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CLOSTRIDIUM PERFRINGENS IN VACUUM PACKAGED MEAT PRODUCTS

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A B S T R A C T

One hundred and fifty random samples of vacuum packaged meat products (salami, frankfurter and cocktail) 50 of each produced by 2 different processing plants determined as A and B (75 0f each) were collected from different supermarkets located in Qalyobia governorate for determination of their contamination with *C. perfringens*. The mean values of total *C. perfringens* counts /g in the examined samples were $3.16\times10^2\pm 0.57\times10^2$& $9.65\times10^2\pm 2.08\times10^2$ for salami, $8.72\times10^2\pm 1.95\times10^2$& $2.81\times10^3\pm 0.44\times10^3$ for frankfurter and $2.43\times10^3\pm 0.38\times10^3$& $7.93\times10^3\pm1.51\times10^3$ for cocktail, respectively. Accurately, *C. bifermentas*, *C. butyricum*, *C. perfrigens*, *C. putrefaciens*, *C. sordelli*, *C. sporogens* and *C. tertium* were isolated from the examined samples of vacuum packaged salami, frankfurter and cocktail produced by both processing plants A and B with different percentages. Typing of lecithinase positive strains of *C. perfringens* indicated the presence of types A, B, C and D in the examined samples of vacuum packaged salami, frankfurter and cocktail of both plants in different rates. The significance of isolated *C. perfringens in* the examined samples of vacuum packaged meat products and possible sources of their contamination as well as some recommendations to improve the quality of such meat items were discussed.

Keywords: Clostridium perfringens, vaccum packaged meat products, Salami, Frankfurter, Cocktail

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1. INTRODUCTION

Anaerobic spore formers are important group of microorganisms responsible for many health hazards to consumers of vacuum packaged meat products. In general, *C. perfringens* is the most important one resulting several diseases to man as well as food poisoning outbreaks (Hobbs, 1987). *Clostridium perfringens* is able to grow at concentration of 3-5% sodium chloride and can gain access during processing or food service operations. Also, the optimal temperature for growth of *C. perfringens* is 43°C- 46°C, while the lowest temperature for its growth is 18°C and the generation time averages 12 minutes and can be as short as 8.5 minutes (Davies and Board, 1998). However, the internal temperature in the meat products must reach 71°C to be lethal for vegetative cells but not for spore formers (Abigail and Dixi, 1994).

Clostridium perfringens organisms was responsible for 7.9% of 62 food poisoning outbreaks affecting 6093 persons in USA in the period between 1971-1980 due to the consumption of meat and its products contaminated with such serious organism (ICMSF, 1996).Therefore, the present study was applied to investigate the contamination of vacuum packaged meat products with *C. perfringens*.

2. MATERIALS AND METHODS

A grand total of 150 random samples of vacuum packaged meat products represented by salami, frankfurter and cocktail (50 0f each) related to 2 different processing plants namely A and B (75 0f each) were collected from different

supermarkets located in Kalyobia governorate to determine their contamination with *C. perfringens*.

2.1. Preparation of the samples:

The samples were prepared according to the technique recommended by ICMSF (1978) to obtain tenfold decimal serial dilutions.

2.2 Monitoring of Clostridium perfringens (ICMSf, 1978):

Two grams of each sample were aseptically inoculated into previously boiled and cooked meat media. The inoculated tubes were incubated an- aerobically at 37°C for 24 hours. Positive tubes show turbidity and gas production. One ml from each of previously prepared serial dilution was spread into *Clostridium perfringens* agar plate media. The plates were then incubated in upright position in anaerobic gar (Mackintosh jar) at 37°C for 48 hours. The suspected plates showing black colonies were selected and counted, and the results were interpreted as colony forming units (CFU) per gram of sample. The suspected isolates of *C. perfringens* were further identified microscopically and biochemically.

2.3 Determination of Clostridium perfringens toxins by dermonecrotic test:

Preparation of toxins and their treatment were carried out according to Bullen (1952 **).** While, application of typing test was applied on Albino Guinea pigs, with an average body weight about 350-450 grams. The animals were kept under observation for two weeks before the beginning of the experiment and the hair of the back of each side was carefully shaved and marked longitudinally onto both sides. On the right side, 0.2 ml of 5 hours trypsinized 48 hour's supernatant of each culture was intradermally injected and the neutralized one was injected into the left side in the same manner and arrangement. The injected Guinea pigs were kept under observation for 48-72 hours to demonstrate any dermal reaction. The results were interpreted by the degree of dermonecrotic reaction and its neutralization according to Sterne and Batty (1975).

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

3. RESULTS

Table (1) declared that the mean values of *C. perfringens* count /g in the examined samples of salami, Frankfurter and cocktail were $3.16\times10^2\pm 0.57\times10^2$, $8.72\times10^2\pm 1.95\times10^2$ &$2.43\times10^3\pm0.38\times10^3$ of plant A, $9.65\times10^2\pm2.08\times10^2$, $2.81\times10^3\pm0.44\times10^3$ and $7.93\times10^3\pm 1.51\times10^3$ for plant B, respectively. Actually, 16%, 28% and 32% of the examined samples of salami, frankfurter and cocktail were contaminated with *C. perfringens* from which 8%, 16% and 20% were lecithinase positive strains, respectively, as shown in table (2).

N.B: % was calculated according to the number of positive samples.

In this respect, table (3) indicated that 12%, 24% and 32% were lecithinase positive strains of *C. perfringens* detected out of 28%, 36% and 48% of the examined samples of salami, frankfurter and cocktail of plant B confirmed to be contaminated with *C. perfringens,* respectively.

N.B: % was calculated according to the number of positive samples.

Table (4) illustrated that the typing of lecithin's positive strains of *C. perfringens* isolated from the examined samples of vacuum packaged meat products of plant A was 1 isolate type A (4%) and 1 isolate type B for the examined salami samples, 1 isolate type A (4%), 1 isolate type C (4%) and 2 isolates type D (8%) for the examined frankfurter samples and 3 isolates type A (12%), 1 isolate type B (4%) and 1 isolate type D (4%) for the examined cocktail samples.

N.B: % was calculated according to the number of positive samples.

Table (1) Statistical analytical results of *C. perfringens* count/g in the examined samples of vacuum packaged meat products (n=25).

Plant / Product	A			B		
	Min.	Max.	Mean ± S.E*	Min.	Max.	Mean ± S.E*
Salami++	1.0×10^2	2.9×10^3	$3.16\times10^2\pm 0.57\times10^2$	1.0×10^2	8.0×10^3	$9.65\times10^2\pm 2.08\times10^2$
Frankfurter	1.0×10^2	6.0×10^3	$8.72\times10^2\pm 1.95\times10^2$	3.0×10^2	2.9×10^4	$2.81\times10^3\pm 0.44\times10^3$
Cocktail	2.0×10^2	1.7×10^4	$2.43\times10^3\pm 0.38\times10^3$	4.0×10^2	5.3×10^4	$7.93\times103\pm 1.51\times10^3$

S.E* = standard error of mean
++ = High significant differences (P<0.01)

Table (2) Lecithinase activity of *C. perfringens* recovered from the examined samples of vacuum packaged meat products produced by plant A (n=25).

Products	Positive samples		Lecithinase +ve *C. perfringens*	
	No	%	No	%
Salami	4	16	2	8
Frankfurter	7	28	4	16
Cocktail	8	32	5	20
Total (75)	19	25.33	11	14.67

Table (3) Lecithinase activity of *C. perfringens* recovered from the examined samples of vacuum packaged meat products produced by plant B (n=25).

Products	Positive samples		Lecithinase +ve *C. perfringens*	
	No.	%	No.	%
Salami	7	28	3	12
Frankfurter	9	36	6	24
Cocktail	12	48	8	32
Total	28	37.33	17	22.67

Table (4) Typing of Lecithinase positive strains of *C. perfringens* isolated from the examined samples of vacuum packaged meat products produced by plant A (n=25).

Toxigenic strains / Product	A		B		C		D		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%
Salami	1	4	1	4	-	-	-	-	2	8
Frankfurter	1	4	-	-	1	4	2	8	4	16
Cocktail	3	12	1	4	-	-	1	4	5	20

Table (5) Typing of Lecithinase positive strains of *C. perfringens* isolated from the examined samples of vacuum packaged meat products produced by plant B (n=25).

Toxigenic strains Products	A		B		C		D		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%
Salami	1	4	-	-	1	4	1	4	3	12
Frankfurter	2	8	1	4	-	-	3	12	6	24
Cocktail	4	16	1	4	2	8	1	4	8	32

Table (5) declared that *C. perfringens* type A was superior to other types isolated from all such examined samples of meat products. The rates of identification were 1 isolate type A (4%),1 isolate type C (4%) and 1isolatetype D (4%) for the examined salami samples, 2 isolates type A (8%), 1 isolate type B (4%) and 3 isolates type D (12%) for the examined frankfurter samples and 4 isolates type A (16%),1 isolate type B (4%),2 isolates type C (8%) and 1 isolate type D (4%) for the examined cocktail samples.

4. DISCUSSION

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 the cooked meat which either served as cold one or insufficiently reheated. The ingested celles survive in the small intestine where they produce their enterotoxins resulting in food poisoning syndrome (Adams and Mossel, 1995).

cause food poisoning as a result of inadequate refrigeration after cooking. On the other side, if the organisms did not possess heat resistant spores, the product may be contaminated through improper handling to a significant level sufficient to cause food poisoning. Thus, the ability of *C. perfringens* to produce heat resistant spores may indicate whether the sanitary precautions are neglected prior to or after cooking.

Generally, *C. perfringens* is widely implicated in many cases of food poisoning outbreaks which usually occurred when large numbers of this organism (10^6/g) were ingested. Furthermore, *C. perfringens* is considered as one of the major spoilage bacteria of various kinds of meat products (Bean and Griffin, 1990).

It is interesting to stipulate that *C. perfringens* type A is of main concern for human being where the organism can survive processing at high temperature for long period of time as compared with the other types of *C. perfringens* (El-Naenaey, 1989). Therefore, improper cooking may contribute to outbreaks of *C .perfringens* if the proper precautions are not taken to inhibit the multiplication of such serious organism in food. Probably of greater concern to state that *C. perfringens* may contaminate the meat products either before processing from the spores which survive heat treatment or after processing during handling of these products (Potter, 2001). Accordingly, if *C. perfringens* was found in the meat products before cooking and possessed heat resistant spores, it may

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مدي تواجد ميكروب الكلوستريديم برفرنجينز في منتجات اللحوم المعبأة تحت التفريغ

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قسم الرقابة الصحية على اللحوم و منتجاتها – كلية الطب البيطرى – جامعة بنها

الملخص العربي

يتزايد الطلب على منتجات اللحوم المعبأة تحت التفريغ لتحقيق أكبر قدر ممكن من الأمان الغذائى، الا أنها قد تكون معرضة هى الأخرى للتلوث بالعديد من الميكروبات الممرضة مثل الكلوستريديم برفرنجينز والذي يشكل خطورة علي صحة المستهلك أثناء تجهيزواعداد تلك المنتجات. لذلك تم جمع عدد مائه وخمسين عينة (150) من منتجات اللحوم المعبأة تحت التفريغ من شركتين مختلفتين (أ) و (ب) بواقع خمس وسبعين(75) عينة من كل شركة. وكانت عينات تلك المنتجات عبارة عن السلامى، الفرانكفورتر والكوكتيل بواقع خمس وعشرين (25) عينة من كل شركة وذلك لدراسة مدى تلوثها بميكروب الكلوستريديم برفرنجينزوكان متوسط العدد الكلى لبكتيريا الكلوستريديمبرفرنجينز(جم)في منتجات السلامي ، الفرانكفورتر والكوكتيل المعباة تحت التفريع هو $3,16\times 10^2$ و $8,72\times 10^2$ و$2,43\times 10^3$ للشركة (أ) و $9,65\times 10^2$ ، $2,81\times 10^3$ و $7,93\times 10^3$ للشركة (ب)، على التوالى. هذا وقد تم عزل الأنواع الأتية من بكتيريا الكلوستريديم:

C.bifermentas, *C.butyricumC.sordelli*, *C.sporogens,C.tertium*, *C.perfrigens and C.putrefaciens*

من عينات السلامى، الفرانكفورتر والكوكتيل المنتجة بواسطة كلتا الشركتين بنسب متفاوتة. كما تم تحديد الأنواع المختلفة للسموم التى يتم افرازها بواسطة بكتيريا الكلوستريديمبرفرنجينز عن طريق الحقن تحت الجلد فى الخنازير الغينية. وكان من بين العترات المعزولة لهذا الميكروب العترات المنتجة للسموم ا، ب، ج، د. وقد وجد أن الاختلافات بين العينات محل الدراسة كانت معنوية كنتيجة للتباين بين شركات الأنتاج من ناحية وبين أنواع منتجات اللحوم محل الدراسة من ناحية أخرى. هذا وقد تم مناقشة الأهمية الصحية لميكروب الكلوستريديم برفرنجينز المعزول من منتجات اللحوم المعبأة تحت التفريغ مع تحديد المصادر المختلفة لتلوثها بالأضافة لوضع بعض التوصيات لتحسين جودة تلك المنتجات والحفاظ على صحة المستهلك.

(مجلة بنها للعلوم الطبية البيطرية: عدد 26(1):49-53, مارس 2014)