997. Meat additive profiles of Egyptian meat products with relation to public health,. Alexandria. Journal of Veterinary Sci., 13: 821-828.
4. Schmidt, V. and Z. Bem, 1978. How should a meat products factory cleaned and disinfected and how should the effects of these processes be controlled. Die Fleischwirschaft, 91: 1442.
5. Costa, F.M., 1979. Microbiological contamination in food processing plant. Preventive measures. Rivista Della Societa Italiana Discienzadella Alimentazion, 8: 258.
6. Stiles, M.E. and L.K. Ng, 1981. Enterobacteriaceae associated with meats handling. Applied Journal of Environment Microbiol., 41: 867- 873.
7. El-Sayed, A.E., 2006. Organoleptic and chemical evalution of some locally manufactured beef burger. M.V. Sc. Thesis, Meat Hygiene, Faculty of Veterinary Medical Moshtohor, Benha University. Egypt.
8. Okolocha, E.C. and L. Ellerbroek, 2005. The influence of acid and alkaline treatments on pathogens and the shelf life of poultry meat. Food Control, 16: 217-225.
9. Cunningham, F.C., 1982. Microbiological aspects of poultry and poultry products. An update. Journal of Food Protection, 45: 1149-1164.
10. Mead, G.C., 1989. Hygenic problems and control of process contamination. In G.C. Mead, (Ed.), processing of poultry: 183-221. London: Elsevier, Applied Science.
11. Libby, T.A., 1975. Meat Hygiene 4th Ed. Lea and Febiger, Philadelphia, U.S.A.
12. Hanninen, M.L., 1980. *E. coli*, Y. Enterocolitica, C. fetus spp. *Jejuni* causing food borne disease. Areview soumer Elainl Alkavilet 86: 175.
13. FAO / WHO, 1983. WHO surveillance programme for control of food borne infection and intoxication in Europe. Inst. Veterinary Medical 2nd report. Berlin.
14. Chesnut, C.M., B.S. Emswiler and A.W. Kotula, 1977. Bacteriological quality of ingredients used in ground beef manufacture. Journal University of Toronto press, Toronto and Buffalo, Canada.
15. Teufel, P., G. Goetz and D. Grossklaus, 1982. Effect of factory hygiene and raw material on the microbiological status of minced meat. Fleischwirtsch, 62: 1404.
16. Nouman, T.F., A. El-Nawawy, A.M. El-Ashmawy and A. Sorur, 1982. The microbial association in cool-stored fresh sausage. Assiut Veterinary Medical Journal, 10: 137.
17. Barnes, E.M., 1985. Isolation methods for anaerobes in foods. International Journal of Food Microbiol., 33: 77-82.

18. Hayes, P.R., 1992. Food Microbiology and Hygiene. 2nd Edition Elsevier Applied Science London, New York.
19. Norris, J.R. and G.L. Pettipher, 1987. Essays in Agricultural and Food Microbiology.
20. Satio, M., 1990. Production of enterotoxin by *C. perfringens* derived from humans, animals, foods and the natural environment in Japan. Journal Food Prot., 53: 115-118.
21. Smith, L.D. and L.V. Holdeman, 1968. The pathogenic anaerobic bacteria. pp: 201-205. 1st Ed., Charles C. Thomas, Spring Field, USA.
22. Koneman, K.W., S.D. Allen, V.R. Dowell and H.M. Sommers, 1992. Color atlas and text book of diagnostic microbiology. 2nd edition, B. Lippicott Co., London.
23. Collee, J.G., A.G. Fraser, B.P. Marmion and A. Simmons, 1996. Practical medical microbiology. 14th education, Churchill Living Stone, New York.
24. Stern, D.H. and I. Batty, 1975. Pathogenic clostridia 1st ed. Butterworth. London, U.K.
25. Giannella, R.A., 1976. Suckling mouse model for detection of heat stable Escherichia coli enterotoxin characteristics of the model. Infect Immune, 14: 95-99.
26. Duncan, C. and D.H. Strong, 1968. Improved medium for sporulation of *C. perfringens.* Applied Microbiol., 16: 82-89.
27. Cruickshank, R., I.P. Duguid, B. Marmion and R.H. Swain, 1975. Medical microbiology. 12th ed., Livingstone, Iowa, New York.
28. APHA, 1992. Standard methods for the examination, of dairy products. American Public Health Association 16th ed. Washington, DC.
29. Garmory, H.S., N. Chanter, N.P. French, D. Bueschel, J.G. Songer and R.W. Tiball, 2000. Occurrence of *C.perfringens* β2 toxin amongst animals, determined using genotyping and subtyping PCR assays. Epidemology Infectious, 124: 61-67.
30. Saint-Joanis, B., T. Garmer and S.T. Cole, 1989. Gene cloning shows the alpha toxin of *C. perfringens* to contain both shipinogomycinase and lecithenase activities. Molecular and General Genetics, 219: 253-260.
31. Meer, R. and J.G. Songer, 1997. Multiplex polymerase chain reaction assay for genotyping Clostridium perfringens. American Journal of Veterinary Reserch, 58: 702-705.
32. Fach, P. and J.P. Guillou, 1993. Detection by in vitro amplification of the alpha- toxin (phospholipase C) gene from *C. perfringens*. Journal of Applied Bacteriol., 74: 61-66.
33. Duncan, C.L., 1973. Time of enterotoxin formation and release during sporulation of *Clostridium perfringens* type A. Journal of Bacteriol., 113: 932-936.
34. Labbe, R.G. and M.P. Doyle, 1989. Clostridium perfringens In: Food borne Bacterial Pathogens, New York and Basel Marcel Decker Inc., New York, pp: 191-227.
35. Labbe, R.G., B.M. Lund, T.C. Baird- Parker and G.W. Gould, 2000. *C. perfringens*. In: The Microbiological Safety and Quality of Food. Vol II. Aspen Pub. Gaithersburg, Maryland, pp: 1110-1135.
36. El-Lawendy, T.M.H., 1996. Occurance of *C. perfringens* in locally produced meat products. M.V.Sc. Thesis (Food hygein and control) zagazig University, Benha Branch.
37. Torky, A.A. and A. Sh, 2004. Trials for inhibition of some food poisoning microorganisms in meat products. Ph. D.V. Thesis Fac. Vet. Med. (Cairo Univ.)
38. Sharma, N.K., S.S. Saini, J.P.S. Gill and M.S. Kwatra, 1993. Occurrence of *C. perfringens* in uncooked cock tail sausage at retail level and its public health significance. Indian Journal of Animal Sci., 63: 112-114.
39. Wen, Q. and B.A. McClane, 2004. Detection of Clostridium perfringens type Aisol*ates in Americ*an Retail Foods. Applied Environment Microbiol., 70: 2685-2691.
40. Khater Khater, F. Dalia, 2004. Studies on microbial toxin in some vacuum packed meat products. Ph.D.Thesis Faculty Veterinary Medicine, Zagazig Univ.
41. Khairy, I.G. Dalia, 2005. Studies on *C.perfringens* in some meat product. Ph.D. Thesis Faculty of Veterinary Medicine, Cairo Univ.
42. Shalaby, B. and A.M. Elmahrouk, 2006. Detection of enterotoxigenic C*losteridium p*erfringens type A in camel meat. Veterinary Medical Journal Giza., 54: 179-188.
43. Hassan, H.F.M., 2001. Study on Gram positive bacteria in meat products. Ph. D. Thesis Veterinary Medical Beni- Suef. Univ.
44. Eleiwa, N.Z., 2003. Effect of chemical preservatives on food poisoning bacteria in some locally manufactured meat products. Ph. D. V. Sc. Thesis, Vet. Medical Zagazig Univ. Benha branch.
45. El-Mossalami, E., 2003. Risk assessment ready prepared meat products. Ph.D. Thesis, Faculty Veterinary Medicine, Cairo Univ.

46. Taman, L.R., 2003. Incidence of anaerobic in canned and cooked meat product. M. V. Sc. Thesis, Faculty Veterinary Medicine Tanta Univ. Kafer El-Sheikh Branch.
47. Miwa, N., T. Nishina, S. Kubo, M. Atsumi and H. Honda, 1998. Amount of enterotoxigenic Clostridium perfringens in meat detected by nest PCR. International Journal of Food Microbiol., 12(42): 195-200.
48. Singh, R.V., K.N. Blilegaonkar and R.K. Agarwal, 2005. Studies on occurrence and characterization of Clostridium perfringens from select meats. Journal of Food Safety, 25: 146-156.
49. Zheng, Y.Y., B.S. Won, Y.K. Kyeong and H.C. Duck, 2010. Rapid Detection of Enterotoxigenic Clostridium perfringens in Meat Samples Using Immunomagnetic Separation Polymerase Chain Reaction (IMS-PCR). Journal Agriculture Food Chem., 58: 7135-7140.