Isolation of *Clostridium perfringens* from Meat Samples Obtained from the University Students' Hostel

Fahim A Shaltout[1]*, Islam M Osman[2], Enas A Kamel[3] and Amira K Abd-Alla[4]

[1]*Food Hygiene Department, Faculty of Veterinary Medicine, Benha University, Egypt*
[2]*Microbiology Department, Animal Health Research, Dokki, Egypt*
[3]*Biochemistry and Nutrition Department, Womens College, Ain Shemes University, Egypt*
[4]*Benha University Hostels, Egypt*

*Corresponding Author: Fahim A Shaltout, Food Hygiene Department, Faculty of Veterinary Medicine, Benha University, Egypt.

Abstract

The microbiological quality of meat usually affected with the length of transport and the time of waiting, in addition to further growth and multiplication of the organisms during bad storage until be used by the consumers. Anaerobic bacteria such as *Clostridium perfringens* constitute an important group of the microorganisms which are responsible for many public health hazards as well as spoilage which may occur in such products.

A grand total of 100 random samples of raw and cooked beef and chicken which were collected from the university student hostel (25 for each) and these samples were examined for evaluation of *Clostridium perfringens* in beef and chicken before and after cooking and the result revealed that *Clostridium perfringens* was isolated from these samples in a percentage of 15% (15 from 100) represented by 24% for raw chicken samples, 12% for cooked chicken samples, 16% for raw beef samples and 8% for cooked beef samples. Also, the incidence of lecithinase +ve strains were 60% (9 from 15) represented by 66.66% for raw chicken samples, 66.66% for cooked chicken samples, 50% for raw beef samples and 50% for cooked beef samples.

All lecithinase +v e strains of *Clostridium perfringens* which isolated from the examined samples were typed as *Clostridium perfringens* type A, while type B, C and D failed to be detected in all examined samples. The sources of contamination and public health hazard of this isolated microorganism, the suggestive hygienic measures to improve the quality of meat meals and methods of prevention of contamination of these meals were discussed.

Keywords: *Meat; Contamination; Clostridium perfringens; Students' Hostel*

Introduction

Food born infection and intoxication outbreaks are increasing day by day in industrial and developing countries. The majority of cases of food borne diseases were due to bacterial agents (Stevenson and Bernard, 1995).

Anaerobic bacteria such as clostridia constitute an important group of the microorganisms which are responsible for many public health hazards as well as spoilage which may occur in such products. They are able to survive the relatively high temperature by production of their resistant spores [1].

Clostridium perfringens strains have been implicated in outbreaks of food poisoning, particularly of cooked meat products, where their spores survive heating at 1000C, *Clostridium perfringens* are of the most important cause of human food borne diseases (Labbe, 1989).

Pathogenic *Clostridium perfringens* bacteria are not invading host cells but inhibit basic function of their target cells by different toxins, which cause cell damage by intracellular enzymatic activities and/or pore-forming activities. The *Clostridium perfringens* toxins are classified in to the so-called major toxins, minor toxins and enterotoxins. The major toxins are alpha, beta, epsilon and iota, while the enterotoxins are toxins that specifically induce epithelial damage. All other toxins belong to the group of minor toxins. Basing on the production of the four major toxins (alpha, beta, epsilon and iota), the species of *Clostridium perfringens* are thus classified into five types (A, B, C, D and E). *Clostridium perfringens* type A is the most predominant type. *Clostridium perfringens* type A strains produce alpha toxin, type B strains produce alpha, beta and epsilon toxin, type C strains produce alpha and beta toxin, type D strains produce alpha and epsilon toxin and type E produce alpha and iota toxin. It has to be stressed that many potential toxins are yet unidentified and that the exact function of major and minor toxins in some diseases is not yet understood. The classification in major and minor toxins is thus for typing but is not necessary related to the severity of the disease and other factors related to pathogenesis [2].

Carcasses may be contaminated with spores of clostridial species from soil, animal feces, knives, hands, clothes of workers as well as water used in washing of carcasses, cutting boards and by un hygienic methods of transportation to butcher's shops [3-5].

To reduce the incidence of food borne illness, consumer and food safety educators need information about behavior that will decrease the exposure to food borne pathogens. The food safety experts ranked behaviors related to keeping food at safety temperature as primary importance in preventing *Clostridium perfringens* (Hillers 2003).

Usually we observe food poisoning out breaks in places which make -high count of meat dishes and don't expose these dishes to sufficient cooking, these places such as restaurants, hospitals, schools, cafeterias and students hostels.

The risk of bacterial food borne disease also increases when meat meals were prepared in kitchens as in student accommodations, youth hostels and shared homes. This increase in risk may be due to the numbers of individuals using the kitchens, the lack of responsibility and difference in the hyagienic standards for the users of these kitchens (Sharp and walker, 2003).

Consumption of healthy food is one of the significant factors affecting the health, so such studies are important and helpful in supervision and control quality of food stuffs especially in university students hostel and there is no doubt that the main task of meat hygienist is the protection of the consumers from the food borne diseases so for this reason my work as a meat hygienist in one of the university students hostels is the main cause to make this study.

So, this work was planned out to investigate and throw the light on the contamination of *C. Perfringens* in meat and chicken served for students in an university hostel.

Material and Methods

Collection of samples

A grand of 100 random beef and chicken meat samples were collected from university student hostel (50 of each) in Kalyobia governorate. They were equally divided into raw and cooked samples (25 for each) Cooking methods were boiling at 1000C for 60 minutes then frying in the oven for chicken meat and boiling only for beef. Both raw and cooked samples were kept in separate sterile plastic bags and transferred directly as soon as possible to the laboratory of Food Hygiene in Animal Health Research Institute, Dokki-Giza in an ice box under complete aseptic conditions without undue delay for the following examination.

Enumeration of viable *Clostridium perfringens* in the examined samples

The results were interpreted as colony forming unites(CFU) per gram of the samples.

Enumeration of *Clostridium perfringens* spores (Weiss and strong, 1967)

Alternatively, apportion of the previously prepared serial dilution was heated at 800C for 15 minutes to destroy the vegetative cells and activate *Clostridium perfringens* spores.

Isolation and identification of *Clostridium perfringens* (Carter and Cole 1990) [6]

suspected colonies were examined for their morphological, culture and biochemical characters.

Test of Lecithenase activity (Neglar's reaction)

*Egg yolk agar media (Neglar's reaction): This test was done according to the method of Willis and Hobbes (1959).

Typing of *Clostridium perfringens* toxin by dermonecrotic test

It was done according to the method of Sterne and batty [7] using dermonecrotic test in guinea pig.

Results

As shown in table 1 results showed that total count of vegetative form of *Clostridium perfringens* in the examined raw and cooked chicken meat samples were 8.1×10^2 to 5.7×10^4 with a mean value $1.15 \times 10^4 \pm 0.72 \times 10^4$ and 2.3×10^2 to 2.4×10^3 with a mean value $2.7 \times 10^2 \pm 0.02 \times 10^2$ cfu/g respectively.

Samples		Chicken meals			Beef meals		
		Before cooking	After cooking	Reduction %	Before cooking	After cooking	Reduction %
Positive Sample	No	15	7		10	5	
	%	60	28		40	20	
Min.		8.1×10^2	2.3×10^2		1.1×10^2	9.7×10	
Max.		5.7×10^4	2.4×10^3		3.6×10^3	5.1×10^2	
Mean ± SE		$1.5 \times 10^4 \pm .72 \times 10^4$	$2.7 \times 10^2 \pm 0.02 \times 10^2$	98.2%	$6.22 \times 10^2 \pm 2.35 \times 10^2$	$1.11 \times 10^2 \pm 0.55 \times 10^2$	82.1%

Table 1: Statistically analytical results of vegetative form of C. Perfringens counts (CFU/g) in e x amined chicken and beef meals in a university student hostel (n = 25).

And also, that total count of vegetative form of *Clostridium perfringens* in the examined raw and cooked beef samples were 1.7×10^2 to 2.50×10^3 with a mean value $6.22 \times 10^2 \pm 2.35 \times 10^2$ and 9.7×10 to 5.1×10^2 with a mean value $1.11 \times 10^2 \pm 0.55 \times 10^2$ cfu/g respectively. And also shows the reduction % in count of vegetative form of *Clostridium perfringens* in chicken and beef meals which were 98.2% and 82.1% respectively.

Results in table 2 indicated that total count of the spore form of *Clostridium perfringens* in the examined raw and cooked chicken meat samples were 2.1×10 to 5.3×10^2 with a mean value $1.58 \times 10^2 \pm 0.66 \times 10^2$ and 1.2×10 to 1.1×10^2 with a mean value $1.4 \times 10 \pm 1.1 \times 10$ cfu/g respectively. And also indicated that total count of the spore form of *Clostridium perfringens* in the examined raw and cooked beef samples were 3.1×10 to 2.4×10^2 with a mean value $4.01 \times 10 \pm 2.50 \times 10$ and 1.4×10 to 2.3×10^2 with a mean value $1.8 \times 10 \pm 0.25 \times 10$ cfu/g respectively. And also shows the reduction % in count of spore form of *Clostridium perfringens* in chicken and meat meals which were 91.1% and 55% respectively.

Samples		Chicken meals			Beef meals		
		Before cooking	After cooking	Reduction %	Before cooking	After cooking	Reduction %
Positive samples	No.	7	4		4	3	
	%	28	16		16	12	
Min.		1.4 x 10	1.2 x 10		3.1 x 10	1.4 x 10	
Max.		5.3 x 10^2	1.1 x 10^2		2.4 x 10^2	2.3 x 10^2	
Mean ± SE		1.58 x 10^2 ±0.66 x 10^2	1.4 x 10±.11 x 10	91.1%	4.01 x 10±2.50 x 10	1.8 x 10±20.51 x 10	55%

Table 2: Statistically analytical results of spore form of C. Perfringens counts (CFU/g) in examined chicken and beef meals in a university student hostel (n = 25).

Results in table 3 illustrated the incidence of *Clostridium perfringens* isolation in examined chicken and meat samples which were 24%, 12%, 16%, 8% for raw chicken, cooked chicken, raw beef and cooked beef respectively.

Samples	C. perfringens	
	No	%
Raw chicken	6	24
Cooked chicken	3	12
Raw beef	4	16
Cooked beef	2	8

Table 3: Incidence of C. perfringens isolation in examined chicken and beef samples (n = 25).

Data in table 4 showed the incidence of Lecithinase positive strains of Clostridium perfringens in the examined chicken and beef samples which were 66.66%, 66.66%, 50%, 50% for raw chicken, cooked chicken, raw beef and cooked beef respectively.

Samples	No of +ve samples	Lecithinase +ve		Lecithinase –ve	
		No	%	No	%
Raw `chicken	6	4	66.66	2	33.33
Cooked Chicken	3	2	66.66	1	33.33
Raw beef	4	2	50	2	50
Cooked beef	2	1	50	1	50

Table 4: Incidence of Lecithinase positive strains of C. perfringens in the examined chicken and beef samples (n = 25) (Naglers reaction).

As shown in table 5 shows typing of Lecithinase +ve *C. perfringens* isolated from examined chicken and beef samples by using dermonecrotic test and all were *Clostridium perfringens* type A.

Samples	No of +ve samples	No of lecithinase +ve samples	Type A		Type B		Type C		Type D	
			No	%	no	%	No	%	No	%
Raw chicken	6	4	4	100	0	0	0	0	0	0
Cooked chicken	3	2	2	100	0	0	0	0	0	0
Raw beef	4	2	2	100	0	0	0	0	0	0
Cooked beef	2	1		100	0	0	0	0	0	0

Table 5: *Typing of Lecithinase +ve C. perfringens isolated from examined chicken and beef samples by using dermonecrotic test (n = 25).*

Discussion

Preparation of meat meals in university student hostels should be subjected to strict hygienic measures to ensure food safety so application of periodical examination of this meals before and after cooking help in the qualification of these meals from the microbiological side.

The safety and hygienic quality of these meals are determined by the presence of microorganisms which are present in nature.

High anaerobic bacterial count indicated most contamination and bad hygienic measure through handling and preparation steps.

Anaerobic bacteria included most pathogenic food poisoning bacteria such as *Clostridium perfringens*.

Clostridium perfringens possess a public health hazards, numerous exotoxins are responsible for food poisoning. Food poisoning caused by *Clostridium perfringens* may occur by consumption of cooked food and held without maintaining adequate heating or refrigeration before serving, where the spores of some strains are thermostable (as high as 100^0C for more than 1hr.) subsequently, spores germinate and grow rapidly in foods (Simjee and Poole, 2007).

The results recorded in table 1 are nearly similar to the results obtained by Abo zaid [8], Ali [9] and El-Melegy [10] whose results were 5.6×10^3 to 4×10^4 with an average $2.2 \times 10^4 \pm 3.8 \times 10^3$ cfu/g but higher than those obtained by Mira., *et al.* [11] while Eman., *et al.* [12] examined 133 samples of chicken meat and chicken meat products and reported that Clostridium perfringens count in 9 samples was at level between 3.2×10 and 7.5×10 organism/gm.

Also, the results recorded also in table 1 about the total count of vegetative form of *Clostridium perfringens* in the examined raw and cooked beef samples are nearly similar to the results obtained by Komber., *et al.* [13] who recorded that the mean value. Clostridium perfringens were 2.75×10^2 and 6.82×10^2 cfu/g from market and butcher's shops respectively and Ali [9] who recorded that the mean value of *Clostridium perfringens* count (vegetative form) of fore and hind quarters of raw cattle meat were $1.7 \times 10^2 \pm 3.5 \times 10$ and $2.1 \times 10^3 \pm 1.1 \times 10^3$ organism/gm respectively.

El-Melegy [10] recorded higher results, the mean value of *Clostridium perfringens* count of raw beef samples was $2.6 \times 10^4 \pm 4.4 \times 10^3$ cfu/g while the mean value of *Clostridium perfringens* count of cooked meat samples was $1.3 \times 10^4 \pm 4.9 \times 10^3$ cfu/g while Taormina., *et al.* [14] found that Population count of vegetative cells did not exceed 1.70 log 10 CFU/g and the average was 1.56 $\log_{10}$ CFU/g.

These results in table 2 agree with those which recorded by Osman [15] and Ali [9] who could isolate *Clostridium perfringens* spores from chicken meat and chicken meat products while Huang [16] mentioned that the factors that contribute to the virulence of *Clostridium perfringens* include the ability of the bacterium to form heat resistant spores so, this chicken meat which contaminated with these spores should be cooked effectively by using high temperature and during sufficient time to avoid the occurrence of food poisoning.

These results which recorded in table 2 about the total count of the spore form of *Clostridium perfringens* in the examined raw and cooked meat samples are in agreement with those recorded by Saleh [17], El-lawendy [18] and Ali [9] who could isolate *Clostridium perfringens* spores from beef and meat products and also in agreement with those recorded by El-Ray [19] who isolated *Clostridium*

perfringens spores from meat products and stated that outbreaks of *Clostridium perfringens* food poisoning were due to meat dishes containing spores of *Clostridium perfringens* which survive the cooking process and can allow spores to germinate then multiply in cooked meat which either served as a cold one or insufficient reheated. The ingested cells (10^6cell/g m) survive in the small intestine where they produce their enterotoxins resulting in food poisoning syndrome, the symptoms appear after 6 - 24 hours after eating the contaminated food and it is usually characterized by acute abdominal pain, diarrhea, nausea, fever, vomiting, short duration, low fatality rate and no immunity developed.

The incidence of isolation of *Clostridium perfringens* in raw chicken meat which were 24%, these results were in agreement with those recorded by Emara [20].

El-Melegy [10] isolated *Clostridium perfringens* in a percentage of 46.66%, while higher results were recorded by Singh., *et al*. (2005) who isolated *Clostridium perfringens* from fresh poultry meat in a percentage 70.4%, Eman., *et al*. [12] who isolated *Clostridium perfringens* from fresh chicken thigh and chicken fillet in a percentage of 57.9% and 46.6% and Ali [9] who isolated *Clostridium perfringens* in a percentage of 60% and 73.33% for breasts and thighs.

The incidence of *Clostridium perfringens* in cooked chicken meat which were 12%, these results are in agreement with those reported by Eman., *et al*. [12] who isolated *Clostridium perfringens* from chicken luncheon samples in an incidence of 8.3% and explained that the low incidence of *Clostridium perfringens* may be attributed to the method of preparing such products.

While these results are lower than those results obtained by Ali [9] who isolated *Clostridium perfringens* in a percentage of 33.4% from chicken luncheon and also lower than those results obtained by El-Melegy [10] who isolated *Clostridium perfringens* in a percentage of 26.66% from cooked chicken meat.

The incidence of *Clostridium perfringens* in raw beef was 16%, are nearly similar to those recorded by Ali [9] who isolated *Clostridium perfringens* in a percentage of 20% and 33.4% from the raw beef (fore and hind quarter) and El-Melegy [10] isolated *Clostridium perfringens* from raw meat in a percentage of 20%, while higher results were recorded by Miki., *et al.* (2008) who could isolate Clostridium perfringens from raw meat in a percentage of 70% and by Singh., *et al*. (2005) who isolated *Clostridium perfringens* from fresh bovine meat in a percentage of 65.7%, but lower results recorded by Cohen., *et al.* [21] who could isolate *Clostridium perfringens* from raw meat in a percentage of 4.3%.

The incidence of *Clostridium perfringens* in cooked meat which were 8%, these results are nearly similar to those obtained by El-Melegy [10] who isolated *Clostridium perfringens* from cooked meat in a percentage of 13.33%, while El-Mossalami [22] and Ali [9] recorded higher results.

Phillips., *et al*. [23] and Hashem [24] did not detect *Clostridium perfringens* in their chicken and meat samples.

The results recorded in table 4 are lower than those obtained by Atwa and Abo El-Rose [25] who found that incidence of toxigenic and non-toxigenic strains were 89.6% and 10.4% and also lower than those of Hamoda [26] who recorded 88.1% toxigenic and 11.9% non-toxigenic, while Ali [9] and El Melegy [10] recorded lower results.

Table 5 shows that +ve lecithinase strains of *Clostridium perfringens* isolates were typed into *Clostridium perfringens* type A in a percentage of 100% for raw chicken, cooked chicken, raw meat and cooked meat samples while *Clostridium perfringens* type B, C and D were not detected.

These results agree with those reported by Deng., *et al*. [27] who isolated 21 strains of *C. perfringens* out of 142 food samples. they stated that the frozen meat having the highest levels (19.6%), followed by fresh meat and fresh meat dumpling (12.2 and 11.9%, respectively) also determined the genotype of the isolated strains and classified all strains as *Clostridium perfringens* type A.

From the previous results, we can observe that the presence of *Clostridium perfringens* in the examined raw samples of chicken and beef served for the university student hostel is due to contamination of this meat or chicken may be during slaughtering, transportation and preparation of them from the hands of workers and from equipment and tools and knives.

While the presence of this m. o in the cooked samples may be due to re contamination or due to inadequate heat treatment during cooking of them or may be due to holding of these cooked meat and chicken until to be served for students in an ambient temperature in which *Clostridium perfringens* spores can vegetate or due to post processing contamination [28].

Bibliography

1. Barnes EM. "Isolation methods for anaerobes in foods". *International Journal of Food Microbiology* 2.1-2 (1985): 81.

2. Frey J and Vileie M. "Molecular genetics of *Clostridium perfringens* toxins". In: protein toxins of the genus clostridium and vaccination. Proceedings of meeting 22 Nd-23 Rd November 2002 of the European Concerted Action QLK2-CT2001-01267 (Duchesnes C., Mainil J., Popoff M and Titball, R, Eds), Press Facultad de Medicina Veterinaria, Ulg, Liege, Belgium (2002): 45-51.

3. Bailey C. "Spray washing lamb carcasses". *Institute Meat Bull* 75 (1972): 3.

4. Skjelkvale RLH and Tjaberg TB. "Incidence of C. Perfringens in meat products". *Nordisk Veterinaermedicin* 26.6 (1974): 389-391.

5. Hussein AZ and Farrag I. "Incidence of *clostridium perfringens* in beef". Journal of the Egyptian Veterinary Medical Association 41 (1981): 57.

6. Carter GR and Cole JR. "Diagnostic procedures in veterinary bacteriology and mycology. 5th Ed", Academic press (1990).

7. Stern M and Batty I. ''Pathogenic Clostridia'' 1st Edition. Butter Worth, London, Boston (1975).

8. Abo Zaied SMA. "Anaerobes in meat and fish products and their ability to toxin production". (Meat Hygiene), Thesis, Faculty of Veterinary Medicine, Benha University (1998).

9. Ali HEH. "Clostridial species and related organisms in meat and meat products". (Meat Hygiene), Thesis, Faculty of Veterinary Medicine, Benha University (2009).

10. El-melegy ASA. "Microbial status of meat and chicken received to university student hostel". M. V. Sc. Thesis (Meat hygiene), Faculty of Veterinary Medicine, Benha University (2015).

11. Mira EK. "Bacteriological quality of animal affected with fascioliasis". M. V. Sc Thesis (Meat Hygien), Faculty of Veterinary Medicine, Cairo University (1998).

12. Eman M N., *et al*. "Enterotoxigincity and typing of *C. Perfringens* isolated from poultry products in Egypt". *Journal of Applied Sciences Research* 3.12 (2007): 1804-1808.

13. Komber U., *et al*. *Clostridium Perfringens* anti-toxin in medicament from kars, Turkey". Faculty of Net medicine Department of food hygeine, University of Kafkas, Kavs. Turkey (2007).

14. Taormina P., *et al*. "Incidence of C. Perfringens in commercially produced cured raw meat product mixtures and behavior in cooked products during chilling and refrigerating storage". *Journal of Food Protection* 66.1 (2003): 72-81.

15. Osman EZ. "Anaerobic Bacteria in chicken meat products". M. V. Sc. Thesis, (Meat Hygiene), Faculty of Veterinary Medicine, Benha University (2005).

16. Huang I Hsu. "*C. perfringens* sporulation and gliding motility". ph. D. Thesis, Orrgon State University, USA (2007).

17. Saleh H. "Incidence and serotyping of *C. perfringens* among animals slaughtered in Cairo and Giza abattoirs". M. V. Sc, Thesis, (Meat Hygiene), Faculty of Veterinary Medicine, Zagazig University, Benha branch (1994).

18. El Lawendy TMH. "Occurrence of C. Perfringens in locally produced meat products". M. V. Sc. Thesis (Meat Hygiene), Faculty of Veterinary Medicine, Zagazig University, Benha Branch (1996).

19. El-Rayes AM A. "Aerobic and an aerobic spore formers in vaccum packaged meat products". D. V.Sc. Thesis (Meat Hygiene) Faculty of Veterinary Medicine, Benha University (2014).

20. Emara MSEMM. "Anaerobic and aerobic microorganisms in human food". M. V. Sc. Thesis (Bacteriology, Immunology, Mycology), Faculty of Veterinary Medicine, Cairo University (2014).

21. Cohen N Ennaja, *et al*. "The bacterial quality of red meat and offals in Casablanca (Morcoo)". Molecular *Nutritional Food Research* 50.6 (2006): 537-562.

22. EL-Mossalami E. "Risk assessment ready prepared meat products". Ph. D. Meat Hygiene. Thesis, Faculty of Veterinary Medicine, Cairo University (2003).

23. Phillips D., *et al*. "A national survey of the microbiological, quality of retail raw meats in Australia". *Journal of Food Protection* 71.6 (2008): 1232-1236.

24. Hashem HMS. "Bacteriological criteria of dressed poultry with special reference to some microbial decontaminators". Thesis Meat Hygiene, Benha University (2015).

25. Atwa EI and El-Roos NA. "Incidence of *clostridium perfringens* in meat products at some Egyptian Governorates". International Journal of Microbiological Research 2.3 (2011): 196-203.

26. Hamoda WSAO. "Study of the effect of some salts on *Clostridium Perfringens* during cooling of raw and cooked ground beef". MV. Sc. Thesis (Bacteriology), Faculty of Veterinary Medicine, Zagazig University (2012).

27. Deng Z., *et al*. "Identification and genotyping of C. perfringens isolated from the food samples". *Journal of Tropical Medicine* 6.6 (2006): 682-684.

28. El- Dally. "Sanitary status of meat serving establishment". Meat hygiene thesis, Faculty of Veterinary Medicine, Cairo University (1983).