Clostridium perfringens in meat and chicken received to University student hostel

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

[1]*Food Hygiene Dep., Fac. of Vet. Med., Benha Uni.,*
[2]*Microbiology Dep., Animal Health Res., Dokki*
[3]*Biochemistry and Nutrition Department Women's College Ain Shams University.*
[4]*Benha Univ. Hostel.*

A B S T R A C T

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 *C. Perfringens* in beef and chicken before and after cooking and the result revealed that *C. 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 and8% for cooked beef samples .Also, the incidence of lecithenase +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 lecithenase +v e strains of *C. Perfringens* which isolated from the examined samples were typed as *C. 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: *C. Perfringens,* Raw and cooked beef and chicken, Lecithenase +ve, *C. Perfringens* Type A.

(http://www.bvmj.bu.edu.eg) **(BVMJ-32(2): 8-13, 2017)**

1. INTRODUCTIN

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. 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 (Barnes, 1985). *Clostridium perfringens* strains have been implicated in outbreaks of food poisoning, particularly of cooked meat products, where their spores survive heating at 100 ^{0}C, *Clostridium perfringens* are of the most important cause of human food borne diseases. Pathogenic *C. 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 *C. 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 *C. perfringens* are thus classified into five types (A, B, C, D and E). *C. perfringens* type A is the most predominant type. *C. 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 (Frey and Vileie, 2003). 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 .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 (Bailey, 1972; Hussein and Farrag, 1981; Skjelkvale and Tjaberg, 1974). 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 *C. perferingens*. 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 student's 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 hygienic standards for the users of these kitchens. 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 a university hostel.

2. MATERIAL AND METHODES

2.1. 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 100 ^{0}C 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

2.2. Enumeration of viable clostridium perfringens in the examined samples

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

2.3. Enumeration of clostridium perfringens spores

Alternatively, apportion of the previously prepared serial dilution was heated at 80^{0}c for 15 minutes to destroy the vegetative cells and activate *clostridium perfringens* spores.

2.4. Isolation and identification of C. perfringens (Carter and Cole, 1990)

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

2.5. Test of Lecithenase activity (Neglar 's reaction)

Egg yolk agar media (Neglar's reaction)

2.6. Typing of C. perfringens toxin by dermonecrotic test

It was done according to the method of Stern and Batty (1975) using dermonecrotic test in guinea pig.

3. RESULTS

As shown in table (1) results showed that total count of vegetative form of *C. Perfringens* in the examined raw and cooked chicken meat samples were $8.1x10^2$ to $5.7x10^4$ with a mean value $1.15x10^4 \pm 0.72x10^4$ and $2.3x10^2$ to $2.4x10^3$ with a mean value $2.7x10^2 \pm 0.02x10^2$ cfu/g respectively. And also that total count of vegetative form of *C. Perfringens* in the examined raw and cooked beef samples were $1.7x10^2$ to $2.50x10^3$with a mean value $6.22x10^2 \pm 2.35x10^2$ and 9.7x10 to $5.1x10^2$ with a mean value $1.11x10^2 \pm 0.55x10^2$ cfu/g respectively. and also shows the reduction % in count of vegetative form of *C. 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 *C. Perfringens* in the examined raw and cooked chicken meat samples were 2.1x10 to $5.3x10^2$ with a mean value $1.58x10^2 \pm 0.66x10^2$ and 1.2x10 to $1.1x10^2$with a mean value 1.4x10±1.1x10 cfu/g respectively. And also indicated that total count of the spore form of *C. Perfringens* in the examined raw and cooked beef samples were 3.1x10 to $2.4x10^2$ with a mean value 4.01x10±2.50x10 and 1.4 x10 to $2.3x10^2$ with a mean value 1.8x10± .25x10 cfu/g respectively. And also shows the reduction % in count of spore form of *C. Perfringens* in chicken and meat meals which were 91.1% and 55% respectively.

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

samples	Chicken meals			beef meals		
	Before cooking	After cooking	Reduction %	Before cooking	After cooking	Reduction %
Positive samples No	15	7		10	5	
Positive samples %	60	28		40	20	
Min.	$8.1x10^2$	$2.3x10^2$		$1.1x10^2$	9.7x10	
Max.	$5.7x10^4$	$2.4x10^3$		$3.6x10^3$	$5.1x10^2$	
mean±SE	$1.5x10^4 \pm .72X10^4$	$2.7x10^2 \pm 0.02X10^2$	98.2%	$6.22x10^2 \pm 2.35x10^2$	$1.11x10^2 \pm 0.55x10^2$	82.1%

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)

samples	Chicken meals			beef meals		
	Before cooking	After cooking	Reduction %	Before cooking	After cooking	Reduction %
Positive samples No.	7	4		4	3	
Positive samples %	28	16		16	12	
Min.	1.4x10	1.2x10		3.1x10	1.4x10	
Max.	$5.3x10^2$	$1.1x10^2$		$2.4x10^2$	$2.3x10^2$	
Mean ±SE	$1.58x10^2 \pm 0.66x10^2$	1.4x10±.11x 10	91.1%	4.01x10 ± 2.50x10	1.8x10 ±20.51x10	55%

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

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

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

Samples	No of +ve samples	Lecithenase +ve		Lecithenase –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 (5): Typing of Lecithenase +ve *C. perfringens* isolated from examined chicken and beef samples by using dermonecrotic test (n=25).

Samples	No of +ve samples	No of lecithenase +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

Results in table (3) illustrated the incidence of *C. 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. Data in table (4) showed the incidence of Lecithinase positive strains of *C. 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. As shown in table (5) shows typing of Lecithenase +ve *C. perfringens* isolated from examined chicken and beef samples by using dermonecrotic test and all were *C. perfringens* type A.

4. 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 *C. perfringens. Clostridium perfringens* possess a public health hazards, numerous exotoxins are responsible for food poisoning. Food poisoning caused by *C. 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 ^{0}c for more than 1hr.) subsequently, spores germinate and grow rapidly in foods. The results recorded in table (1) are nearly similar to the results obtained by Abo Zaied (1998))Ali (2009) and El-melegy (2015) whose results were $5.6x10^3$ to $4x10^4$ with an average $2.2x10^4 \pm 3.8x10^3$ cfu/g but higher than those obtained by Mira (1998). While Eman et al. (2007) examined 133 samples of chicken meat and chicken meat products and reported that *C. perfringens* count in 9 samples was at level between 3.2 x10 and 7.5 x 10 organism/gm. Also the results recorded also in table (1) about the total count of vegetative form of *C. Perfringens* in the examined raw and cooked beef samples are nearly similar to the results obtained by Komber et al. (2007) who recorded that the mean value. *C. perfringens* were 2.75 x 10^2 and 6.82 x 10^2 cfu/g from market and butcher's shops respectively and Ali (2009) who recorded that the mean value of *C.perfringens* count (vegetative form) of fore and hind quarters of raw cattle meat were $1.7x10^2 \pm 3.5x10^2$ and $2.1x10^3 \pm 1.1x10^3$ organism/g m respectively. El-melegy (2015) recorded higher results, the mean value of *C. Perfringens* count of raw beef samples was $2.6x10^4 \pm 4.4x10^3$ cfu/g, while the mean value of *C. Perfringens* count of cooked meat samples was $1.3x10^4 \pm 4.9x10^3$ cfu/g. While Taormina et al. (2003) 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 (2005) and Ali (2009) who could isolate *C. perfringens* spores from chicken meat and chicken meat products while Huang (2007) mentioned that the factors that contribute to the virulence of *C. 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 *C. Perfringens* in the examined raw and cooked meat samples are in agreement with those recorded by El Lawendy (1996); Saleh (1994) and Ali (2009) who could isolate *C. perfringens* spores from beef

and meat products and also in agreement with those recorded by El-Rayes (2014) who isolated *C. perfringens* spores from meat products and stated that outbreaks of *C .perfringens* food poisoning were due to meat dishes containing spores of *C. 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 *C. perfringens* in raw chicken meat which were 24%, these results were in agreement with those recorded by Emara (2014). El-melegy (2015) isolated *C. perfringens* in a percentage of 46.66%, while higher results were recorded by Taormina et al. (2003) who isolated *C. perfringens* from fresh poultry meat in a percentage 70.4%. Eman et al. (2007) who isolated *C. Perfringens* from fresh chicken thigh and chicken fillet in a percentage of 57.9% and 46.6% and Ali (2009) who isolated *C. perfringens* in a percentage of 60% and 73.33% for breasts and thighs. The incidence of *C. perfringens* in cooked chicken meat which were 12%, these results are in agreement with those reported by Eman et al. (2007) who isolated *C. Perfringens* from chicken luncheon samples in an incidence of 8.3% and explained that the low incidence of *C. perfringens* may be attributed to the method of preparing such products. While these results are lower than those results obtained by Ali (2009) who isolated *C. perfringens* in a percentage of 33.4% from chicken luncheon and also lower than those results obtained by El-melegy (2015) who isolated *C. perfringens* in apercentage of 26.66% from cooked chicken meat. The incidence of *C. perfringens* in raw beef was 16%, are nearly similar to those recorded by Ali Ali (2009) who isolated *C. perfringens* in a percentage of 20% and 33.4% from the raw beef (fore & hind quarter) and El-melegy (2015) isolated *C. Perfringens* from raw meat in a percentage of 20%, while higher results were recorded by Mira (1998) who could isolate *C. Perfringens* from raw meat in a percentage of 70% and isolated *C. perfringens* from fresh bovine meat in a percentage of 65.7%, but lower results recorded by Cohen et al. (2006) who could isolate *C. Perfringens* from raw meat in a percentage of 4.3%. The incidence of *C. perfringens* in cooked meat which were 8%, these results are nearly similar to those obtained by El-melegy (2015) who isolated *C. Perfringens* from cooked meat in a percentage of 13.33%. While EL-Mossalami (2003) and Ali (2009) recorded higher results. Phillips et al. (2008) and Hashem (2015) did not detect *C.perfringens* in their chicken and meat samples . The results recorded in table (4) are lower than those obtained by Atwa and El-Roos (2011) who found that incidence of toxigenic and non-toxigenic strains were 89.6% and 10.4% and also lower than those of Hamoda (2012) who recorded 88.1% toxigenic and 11.9% non-toxigenic. While Ali (2009) and El-melegy (2015) recorded lower results. Table (5) shows that +ve lecithinase strains of *C. perfringens* isolates were typed into *C. Perfringes* type A in a percentage of 100% for raw chicken, cooked chicken, raw meat and cooked meat samples while *C.perfringens* type B, C and D were not detected. These results agree with those reported by Deng et al. (2006) 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 *C. perfringens type A.*

From the previous results we can observe that the presence of *C. 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 *C. perfringens* spores can vegetate or due to post processing contamination.

5. REFERENCE

Abo Zaied, S.M.A., 1998. Anaerobes in meat and fish products and their ability to toxin production. PhD. Sci (Meat Hygiene). Thesis, Fac. Vet. Med., Benha Univ.

Ali, H.E.H., 2009. Clostridial species and related organisms in meat and meat products. PhD V.Sc. (Meat Hygiene). Thesis, Fac.Vet. Med., Benha Univ.

Atwa, E.I., El-Roos, N.A., 2011. Incidence of clostridium perfringens in meat products at some Egyptian Governorates. International Journal of Microbiological Reasearch 2, 196-203.

Bailey, C., 1972. Spray washing lamb carcasses. Inst. Meat Bull 75, 3.

Barnes, E.M., 1985. Isolation methods for anaerobes in foods. Int. J. Food Microbial 2, 81.

Carter, G.R., Cole, J.R., 1990. Diagnostic procedures in veterinary bacteriology and mycology. 5th Ed., Academic press., Harcout. Boace Jov., Publishers, New York, Boston, Tokyo, Toronto.

Cohen, N., Ennaja, H., Hassar, M., Karib, H., 2006. The bacterial quality of red meat and offals in Casablanca (Morcoo). Mol Nutritional food Reseach 50, 537-562.

Deng, Z., Li, X.C., Zhang, J., Zhang, X., Pang, X., Zhang, Y., Mo, M., 2006. Identification and geno typing of *C. perfringens* isolated from the food samples. J. Trop. Med. 6, 682-684.

El-melegy, A.S.A., 2015. Microbial status of meat and chicken received to university student hostel. M.V.Sc. Thesis (Meat hygiene), Fac. Vet. Med, Benha Univ.

EL-Mossalami, E., 2003. Risk assessment ready prepared meat products. PhD. Meat Hygiene. Thesis, Fac. Vet. Med., Cairo Univ.

El-Rayes, A.M.A., 2014. Aerobic and an aerobic spore formers in vaccum packaged meat products. M.D.V.Sc. Thesis (Meat Hygiene) Fac. Vet. Med, Benha Univ.

El Lawendy, T.M.H., 1996. Occurrence of *C. Perfringens* in locally produced meat products. M.V.Sc. Thesis (Meat Hygiene), Fac. Vet. Med., Zagazig Univ., Benha Branch.

Eman, M.N., Hala, A.A., Amal, A.S., 2007. Enterotoxigincity and typing of C. Perfringens isolated from poultry products in Egypt. J. Appl. Sci. Res. 3, 1804-1808.

Emara, M.S., 2014. Anaerobic and aerobic microorganisms in human food. M.V.Sc. Thesis (Bacteriology, Immunology, Mycology), Fac. Vet. Med. Cairo Univ.

Frey, J., Vileie, M., 2003. 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), pp 45-51, Presses Fac. Med. Vet. Ulg, Liege, Belgium.

Hamoda, W.S., 2012. Study of the effect of some salts on Clostridium Perfringens during cooling of raw and cooked ground beef. M.V.Sc. Thesis (Bacteriology), Fac. Vet. Med., Zagazig Univ.

Hashem, H.M.S., 2015. Bacteriological criteria of dressed poultry with special reference to some microbial decontaminantors. Thesis Meat Hygiene, Benha Univ.

Huang, I.H., 2007. C. perfringens sporulation and gliding motility. Ph.D. Thesis, Orrgon State Univ., USA.

Hussein, A.Z., Farrag, I., 1981. Incidence of clostridium perfringens in beef. J. Egypt. Vet. Med. Assoc. 41, 57.

Komber, U., Gokce, H.T., Elmali, M., 2007. Clostridium Perfringens anti toxin in medicament from kars,Turkey. Faculty of net medicine Dept. of food hygeine, Univ. of Kafkas, Kavs. Turkey.

Mira, E.K., 1998. Bacteriological quality of animal affected with fascioliasis. M.V.Sc Thesis (Meat Hygien), Fac. of Vet. Med., Cairo Univ.

Osman, E.Z., 2005. Anaerobic Bacteria in chicken meat products. M.V.Sc. Thesis, (Meat Hygiene), Fac. Vet. Med., Benha Univ.

Phillips, D., Jordan, D., Morris, S., Jenson, I., Sumner, J., 2008. Anational survey of the microbiol,quality of retail raw meats in Australia. J. Food prot. 71, 1232-1236.

Saleh, H., 1994. Incidence and serotyping of C. perfringens among animals slaughtered in Cairo and Giza abattoirs. M.V.Sc, Thesis, (Meat Hygiene), Fac. Vet. Med., Zagazig Univ. Benha branch.

Skjelkvale, R.L.H., Tjaberg, T.B., 1974. Incidance of C. Perfringens in meat products. Nord. Vet. Med. 26, 389-391.

Stern, M., Batty, I., 1975. Pathogenic Clostridia " 1st Edt. Butter Worth, London, Boston.

Taormina, P.J., Bartholomew, G.W., Dorsa, W.J., 2003. Incidence of C. Perfringens in commercially produced cured raw meat product mixtures and behavior in cooked products during chilling and refrigerating storage. J. Food Project 66, 72-81.