Incidence of some anaerobic bacteria isolated from chicken meat products with special reference to *Clostridium perfringens*

Shaltout, F. A.[1]; Zakaria, I. M.[2]; Nabil, M.E.[3].

[1]Food hygiene Dept., Faculty of Veterinary Medicine, Benha University; [2]Animal Health Research Institute (El-Dokki - Bacteriology Dept.); [3]Animal Health Research Institute (Benha Branch – Food Control Dept.)

A B S T R A C T

Anaerobic spore formers, especially *Clostridium perfringens*, represent one of the most prevalent bacterial food poisoning outbreaks which mostly related to consumption of contaminated meat and meat products, therefore, a total of 125 random raw and half cooked chicken meat samples represented by (breast, thigh, nuggets, panée and frankfurter "25 of each") were collected from various retail stores and supermarkets in Qualyubia governorate. Results illustrated that, raw thigh samples were the most contaminated with anaerobic bacterial counts in incidence of 84%. The identified strains were *C. perfringens, C. sporogenes, C. bifermenants, C. butyricum and C. sordelli* in 21.6, 16, 8, 3.2 and 3.2%, respectively. Regarding to the incidence of vegetative and spore of *C. perfringens* were 24, 32, 20, 16, 16% and 16, 20, 16, 8, 8% in examined raw breast, raw thigh, nuggets, panée and frankfurter, respectively. 33.3% of isolates were lecithinase positive strains and typed as *C. perfringens* type A (6.4%), type D (0.8%); in absence of neither type B nor D. Experimental heat resistant *C. perfringens* spores were six heat resistant strains; where all isolates were of type A. The high incidence of these food poisoning microorganisms in chicken meat may indicate defects in sanitary conditions and handling in processing plant.

Keywords: chicken meat, *Clostridium perfringens*, heat resistant spores, Nagler's reaction, other anaerobes.

1. INTRODUCTION

Rapid reproductive cycle, high acceptability of poultry meat due to its high biological value, palatability and many production processing variables; made poultry production one of the major worldwide food industry. Selection of broiler chickens has been primarily directed at economic traits which have reduced costs of production.

In Egypt, Chicken represents the major brand of production and consumption among poultry. Chicken meat becomes the most popular meat eaten due to its reliable price, health benefits and good flavor. Chicken meat is easily prepared, consistent quality and wide ranged pre-packed, raw and ready to eat products (Shedeed, 1999).

Poultry and poultry products are subjected to contamination with several types of microorganisms from different sources from the time of rearing, slaughtering till consumption. Such contamination may render the product inferior quality or even contributed in public health hazards.

Any defect of the hygienic measures in the slaughtering houses and/or processing plants leads to microbial contaminations, which cause serious diseases for the consumer. Thus, raw poultry products are reported to be responsible for a significant number of cases of human food poisoning (Georgnaras *et al.,* 1995).

In processing plants, contamination of poultry meat products may be recorded throughout initial processing, packaging and storage until the product is sufficiently cooked and consumed. Heavy bacterial loads enter the processing operations with the living birds or raw materials can be disseminated throughout the plant during processing. Food poisoning may occur when these products not properly cooked or due to post-processing contamination (Zhang *et al.,* 2001).

Regarding to slaughtering abattoirs and processing plants hygiene, the presence of pathogenic and spoilage microorganisms in poultry meat and its products represent a significant concern for suppliers, consumers and public health officials worldwide. Bacterial contamination of food products is undesirable but unavoidable; it depends on the initial bacterial load of the fresh raw materials, hygienic practices during manufacturing and on time/temperature factor (El-Bassuony, 2008).

Foodborne infection and intoxication outbreaks are increasing especially in industrial and developing countries, where bacterial foodborne infection is the major reported cases (Stevenson and Bernard, 1995); where anaerobic spore formers bacteria are considered as one of the causative agents of poultry meat borne infection. Clostridia have been incriminated in many anaerobic infections by producing toxins that are able to damage tissues of the nervous system as well as lead to inflammation and even destroy the wall of the large and small intestine, this condition is called necrotizing enterirtis, this infection may be occurred as an isolated case or may be considered as outbreaks caused by consumption of contaminated meat (Varnam and Evans, 1991).

C. perfringens is a ubiquitous pathogen and natural intestinal inhabitant of poultry, different stages of poultry processing line can add a contamination source even starting from the hatchery. Chicken carcass and meat cuts may also be contaminated with *C. perfringens* from intestinal contents during slaughterhouse process especially during evisceration (Voidarou *et al.,* 2011).

Moreover, *C. perfringens* is a common foodborne pathogen associated with food poisoning, gas gangrene, and infectious diarrhea in human. Because of its ability to form a spore, this microorganism is able to survive adverse conditions such as aerobic and food processing procedures. *C. perfringens* causes food poisoning post-ingestion, because a large number of vegetative cells can survive acidic pH of the stomach, then sporulate and produce an enterotoxin in the small intestine (Santos *et al.,* 2002).

Therefore, the current study was plamed for monitoring of anaerobic spore formers especially *C. perfringens* in raw and half cooked chicken meat products.

2. MATERIAL AND METHODS

2.1. Collection of samples:

A total of 125 random samples of fresh raw and partially cooked chicken meat products represented by chicken breast, chicken thigh, chicken nuggets, chicken panée and chicken frankfurter (25 of each), these were collected from different retail groceries and supermarkets in Qalyubiya governorate

for bacteriological examination. All the collected samples were subjected to the following examination.

2.2. Preparation of the samples:

It was done according to (APHA, 1992)

2.3. Determination of total anaerobic bacterial count:

It was done according to (Roberts et al., 1995) using reinforced clostridial agar media.

2.4. Determination of viable Clostridium perfringens:

It was done according to (ISO, 2004) using TSC media.

2.5. Determination of Clostridium perfringens spores:

It was done according to (Weiss and Strong, 1967) using Clostridium perfringens agar plate media.

2.6. Isolation of Clostridium perfringens:

It was done according to (Carter and Cole, 1990) using cooked meat media and 10% sheep blood agar.

2.7. Identification of Clostridium perfringens:

It was done according to (MacFaddine, 1980 and Cato et al. 1986).

2.7.1. Staining:

It was done according to (Cruickshank et al., 1975).

2.7.2. Cultural characteristics:

It was done according to (Cruickshank et al., 1975):

2.7.2.1.Cooked meat media.

2.7.2.2. Sheep blood agar media.

2.7.2.3. Egg yolk agar media (Nagler's reaction).

2.7.2.4. Nutrient gelatin media.

2.7.3. Biochemical reactions.

2.7.3.1. Nitrate reduction test:

It was done according to (Willis, 1977).

2.7.3.2. Zinc Test:

2.7.3.3. Indole production test:

It was done according to (MacFaddine, 1980)

2.7.3.4. Hydrogen sulphid test:

It was done according to (MacFaddine, 1980).

2.7.3.5. Sugar fermentation test;

It was done according to (Willis, 1977).

2.7.4. Neutralization test in Swiss mice:

It was done according to (Smith and Holdeman, 1968).

2.7.5. Determination of C. perfringens toxin by dermonecrotic test:

It was done according to (Sterne and Batty, 1975).

2.7.5.1.*Preparation of toxin and their treatment:*

It was done according to (Bullen, 1952).

2.7.5.2.Application of the typing test:

It was done according to (Oakley and Warrack, 1953): the results were interperitated by the degree of dermonecrotic reaction and its neutralization according to Sterne and Batty (1975).

2.8. Detection of C. perfringens heat resistant spores:

2.8.1. Preparation of *C. perfringens* spore suspension:

It was done according to (Ellner, 1956).

2.8.2. Determination of heat spore resistance:

It was done according to (Hussein, 1997).

2.9. Statistical analysis:

The obtained results were statistically evaluated by application of Analysis of Variance (ANOVA) test according to Feldman et al. (2003).

3. RESULTS

Results as tabulated in table (1) revealed that examined raw thigh samples were the most contaminated with anaerobic bacterial count in prevalence of 84%, followed by breast, nuggets, panée and frankfurter in 76, 48, 48 and 40%, respectively.

Also, results demonstrated in table (2) showed the incidence of isolation and identification of anaerobic isolates revealed detection of C. perfringens, C. sporogenes, C. bifermenants, C. butyricum and C. sordelli in 21.6, 16, 8, 3.2 and 3.2% of examined samples, respectively.

As shown in table (3) results illustrated that the incidence of vegetative form C. perfringens were 24, 32, 20, 16 and 16%; while in table (4) that in spore form C. perfringens was 16, 20, 16, 8 and 8% in examined raw breast, raw thigh, nuggets, panée and frankfurter samples, respectively. From these isolates 33.3% were lecithinase positive strains as recorded in table (5). There were significant differences between breast and thigh as raw samples; and between raw examined samples and half cooked samples. In reference to (EOS, 2005); 20 and 24% of examined nuggets and panée samples were rejected those were exceeding the permissible limits of total anaerobic counts. 8, 28, 20, 16 and 16% of examined breast, thigh, nuggets, panée and frankfurter were rejected for C. perfringens cell counts.

Typing of toxigenic C. perfringens isolates results were recorded in table (6) proved C. perfringens type A in incidence of 6.4% followed by type D in incidence of 0.8%; in absence of neither type B nor D basing on classical bioassay.

Regarding to detection of heat resistant spores of C. perfringens isolated, results showed in table (7) revealed that six heat resistant strains were detected in prevalence of 4.8%; where all isolates were classified as type A.

Table (1) total anaerobic count/g of the examined chicken meat product samples (n=25).

Sample	Positive samples		Count of cfu/g			EOS, 2005	Rejected samples	
	NO.	%	Min.	Max.	Mean ± SE*		No.	%
Raw chicken meat								
Chicken breast	19	76	$1.4x10^3$	$2.3x10^4$	$1.05x10^4 \pm 1.4x10^{3b}$	-	-	-
Chicken thigh	21	84	$2.5x10^3$	$6.8x10^4$	$2.8x10^4 \pm 4.0x10^{3a}$	-	-	-
Half cooked chicken meat products								
Chicken nuggets	12	48	$1.5x10^2$	$1.8x10^3$	$8.4x10^2 \pm 9.2x10^c$	10^2	5	20
Chicken panée	12	48	$1.6x10^2$	$1.4x10^3$	$6.8x10^2 \pm 7.0x10^c$	10^2	6	24
Chicken frankfurter	10	40	$2.0x10^2$	$9.8x10^2$	$5.3x10^2 \pm 4.9x10^c$	10^2	0	0
Total	74	59.2	-	-	-	-	11	8.8

a.b.c.= significant difference sympols (p>0.05).
EOS, 2005: No. 1651 for chilled raw poultry and rabbit meat, No. 3492 for chicken frankfurter, and No. 3493 for heat treated poultry meat products.

Table (2): Incidence of anaerobic spore former other than *Clostridium perfringens* in examined chicken meat products (n=25).

Clostridia species / Samples	*C. sporogenes*		*C. bifermenants*		*C. butyricum*		*C. sordelli*	
	No.	%	No.	%	No.	%	No.	%
Raw chicken meat								
Chicken breast	3	12	2	8	1	4	0	0
Chicken thigh	5	20	4	16	2	8	1	4
Ready to cook								
Chicken nugget	5	20	1	4	0	0	1	4
Chicken pane	4	16	2	8	0	0	2	8
Chicken frankfurter	3	12	1	4	1	4	0	0
Total	20	16	10	8	4	3.2	4	3.2

Table (3): Statistical analysis of *Clostridium perfringens* (vegetative form) count/g of the examined chicken meat product samples (n=25).

Samples	Positive samples		Count of cfu/g			EOS, 2005	Rejected samples	
	NO.	%	Min.	Max.	Mean±SE*		No.	%
Raw chicken meat								
Chicken breast	6	24	$5.2x10^2$	$2.07x10^4$	$9.1x10^3 \pm 2.7x10^{3b}$	10^3	2	8
Chicken thigh	8	32	$1.2x10^3$	$5.03x10^4$	$2.5x10^4 \pm 5.7x10^{3a}$	10^3	7	28
Half cooked chicken meat products								
Chicken nuggets	5	20	$2.4x10^2$	$1.2x10^3$	$5.6x10^2 \pm 1.7x10^{2ab}$	Free	5	20
Chicken panée	4	16	$1.9x10^2$	$1.1x10^3$	$6.9x10^2 \pm 2x10^{2ab}$	Free	4	16
Chicken frankfurter	4	16	9x10	$7.5x10^2$	$4.1x10^2 \pm 1.4x10^{2ab}$	Free	4	16
Total	27	21.6	-	-	-	-	22	17.6

a.b.ab.= significant difference sympols (P>0.05).

EOS, 2005: No. 1651 for chilled raw poultry and rabbit meat, No. 3492 for chicken frankfurter, and No. 3493 for heat treated poultry meat products.

Table (4): Statistical analysis of *Clostridium perfringens* (spore form) count/g of the examined chicken meat product samples (n=25).

Samples	Positive samples		Count of cfu/g		
	NO.	%	Min.	Max.	Mean ± SE.
Raw chicken meat					
Chicken breast	4	16	$1.2x10^2$	$2.2x10^3$	$9.7x10^2 \pm 4.4x10^2$
Chicken thigh	5	20	$1x10^2$	$1.9x10^3$	$8.4x10^2 \pm 3x10^2$
Half cooked chicken meat products					
Chicken nuggets	3	16	3.2x10	$2.3x10^2$	$1.4x10^2 \pm 5.9x10$
Chicken panée	2	8	3.6x10	$1.5x10^2$	9.3x10±5.7x10
Chicken frankfurter	2	8	1.9x10	$1.1x10^2$	6.4x10±4.5x10
Total	16	12.8	-	-	-

Table (5): Incidence of Lecithinase positive strains of *C. perfringens* in the examined chicken meat product samples (n=25).

samples	Number of isolates		Lecithinase positive		Lecithinase negative	
	NO.	%	NO.	%	NO.	%
Raw chicken meat						
Chicken breast	6	24	2	33.3	4	66.6
Chicken thigh	8	32	3	37.5	6	62.5
Half cooked chicken meat products						
Chicken nuggets	5	20	1	20	4	80
Chicken panée	4	16	2	50	2	50
Chicken frankfurter	4	16	1	25	3	75
Total	27	21.6	9	33.3	18	66.6

Table (6): serotyping of toxigenic *Clostridium perfringens* strains isolated from chicken meat product samples.

Poultry meat product samples	No. of toxigenic isolates	Types of isolates							
		A		B		C		D	
		No.	%	No.	%	No.	%	No.	%
Raw chicken meat									
Chicken breast	2	2	100	0	0	0	0	0	0
Chicken thigh	3	2	66.6	0	0	0	0	1	33.3
Half cooked chicken meat products									
Chicken nuggets	1	1	100	0	0	0	0	0	0
Chicken panée	2	2	100	0	0	0	0	0	0
Chicken frankfurter	1	1	100	0	0	0	0	0	0
Total	9	8	6.4*	0	0	0	0	1	0.8*

** incidence of toxigenic strains in relation to total number of samples (125).*

Table (7): Incidence of heat resistant strains of *C. perfringens* isolates and its typing (n=25).

Samples	Heat resistant positive samples		Typing of heat resistant C. perfringens isolates							
			A		B		C		D	
	No.	%	No.	%	No.	%	No.	%	No.	%
Raw chicken meat										
Chicken breast	1	4	1	100	0	0	0	0	0	0
Chicken thigh	2	8	2	100	0	0	0	0	0	0
Half cooked chicken meat products										
Chicken nuggets	0	0	0	0	0	0	0	0	0	0
Chicken panée	2	8	2	100	0	0	0	0	0	0
Chicken frankfurter	1	4	1	100	0	0	0	0	0	0
Total	6	4.8	6	100	0	0	0	0	0	0

4. DISCUSSION

The modern revolutionary poultry industry made poultry meat available for large population of consumers, and considered a major source of animal protein supplement especially due to its nutritional, sensory, economic and consumer profitability characteristics. However, poultry meat may harbor different types of pathogenic microorganisms during different processing procedures. Anaerobic spore formers are one of implicated microorganisms in worldwide foodborne outbreaks especially C. perfringens which associated mainly to consumption of meat, poultry and its products.

Results illustrated in table (1) were in a great reliable to (Nabil et al., 2014) ($4.8x10^2$ cfu/g in frankfurter); and (Sobhy, 2016) ($5.6x10^3$ to $5.1x10^4$ cfu/g, with incidence of 40-66% in chicken meat). While recorded higher results than (Zakaria, 2005) results who reported the total anaerobic counts of examined chicken meat products were ranged from $2.3x10^2$-$5.5x10^3$cfu/g.

Microscopical and biochemical identification of other than C. perfringens isolates as illustrated in table (2) were recorded to be found in different examined chicken meat products as reported by (Zakaria, 2005) who detected C. sporogenes, C. butyricum, C. subterminalis in different examined chicken meat products; and (Sathish and Swaminathan, 2009) who isolated C. bifermentans from 40% of examined chicken meat samples.

Clostridium perfringens is considered as foodborne pathogen of public health importance due to its ability to produce many lethal and enterotoxins. C. perfringens food poisoning may occur after consumption of improper hot held cooked food or slowly cooled after preparation; where some heat resistant spores (100OC for more than 1h) can survive, subsequently spore germination and rapid multiplication leading to food poisoning (Simjee and poole, 2007).

Table (3&4) results were in agree with (Edris et al., 1992) who reported the highest C. perfringens prevalence in examined thigh samples followed by breast and frankfurter samples; (Zakaria, 2005) who recorded isolation of C. perfringens (vegetative and spore form) in examined chicken breast, thigh and frankfurter samples in prevalence of 25, 35, 10%, respectively; (Emara, 2014) (30% in examined fillet); (Nabil et al., 2014) (13.3% in frankfurter with count of 3.6x102 cfu/g); (Kamal, 2017) who detected C. perfringens (vegetative and spore form) in count of 1.5x104 and 1.58x102 cfu/g, respectively in chicken meat. On contrast, results were lower than that reported by (Salah El-din et al., 2015) who detected C. perfringens in 79.6% of examined samples; while, higher than those reported by (Thangamani and Subramanian, 2012) who detected C. perfringens in 3.81% of examined samples. Moreover, reported results were disagreed with (Hashem, 2015) and (Ibrahim-Hemmat et al., 2015) who failed to detect C. perfringens in any examined chicken meat sample.

Differences may be attributed to difference in effectiveness of hygienic measures during processing practices, handling from production to consumption; high contamination of raw materials; addition of additives, spices

and preservatives as well as the conditions occurred before and after slaughtering of the birds affect the bacterial load in these products.

Only C. perfringens type A produces the alpha-toxin and phospholipase C (PLC). This exotoxin has the distinction of being the first bacterial toxin to which an enzymatic activity, lecithinase enzyme; inoculation of C. perfringens type A with lecithinase activity on egg yolk agar produce an opalescent change around the colonies due to enzymatic action of lecithin in the medium. Those producing a lipase cause a pearly layer or iridescent film that can cover the colonies and in some cases, extend into the surrounding agar (Markey et al., 2013).

Lecithinase activity of C. perfringens isolates as tabulated in table (5) were nearly similar to (Sobhy, 2016) who reported 27.2% of C. perfringens isolates were lecithinase positive, while (Kamal, 2017) reported higher results where 66.6% of examined isolates were lecithinase positive.

Prevalence and typing of toxigenic C. perfringens results as typed in tables (6) were in agree with (Torky and Hassan, 2014) who recorded that traditional typing of C. perfringens isolates revealed 8 (6.4%) of type A and 1 (0.8%) of type D, while failed to detect either type B or C.

Clostridium perfringens type (A) is usually contributed in worldwide food poisoning outbreaks. Symptoms appear within 6 to 24 hours after consumption of contaminated food characterized by acute abdominal cramps, watery diarrhea, nausea, and rarely fever with vomiting especially in children and elderly persons. Furthermore, chicken dishes are commonly involved in such outbreaks particularly when prepared and held long period before consumption, so the hot cooking of such food is usually presumably inadequate to destroy the heat resistance endospores leading to release of enterotoxin by C. perfringens cells undergoing sporulation in the lower part of gastrointestinal tract (Mossel et al., 1995); (McClane and Rood, 2001). However, C. perfringens type A common contribution in food poisoning, type (D) has been implicated in food poisoning cases which produce symptoms resembled that produced by other food poisoning pathogens as recorded by (Kohn and Warrack, 1955).

Table (7) discussed number and prevalence of heat resistant C. perfringens spores isolates; results were in agree with (Kudaka et al., 2005) who reported that food poisoning C. perfringens spores differed from those vegetative cells in respect to its heat resistance; where they can survive cooking at high temperature (100OC for >2h); while lower than (Zakaria, 2005) who notified heat resistant C. perfringens in 15% of examined isolates, where C. perfringens type A was predominant (66.6%) followed by type D (33.3%).

Poultry meat and meat products may be considered as a major source of anaerobic bacteria especially C. perfringens, which may get contamination through many different ways; raw poultry meat samples exhibited higher C. perfringens contamination levels starts with thigh sample, followed by breast, nuggets, panée and frankfurter samples, respectively. High counts of anaerobic spore forming bacteria especially C. perfringens may render these types of

food of inferior quality or even become harmful for the consumers, so restrict hygienic measures should be applied during different stages of chicken processing till consumption.

5. REFERENCES

American Public Health Association (APHA), 1992. "Compendium of methods for the microbiological examination of foods. 3rd Ed. APHA Technical Committee on Microbiological Methods for Foods. Washington, D. C. USA.

Bullen, J.J. 1952. "*Clostridium welchii type D* in the alimentary tract of normal sheep". J. Path. Bact., 64:201- 210.

Carter, G.R.; Cole, J.R. 1990. "Diagnostic procedures in veterinary bacteriology and mycology". 5th Ed., Academic Press, Harcourt, Boace. J., New York, Boston, Tokyo, Toronto.

Cato, E.P.; George, W.L.; Finegold, S.M. 1986. "Bergeys manual of systematic bacteriology, Vol.2. Williams and Wilkins, Baltimore, USA.

Cruickshank, R.; Duguid, J.P.; Marmion, B.P.; Swain, R.H. 1975. "Medical Microbiology". 2nd Ed., Vol.2. The Practice of Medical microbiology, Churchill Living Stone Edinburgh.

Edris, A. M.; Abdel Rahman, M.; Mousa, M.M. 1992. "Microbial evaluation of some related cut-up chicken and poultry meat product". Benha Vet. Med. J., 3(1): 154-165.

Egyptian Standard Specifications (EOS) 2005. No. 1651 for chilled raw poultry and rabbit meat, No. 3492 for chicken frankfurter, and No. 3493 for heat treated poultry meat products.

El-Bassuony, R.A. 2008. "Bacterial evaluation of frozen poultry and some poultry products in Luxor City". J. Egypt. Vet. Med. Assoc., 68 (4): 295-307.

Ellner, P.D. 1956. "A medium promoting rapid quantitative sporulation in *Clostridium perfringens*". J. Bact., 71: 495-596.

Emara, M.S. 2014. "Anaerobic and aerobic microorganisms in human food". M. V. Sc. Thesis, Fac. Vet. Med., Cairo Univ., Egypt.

Feldman, D.; Ganon, J.; Haffman, R.; Simpson, J. 2003. "The solution for data analysis and presentation graphics. 2nd Ed., Abacus Lancripts, Inc., Berkeley, USA.

Geornaras, I.A.; De Jesus, E.V.; Von Holy, A. 1995. "Microbiological survey of a South African poultry processing plant". J. Basic Microbiol, 35: 73-82.

Hashem, H.M. 2015. "Bacteriological criteria of dressed poultry with special reference to some microbial decontaminators". M. V. Sc. Theis, Fac. Vet. Med., Benha Univ., Egypt.

Hussein, A.Z. 1997. "The physical condition of cattle before slaughtering and its relationship to probable isolation of Clostridium perfringens from carcasses".

Ibrahim-Hemmat, M.; Amin-Reham, A.; El-Shater, M.A.; Hafez-Salwa, M. 2015. "Bacteriological evaluation of freshly slaughtered chicken carcasses". BVMJ, 28(2): 74-82.

ISO "International Standard Organization" 2004. "Microbiology of food and animal feeding stuffs-horizantal method for the enumeration of *Clostridium perfringens*-colony count technique". Ref. No. ISO 7937:2004.

Kamal, A. 2017. "*Clostridium perfringens* in meat and chicken received in University hostel". M. V. Sc. Thesis, Fac. Vet. Med., Benha Univ., Egypt.

Kessel, A.S.; Gillespie, I.A.; O′ Brien, S.J.; Adak, G.K.; Humphrey, T.J.; Ward,

L.R. 2001. "General outbreaks of infectious intestinal disease linked with poultry, England and wales, 1992-1999". Common Dis. Public Health, 4 (2):171-177.

Kohn, J.; Warrack, G.H. 1955. "Recovery of *Clostridium perfringens type D* from man". Lancet, 1: 385-390.

Kudaka, J; Horikawa, K; Uryu, K; Matsuyuki, S; Ogata, K; Kawano, K; Yamaguchi, Y; Yamasaki, S; Watanabe, H.; Iwanaga, M. 2005. "Symptoms of foodborne diseases and gastroenteritis in Kyushu, Japan". Kansenshogaku Zasshi, 79: 864-870.

MacFaddine, F. 1980. "Biochemical tests for identification of medical bacteria". 2nd Ed., Williams and Wilkins, Baltimore, USA.

Markey, B.K.; Leonard, F.C.; Archambault, M.; Cullinane, A.; Maguire, D. 2013. "Clinical Veterinary Microbiology". 2nd Ed., Mosby Elsevier Press, London, New York, Oxford; p. 217-220.

McClane, B.A.; Rood, J.I. 2001. "Clostridial toxins involved in human enteric and histotoxic infection". In: *Clostridia*: Biotechnology and Medical Applications. Bahl H, Dürre P. (Eds.), Wiley-VHC Weinheim, p. 169-220.

Mossel, T.A; Corry, J.L.; Struijk, C.B.; Beird, R.N. 1995. "Essentials of microbiology of foods". John, New York.

Nabil, M.E.; Edris, A.M.; Shaltout, F.A.; Zakaria, I.M. 2014. "Effect of some preservatives on bacterial load of some poultry meat products". Benha Vet. Med. J., 26(1): 94-103.

Oakley, A.; Warrack, S. 1953. Routine typing of *Clostridium welchii".*J. Hyg. Gamb., 51:102- 107.

Roberts, D; Hooper, W.; Greenwood, M. 1995. "Practical food microbiology". Puteler and Tanar, London.

Salah-Eldin, A.H.; Fawzy, E.H.; Aboelmagd, B.A.; Ragab, E.A.; Bedawy, S. 2015. "Clinical and laboratory studies on chicken isolates of *Clostridium Perfringens* in El-Behera, Egypt". J. World's Poult. Res., 5(2): 21-28.

Santos, G.; Mirna, A.; Marivael, G.; Nerma, H. 2002. "Inhibition of growth enterotoxin production, and spore formation of *C. perfringens* by extracts of medicine plants". J. Food Prot., 65(10): 1667-1669.

Sathish, S.; Swaminathan, K. 2009. "Genetic diversity among toxigenic clostridia isolated from soil, water, meat and associated polluted sites in South India". Indian J. Med. Microbiol., 27(4): 311-320.

Shedeed, N.A. 1999. "Evaluation of microwave cooking of chicken meat". M. Sc. Thesis Fac. of Agric., Cairo University.

Simjee, S.; Poole, T.L. 2007. "Foodborne diseases". Human Press, Totowa, New Jersy.

Smith, W.P.; Holdeman, L. 1968. "The Pathogenic Anaerobic Bacteria". 1st Ed. Pp. 201-255.

Sobhy, A.E. 2016. "Microbial status of meat and chicken received to university hostel". M. V. Sc. Thesis, Fac. Vet. Med., Benha Univ., Egypt.

Sterne, M.; Batty, I. 1975. Pathogenic Clostridia. Buthogenic, London, Baston.

Stevenson, K.E.; Bernard, D.T. 1995. "Establishing hazard analysis critical control point programs". A work shop manual, 2nd Ed., p. 4-15, 4-20. The Food Processors Institute, Washington. D. C.

Thangamani, A.; Subramanian, S. 2012. "Prevalence of *Clostridium perfringens* in the chicken meat rendered at retail outlets of Namakkal, Tamilnadu". Journal of Advanced Veterinary Research, 2: 157-159.

Torky, H.A.; Hassan, M.A. 2014. "Sequence analysis of alpha toxin produced by *C. perfringens* isolated from different

sources". Nature and Science, 12(1): 55-61.

Varnam, P.; Evans, M. 1991. "Food borne pathogens". An illustrated text book. Wolf publishing Ltd., New York, App., 101-108.

Voidarou, C.; Vassos, D.; Rozos, G.; Alexopoulos, A.; Plessas, A.; Tsinas, A.; Skoufou, M.; Stavropoulou, E.; Bezirtzoglou, E. 2011. "Microbial challenges of poultry meat production". Anaerobe, 17: 341-343.

Weiss, D.; Strong, H. 1967. "Some properties of heat resistant and heat sensitive strains of *Clostridium perfringens*". J. Bacterial., 93:21.

Willis, A.T. 1977. "Anaerobic Bacteriology Practice". 3rd Ed. Butter Worths, London.

Zakaria, I.M. 2005. "Anaerobic bacteria in chicken meat products". M.V.Sc. Thesis, Fac. Vet. Med., Zagazig University, Benha branch.

Zhang, L.; Davis, M.A.; Conner, D.E. 2001. "Poultry-borne pathogens: plant consideration". Poultry Meat Processing, ch. 9. ISBN 0-8493-0120-3, CRC Press LLC, New York, USA.