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Incidence of Some Anaerobic Bacteria Isolated from Chicken Meat Products with Special Reference to *Clostridium perfringens*

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Abstract

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, Egypt. Results illustrated that, raw thigh samples were the most contaminated with anaerobic bacterial countsin 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 andtypedasC. perfringens type A(6.4%), type D (0.8%); in absence of neither type B nor D. Experimental heat resistant *C. perfringensspores* 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 insanitary conditions and handling in processing plant.

Keywords: *Chicken meat; Clostridium perfringens,heat resistant spores; Clostridium species*

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Introduction

Rapid reproductive cycle, high acceptabilityof 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 Knowles., *et al*. (2008). 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 Rouger., *et al*. (2017).

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 occurred 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 cases or may be considered as outbreaks caused by consumption of contaminated meat Frey and Vileie (2003).

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 planned for monitoring of anaerobic spore formers especially *C. perfringens* in raw and half cooked chicken meat products.

Materials and Methods

Collection of samples

A total of 125 random samples of fresh raw and half 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, Egypt. All the collected samples were subjected to the following examination.

Preparation of the samples it was done according to APHA (1992)

Determination of total anaerobic bacterial count it was done according to Roberts., et al. (1995) using reinforced clostridial agar media.

Determination of viable Clostridium perfringens it was done according to ISO (2004) using Tryptose Sulphate Cycloserinemedia.

Determination of Clostridium perfringens spores it was done according to Weiss and Strong (1967) using Clostridium perfringens agar plate media.

Isolation of Clostridium perfringens it was done according to Carter and Cole (1990) using cooked meat media and 10% sheep blood agar.

Identification of Clostridium perfringens it was done according to Koneman., *et al.* (1992).

Staining it was done according to Cruickshank., *et al*. (1975).

Cultural characteristics it was done according to Cruickshank., *et al*. (1975)

Cooked meat media

Sheep blood agar media

Egg yolk agar media (Nagler's reaction)

Nutrient gelatin media

Biochemical reactions ISO 7937:2004

Nitrate reduction test it was done according to Willis (1977)

Zinc Test

Indole production test it was done according to Mac Faddine (1980)

Hydrogen sulphid test it was done according to Mac Faddine (1980)

Sugar fermentation test it was done according to Willis (1977)

Neutralization test in Swiss mice it was done according to Smith and Holde man (1968)

Determination of *C. perfringens* toxin by dermonecrotic test it was done according to Sterne and Batty (1975)

Preparation of toxin and their treatment it was done according to Bullen (1952)

Application of the typing test it was done according to Oakley and Warrack (1953): the results were interpertatedby the degree of dermonecrotic reaction and its neutralization according to Sterne and Batty (1975).

Detection of *C. perfringens* heat resistant spores

Preparation of *C. perfringensspore* suspension *it was done according to Ellner (1956).*

Determination of heat spore resistance it was done according to Hussein (1997).

- **Statistical analysis:** The obtained results were statistically evaluated by application of Analysis of Variance (ANOVA) test according to Feldman., *et al*. (2003).

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.

Sample	Positive samples		Count of cfu/g			EOS, 2005	Rejected samples	
	NO.	%	Min.	Max.	Mean ± SE*		No.	%
Raw chicken meat								
Chicken breast	19	76	$1.4x10^3$	2.3×10^4	$1.05 \times 10^4 \pm 1.4 \times 10^{3b}$	-	-	-
Chicken thigh	21	84	2.5×10^3	6.8×10^4	$2.8 \times 10^4 \pm 4.0 \times 10^{3a}$	-	-	-
Half cooked chicken meat products								
Chicken nuggets	12	48	1.5×10^2	1.8×10^3	$8.4 \times 10^2 \pm 9.2 \times 10^c$	102	5	20
Chicken panée	12	48	1.6×10^2	1.4×10^3	$6.8 \times 10^2 \pm 7.0 \times 10^c$	102	6	24
Chicken frankfurter	10	40	2.0×10^2	9.8×10^2	$5.3 \times 102 \pm 4.9 \times 10^c$	102	0	0
Total	74	59.2	-	-	-	-	11	8.8

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

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. sordelliin* 21.6, 16, 8, 3.2 and 3.2% of examined samples, respectively.

Clostridia species	*C. sporogenes*		*C. bifermenants*		*C. butyricum*		*C. sordelli*	
Samples	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

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).*

As shown in Table (3) illustrated that the incidence of vegetative form *C. perfringens* were24, 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 isolates33.3% 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); 20and 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.

Samples	Positive samples		Count of cfu/g			EOS, 2005	Rejected samples	
	NO.	%	Min.	Max.	Mean ± SE*		No.	%
Raw chicken meat								
Chicken breast	6	24	5.2×10^2	2.07×10^4	$9.1 \times 10^3 \pm 2.7 \times 10^{3b}$	103	2	8
Chicken thigh	8	32	1.2×10^3	5.03×10^4	$2.5 \times 10^4 \pm 5.7 \times 10^{3a}$	103	7	28
Half cooked chicken meat products								
Chicken nuggets	5	20	2.4×10^2	1.2×10^3	$5.6 \times 10^2 \pm 1.7 \times 10^{2ab}$	Free	5	20
Chicken panée	4	16	1.9×10^2	1.1×10^3	$6.9 \times 10^2 \pm 2 \times 10^{2ab}$	Free	4	16
Chicken frankfurter	4	16	9 x 10	7.5×10^2	$4.1 \times 10^2 \pm 1.4 \times 10^{2ab}$	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 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		
	NO.	%	Min.	Max.	Mean ± SE.
Raw chicken meat					
Chicken breast	4	16	1.2×10^2	2.2×10^3	$9.7 \times 10^2 \pm 4.4 \times 10^2$
Chicken thigh	5	20	1×10^2	1.9×10^3	$8.4 \times 10^2 \pm 3 \times 10^2$
Half cooked chicken meat products					
Chicken nuggets	3	16	3.2 x 10	2.3×10^2	$1.4 \times 10^2 \pm 5.9 \times 10$
Chicken pane	2	8	3.6 x 10	1.5×10^2	$9.3 \times 10 \pm 5.7 \times 10$
Chicken frankfurter	2	8	1.9 x 10	1.1×10^2	$6.4 \times 10 \pm 4.5 \times 10$
Total	16	12.8	-	-	-

Table 4: *Statistical analysis of Clostridium perfringens (spore form) count/g of 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 pane	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 5: *Incidence of Lecithinase positive strains of C. perfringens in the examined chicken meat product samples (n = 25).*

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 pane	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 6: *Serotyping of toxigenic Clostridium perfringens strains isolated from chicken meat product samples.*

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.

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 pane	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

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

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, and economic and consumer profitability characteristics Zakaria (2005). 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 (ref??).

Results illustrated in Table (1) were in a great reliable to Nabil., *et al*. (2014) (4.8 x 10^2 cfu/g in frankfurter); and Sobhy (2016) (5.6 x 10^3 to 5.1 x 10^4 cfu/g, with incidence of 40-66% in chicken meat). While recorded higher results than Zakaria (2005) who reported the total anaerobic counts of examined chicken meat products were ranged from 2.3 x 10^2 to 5.5 x 10^3 cfu/g.

Microscopical and biochemical identification of other than *C. perfringensisolates* as illustrated in Table (2) were recorded to be found in different examined chicken meat products as reported by Zakaria (2005) whodetected *C. sporogenes, C. butyricum, C. subterminalisin* different examined chicken meat products; and Sathish and Swaminathan (2009) whoisolated *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 (100°C for more than 1h) can survive, subsequently spore germination and rapid multiplication leading to food poisoning Simjee and poole (2007).

Tables (3 & 4) 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% infrankfurter with count of 3.6 x 10^2 cfu/g); Kamal (2017) who detected *C. perfringens* (vegetative and spore form) in count of 1.5 x 104 and 1.58 x 102 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 Kamal (2017).

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 one ggyolkagar. produceanopalescentchangearoundthecoloniesduetoenzymaticactionoflecithininthemedium. Those producing alipase causeap early layer or iridescent film that can cover the colonies and in some case sext end in to 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& 7) 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 Ohtani., *et al*. (2013). Symptoms appear within 6 to 24 hoursafter consumption of contaminated food characterized by acute abdominal cramps, watery diarrhea, nausea, and rarely fever with vomiting especially in children and elderly persons Lindström., *et al.* (2011). 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 Sayeed., *et al*. (2005).

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 (100°C 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%).

Conclusion

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. perfringensmay* rendering 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.

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