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PREVALENCE OF *BACILLUS CEREUS* AND ITS ENTEROTOXIN IN SOME COOKED AND HALF COOKED CHICKEN PRODUCTS

(With 4 Tables)

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مدى تواجد ميكروب الباسلس سيرس وسمومه فى بعض منتجات الدواجن المطهية والنصف مطهية

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أجريت هذه الدراسة على خمسة أنواع من منتجات الدواجن (ناجتس، فيليه صدور، فاهيتا، دبوس وأجنحة) وكانت هذه الأنواع عبارة عن منتجات دواجن جاهزة للأكل تم تجميعها من محلات الوجبات السريعة ومنتجات دواجن نصف مطهية مجمدة تم تجميعها من السوبر ماركت وكذلك نفس العينات المجمدة النصف مطهية التى تم إعدادها طبقا لطريقة الطهى المدونة على الغلاف الخارجى لكل منتج. وتم فحص جميع العينات المجمعة للكشف عن تواجد ميكروب الباسلس سيرس وقدرة العترات المعزولة على إفراز السموم المعوية. وأثبتت نتائج الدراسة تواجد ميكروب الباسلس سيرس فى جميع أنواع العينات بنسب مختلفة وكانت اعلى نسبة عزل للميكروب من عينات الوجبات الجاهزة يليها المنتجات النصف مطهية المجمدة فى حين أثبتت الدراسة أن إتباع طرق الطهى للعينات النصف مطهية المدونة على العبوات أنقصت من نسبة تواجد الميكروب بالعينات. بالنسبة لقدرة العترات المعزولة من ميكروب الباسلس سيرس على إنتاج السموم المعوية باستخدام *B. cereus* enterotoxin reversed passive latex agglutination kit أظهرت نتائج البحث أن ١٣ عترة من ٣٣ عترة (٣٩,٤%) و ٦ عترات من ٣٠ (٢٠%) ايجابية لإفراز السموم المعوية بعينات منتجات الدواجن المطهية الجاهزة والعينات المجمدة النصف مطهية على التوالى بينما وجدت عترتان فقط من ٦ عترات معزولة من العينات المطهية طبقا للطرق المدونة لها القدرة على إفراز السموم.

SUMMARY

Five types of chicken products (Nuggets, Tenders, Fajieta, Drum sticks and Wings) were used in this study. These types included ready-to-eat chicken products which were collected from fast food shops and frozen half cooked chicken products collected from supermarkets.Some samples of frozen half cooked products were cooked according to the cooking instruction printed on the package label. All samples were examined for the detection of *B. cereus* and the ability of the isolated strains to produce diarrheal enterotoxin. *B. cereus* was isolated from all the types of the examined samples with different percentages. Ready-to-eat chicken product samples recorded the highest incidence of isolation followed by frozen half cooked chicken products. While applying cooking instruction reduced the incidence of *B. cereus*.

By using *B. cereus* enterotoxin reversed passive latex agglutination kit, Bacillus diarrheal enterotoxin was detected from 13 out of 33 strains (39.4%) isolated from ready-to-eat samples, and 6 out of 30 isolates (20%) from frozen half cooked samples. Whereas, only 2 out of 6 isolates were diarrheal enterotoxigenic producing strains isolated from cooked chicken product samples.

Key words: *Cereus, chicken products, B. cereus enterotoxin, passive latex agglutination technique.*

INTRODUCTION

Bacillus cereus is an aerobic spore-forming bacterium commonly found in the environment. It is likely to be found in food because of its widespread occurrence and because of certain factors that favor its survival and presence in food production environments.

B. cereus is an important foodborne pathogen, and its strain can be characterized as mesophilic or psychrotrophic. Mesophilic strains have a growth range of 15-55 ° C and their spores tends to be more heat resistant. Whereas, psychrotrophic strains have a growth range of 4 to 35 °C and their spores tend to be less heat resistant (Choma *et al.* 2000 and Granum *et al.* 2000).

Human gastroenteritis attributed to *B. cereus* is a food intoxication after ingestion of food containing preformed enterotoxins rather than a result of colonization or infection of host.

B. cereus food poisoning was attributed to two enterotoxins, diarrhoeal which has an onset period of 10-15 hours after ingestion and mainly associated with proteinaceous food such as meat products and milk, and emetic which has a short onset period of 1-5 hours after ingestion and it is mainly associated with farinaceous foods, particularly rice, and cereal derivatives such as flour (Kramer and Gilbert, 1989).

A variety of different chicken product types, breaded, half or fully cooked are widely spread in Egyptian markets and most preferable especially by children and youth. Poultry is likely to be contaminated with *B. cereus* during grow out, from dusty housing condition, from contaminated chicks, or from feed. Hatchery environments, facilities and equipment have been shown to be contaminated with cells, which could be contaminating chicks (Willinghan *et al*. 1996).

Spores survive feed manufacture and readily colonize the gut of the chicken (Jadamus *et al*. 2001). As would be expected *B. cereus* has been found in meat and poultry products (Hatakka, 1998, Fang *et al*. 2002; Tessi *et al*. 2002).

Ingredients typically added to meat products, such as spices, seasoning and protein supplements, have been found to contain *B. cereus* (Konuma *et al*. 1998 and Te Giffel *et al*. 1996). Similarly, ingredients typical of those used in breaded coatings for chickens, such as wheat products and flour, have been shown to contain *B. cereus* (Te Giffel *et al*. 1996). Food packaging proper has been reported to harbor *B. cereus* (Vaisanen *et al*. 1991), which could lead to post cook exposure.

Therefore the aim of this study was to determine whether *B. cereus* or its enterotoxin could be detected in several types of retail chicken products.

MATERIALS and METHODS

Samples:

This study was performed on five types of chicken products (Nuggets, Tenders, Fajieta, Drum-sticks and Wings) where ten samples of each item were obtained.

First: A survey was conducted on the ready-to-eat aforementioned products served in Giza Governorate restaurants, a total of 50 samples (10 samples of each product were obtained).

Second: 50 samples of frozen half cooked chicken products (the same types of the products) were collected from supermarkets and divided into two groups. The first group was examined without cooking whereas the second group of the samples was cooked according to the cooking instructions written on the package labels.

All samples were subjected to bacterial analysis for isolation and identification of *B. cereus.*

- Isolation of *B. cereus* was performed as described in the Bacteriological Analytical Manual (US FDA, 1995) by using 25 gm of the sample enriched in 225 ml of trypticase soy-polymixin broth which stomachered for 1 min and incubated for 18-24 h at 30 ° C. then 0.1 ml was plated on mannitol-egg yolk-polymixin and incubated at 30 ° C for 18-24 h.

- Suspected *B. cereus* colonies were identified morphologically and biochemically according to Cowan and Steel, (1974).
- Detection of *B. cereus* enterotoxin was done by reversed passive latex agglutination technique (BCET-RPLA SEIKEN, Japan, Tokyo, 103)

RESULTS

Table 1: Incidence of *B. cereus* isolated from ready-to-eat chicken product samples

Samples	No. of samples	Positive samples	
		No.	%
Nuggets	10	8	80
Tender	10	6	60
Fajita	10	5	50
Drumsticks	10	8	80
Wings	10	6	60

Table 2: Incidence of *B. cereus* isolated from frozen half cooked chicken product samples

Samples	No. of samples	Positive samples	
		No.	%
Nuggets	10	8	80
Tender	10	4	40
Fajita	10	4	40
Drumsticks	10	8	80
Wings	10	6	60

Table 3: Incidence of *B. cereus* isolated from chicken product samples after application of cooking instructions.

Samples	No. of samples	No of +ve samples	Reduction %
Nuggets	10	0	100
Tender	10	0	100
Fajita	10	0	100
Drumsticks	10	4	60
Wings	10	2	80

Table 4: Incidence of enterotoxigenic strains of *B. cereus* isolated from ready- to- eat, frozen half cooked and cooked chicken product samples:

Samples	Ready -to-eat			Frozen half cooked			Cooked		
	No. of +ve	Toxin production	%	No. of +ve	Toxin production	%	No. of +ve	Toxin production	%
Nuggets	8	2	25	8	2	25	0	0	0
Tender	6	1	16	4	-	-	0	0	0
Fajita	5	3	60	4	-	-	0	0	0
Drumsticks	8	5	62.5	8	2	25	4	2	50
Wings	6	2	33.3	6	2	33.3	2	0	0
Total	33	13	39.4	30	6	20	6	2	33.3

DISCUSSION

A survey was conducted on ready to eat five chicken products (Table 1) indicated that *B. cereus* was isolated from all the examined samples. The highest incidence of *B. cereus* was found in nuggets and drumsticks (80%) followed by tenders and wings (60%) then fajieta (50%).

Nearly similar results were obtained by Harmon and Kautter (1991); Tessi *et al.* (2002) and Murindamombe *et al.* (2005), while lower percentages of occurrence were reported in other studies (Mosupye and Von Holy, 1999, 2000 and Umoh and Odoba, 1999).

The high incidence of *B. cereus* isolation may be due to the temperature of holding the chicken product, environmental contamination, surviving spores or chicken under cooking. Monitoring of cooking temperature on site was impossible, since shops served ready to eat fast food didn't consent to have their preparation examined.

Smith *et al.* (2004) reported that some types of cooked products are possible to mishandling and temperature abuse, which could potentially lead to the growth of *B. cereus* and toxin production, sometimes ready to eat chicken products are held at room or outdoor temperatures before served. During which time large population of bacteria, including pathogens, can proliferate, (Bryan *et al.*, 1982a,b). This practice potentially hazardous for products that support the growth of pathogenic bacteria and should be prevented.

Results in Table (2) shows that *B. cereus* was isolated from all types of frozen half cooked chicken products samples, the highest incidence was from nuggets and drum stick (80%) followed by wings (60%) then, tenders and fajieta (40%).

The presence of *B. cereus* in frozen half cooked products may be attributed to spore surviving from raw poultry, post processing contamination with either spices, seasoning or protein supplements that may contain *B. cereus* (Te Giffel, 1996 Konuma *et al*., 1998 and Smith *et al*., 2004).Wheat products and flour used in breaded coatings have been also shown to contain *B. cereus* (Te Giffel *et al*. 1996). Similarly, food packaging has been reported to harbor *B. cereus* (Vaisanen *et al*., 1991).

The effect of preparing chicken products samples (cooking)- according to the instruction printed on labels- on the incidence of *B. cereus* isolation is illustrated in Table (3). The results indicated that *B. cereus* failed to be isolated from nuggets, tenders and fajieta samples. This result indicated that cooking process was efficient to achieve 100% reduction of *B. cereus* isolated from samples before the cooking process and lead to conclude that the proper time-temperature exposure can be effective in killing *B. cereus* (Fruin and Guthertz, 1982 and ICMSF, 1996). Although, the cooking instruction for drum stick and wings samples were applied, *B. cereus* could survive and be isolated after cooking with reduction rate 60% and 80% respectively.

Patterson and Gibbs (1973) illustrated that cooking can kill heat sensitive microbes while allow heat resistant forms including *B. cereus* to survive. In addition, ICMSF (1996) reported that vegetative forms of pathogens in portions that are still frozen, in thick masses and in cavities or layers insulated by stuffing survive cooking temperature. Smith *et al*. (2004) found that chicken skin harbor *B. cereus* which may explain their presence in drumsticks and wings samples after cooking.

From the results achieved in Table (4), the enterotoxin analysis of the isolates in which the production of the diarrheal toxin was confirmed by the reversed passive latex agglutination test demonstrated that, 25, 16, 60, 62.53 and 33.3% of the isolates recovered from the five ready to eat samples elaborated the diarrheogenic toxin, respectively. While frozen half cooked nuggets, drum sticks and wing samples contained 2 (25%) of 8, 2 (25%) of 8 and 2 (33.3%) of 6, isolates that were shown to be enterotoxin producers, respectively.

Strains of *B. cereus* isolated from drumsticks and wings after applying the cooking instructions revealed that only 2 out of 4 isolates recovered from drumsticks samples had the ability to produce the diarrheogenic enterotoxin.

Several studies proved that *B. cereus* isolated from frozen, cooked and ready to eat chicken products had the ability to produce enterotoxin. The results achieved by Smith *et al.* (2004) and Murindamombe *et al.* (2005) were approximately near to that obtained in the present study. While Choma *et al.* (2000) recorded that *B. cereus* isolates were enterotoxigenic in slightly higher percentage.

Therefore, finding indicated that at least for the types of products represented by these samples, control measures should be directed at preventing the germination and out growth of spores in cooked food. Heat-resistant bacterial spores especially *B. cereus* can be reduced by proper cooking using suitable time-temperature to ensure safety. Also, by storing the cooked food at high temperature or keeping it at refrigeration temperature until use since *B. cereus* don't have the ability to grow and produce toxin at temperature below 4 °C (Van Netten *et al.*, 1990).

Further researches are required to determine the scope of the problem and the source of contamination.

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