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RESEARCH ARTICLE

Multiplex Polymerase Chain Reaction for Detection of Toxin Genes of *Bacillus cereus* Group Isolated from Meat and Chicken Products

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Abstract

Bacillus cereus sensu lato (*B. cereus s. l.*) is a significant cause of food spoilage issue owing to the activity of certain hydrolytic enzymes. This study aimed to detect the incidence and contamination level of meat and chicken products with *B. cereus* group in Sharkia Governorate, Egypt, with reference to their enterotoxin genes` profiles. Overall, 43 out of 200 (21.5%) examined samples were contaminated by *B. cereus* group, with identification of only one species, *B. cereus*. *B. cereus* group isolates were frequent in chicken samples (25.71%), with the highest incidence in chicken meat (30%) followed by chicken sausage and chicken luncheon (25% each). Meanwhile, they were isolated from 19.23% of examined meat products, which predominated in meat burger (25%), followed by each of meat kofta, shawarma, and luncheon (20% each), minced meat (17.14%) and meat sausage (15%). Of interest, the highest *B. cereus* count (>10^4 colony forming units (CFU)/ g) was found in 2% of positive samples, with a higher percent in meat sausage (33.33%). Whereas 15.5% of positive samples harbored *B. cereus* with counts ranging from >1x10^3-10^4 CFU/g. Molecular analysis of *B. cereus* enterotoxin genes using multiplex polymerase chain reaction (PCR) revealed that both *ces* and *nhe* genes were detected in 100% of the examined isolates, while *cytk* and *hbl* genes were present only in 9.52% and 23.8% of analyzed isolates, respectively. These findings, involving a higher occurrence of *B. cereus* and their toxin genes in meat and chicken products represent a serious public health concern in Egypt.

Keywords: *B. cereus*; Enterotoxin genes; Foodborne infection; Multiplex PCR

Introduction

Bacillus cereus (*B. cereus*) group comprises eight species; *B. pseudomycoides*, *B. mycoides*, *B. weihenstephanensis*, *B. cytotoxicus*, *B. toyonensis*, *B. cereus sensu stricto*, *B. anthracis* and *B. thuringiensis* [1].The species of this group are simply differentiated from other members of the aerobic spore-forming bacteria by their incapability to ferment the mannitol sugar and their lecithinase production; but it is too hard to be differentiated from each other [2]. *B. cereus*, Gram positive motile rods and beta hemolytic, are dangerous to humans causing foodborne illness [3]. Other strains could be used as probiotics for animals [4]. *B. cereus* foodborne intoxication leads to two forms of illness; diarrheal and emetic (vomiting) [5]. Meat, milk, fish, vegetables, pudding, soup and sauce have been recorded as the predominant food types associated with the diarrheal syndrome. However, rice products, potato, pasta and cheese products are the most common food associated with the emetic syndrome [6, 7]. The diarrheal syndrome supposed to be a toxicoinfection occurred by the vegetative cells, which are consumed as spores or viable cells secreting enterotoxin proteins in the small intestine [8, 9]. It is associated with diarrhea, abdominal spasms and gastrointestinal pain 8-16 h after consumption of contaminated food [10]. The emetic disease is an intoxication triggered by the cereulide toxin, which is performed in food. It is mainly characterized by nausea and

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vomition within 1-5 h after consumption of contaminated food [10, 11].

The illnesses associated with this organism are probably mediated by the synergistic effects of several virulence products and spores [12]. The cereulide, a hydrophobic non-ribosomally peptide synthetase (NRPS) system, is a small cyclic emetic toxin of a 1.2 kD molecular weight [13]. It is encoded by the cereulide synthetase (*ces*, 24-kb) gene cluster [14, 15]. It is highly heat stable (121ºC for 90 min), inactivated upon exposure to pH from 2 to 11 and the proteolytic activities of pepsin and trypsin [16, 17]. Cereulide is not associated with sporulation and is performed in improper refrigerated foods resulting in vomition when ingested at concentrations about 10 µg/kg body weight [18, 19]. Diarrheal toxins are heat-labile chromosomally mediated enterotoxins as hemolysin BL (HBL; encoded by *hblABCD)*, non-hemolytic enterotoxin (NHE; *nheABC*), cytotoxin K (*cytK*), cereolysin (*cerAB*) and enterotoxin FM (*entFM*) produced during the exponential phase of vegetative evolution in the small intestine [20-22]. The CytK, Hbl and Nhe pore forming toxins possess cytotoxic and hemolytic activities on host cell membrane [23, 24]. The enterotoxin FM (*entFM*), hemolysins (*hly*), putative enterotoxin (ent ABC), degradative enzymes and phospholipases C could not produce direct cytotoxic activity, but they contribute to cytotoxic and hemolytic activities of *B. cereus* group a well as attachment to the host epithelial cells [25,26].

World Health Organization listed *B. cereus* as one of 22 foodborne pathogens for evaluating the volume of foodborne illnesses [27]. *B. cereus* infections with vomition and diarrhea have been previously detected in Finland [28], Belgium [29], Thailand [30], United Kingdom (UK) [31, 32] and United States (USA) [33].

In Egypt, foodborne *B. cereus* outbreaks were not locally proved. The lack of correct documents may be related to the similarity of the symptoms with other foodborne pathogens [34]. Hence, to stand upon the Egyptian outbreak incidence, this study aimed to determine the incidence and contamination level with *B. cereus* group isolates in different meat and chicken products in Sharkia Governorate, Egypt. Moreover, concurrent determination of *B. cereus* enterotoxin genes using multiplex PCR was performed to verify whether the isolated *B. cereus* group could be a significant foodborne pathogen.

Materials and Methods

Samples

In all, 200 meat and chicken products` samples were collected from various supermarkets and restaurants in Zagazig City, Sharkia Governorate, Egypt. Meat products (n= 130) included frozen minced meat (n= 35), fresh and frozen sausage (n= 20), frozen burger (n= 20), cooked shawarma (n= 10), chilled luncheon (n= 25) and frozen kofta (n= 20). While chicken products (n= 70) comprises fresh and frozen chicken meat (n= 20), frozen sausage (n= 20), chilled luncheon (n= 20) and cooked shawarma (n= 10). The collected samples were transported in a cool container under complete aseptic conditions in an ice box as soon as possible to the laboratory of Microbiology, Faculty of Veterinary Medicine, Zagazig University for bacteriological analysis on the same day of collection.

Isolation of B. cereus group

Twenty-five milligrams of each meat and chicken product sample was blended with 225 mL of buffered peptone water (PBW; Oxoid, UK) for 1 min [35]. Tenfold serial dilution was prepared, and 10 µL of each diluted sample was cultivated onto polymyxin egg yolk mannitol bromothymol blue (PEMBA; Oxoid, UK) medium**,** followed by incubation at 30°C for 24-48 h. Ideal colonial appearance of *B. cereus* group isolates is crenate with a characteristic turquoise to peacock blue color, surrounded by a same color precipitate of hydrolyzed lecithin with the failure to utilize mannitol (Nagler's reaction). The total viable count of presumptive colonies was obtained and the $\log_{10}$ colony-forming unit (CFU)/g of sample was then calculated as previously

described [36]. Three independent experiments were performed for each sample. Moreover, one ideal colony of the supposed *B. cereus* group was subcultured onto brain heart infusion (BHI; Sigma-Aldrich, USA) agar followed by incubation at 30°C for 24 h. Thereafter, a single colony of each pure culture was preserved into an Eppendorf tube filled with sterile trypic soya broth (TSB; Difco, USA). The cultures were overnight-incubated then frozen at −20°C with glycerol 30%.

Confirmation of B. cereus group isolates

Suspected *B. cereus* colonies were subjected to Gram staining then examined for catalase production, hemolysis, motility, rhizoid growth, citrate utilization, protein toxin crystals production and psychrotolerance for the species identification as documented elsewhere [12, 37, 38].

DNA extraction

Typical colonies were picked up from presumptive *B. cereus* isolates, inoculated in 5 mL BHI broth (Sigma-Aldrich, USA) then incubated at 35 °C overnight. DNA extraction was applied from the broth culture using DNeasy Mini Kit (Qiagen, Germany) following the supplier protocol.

PCR identification of B. cereus group and their toxin genes

B. cereus group isolates were identified at genus level using the *groEL* gene in a conventional PCR (cPCR) assay. Multiplex PCR was then performed for the simultaneous detection of *B. cereus* group toxin genes (*hbl*, *nhe*, *ces*, and *cytk*) using specific primers (Table 1) [39-41]. PCR amplification reactions were applied in the MJ Research PTC-100 thermal cycler (Bio-Rad, USA) using 50 µL reaction volume comprising 25 µL of Dream *Taq* Green Master Mix (2X) (Fermentas, USA), 1 µL of each primer (20 pmole) (Sigma-Aldrich, USA), 5 µL template DNA and the volume was completed to 50 µL by nuclease-free water. Oligonucleotide primers used for PCR assays and their cycling programs are depicted in Table 1. PCR products were electrophoresed in 1.5 % agarose gel stained with ethidium bromide at a concentration of 0.5 µg/mL, viewed by ultraviolet transilluminator (Spectroline, Westbury, USA) and analyzed using Gel documentation system (Alpha Innotech, USA) [42].

Table (1): Primer sets and cycling conditions used to amplify *B. cereus* group isolates and their toxin genes

PCR type	Target gene	Primer name	Primers sequence (5`-3`)	Cycling conditions	Amplified product (bp)	Reference
Uniplex PCR	*groEL*	balF	TGCAACTGTATTAGCACAAGC T	One cycle at 94 °C for 5 min, 35 cycles of 94 °C for30 sec; 55 °C for40sec, and 72°C for 45 sec and finally72ºC for 10 min.	533	[39]
		balR	TACCACGAAGTTTGTTCACTACT			
Multiplex PCR	*hbl*	HD2F	GTA AAT TAI GAT GAI CAA TTTC	One cycle at 95 °C for 15 min, 30 cycles of 95 °C for 30 sec ; 49°C for 30 sec, and 72°C for 1min and finally72ºC for 2 min.	1091	[40]
		HA4R	AGA ATA GGC ATT CAT AGA TT			
	nhe	NA2F	AAG CIG CTC TTC GIA TTC		766	[40]
		NB1R	ITI GTT GAA ATA AGC TGT GG			
	cytk	ckF2	ACA GAT ATC GGI CAA AAT GC		421	[40]
		ckR5	CAA GTI ACT TGA CCI GTT GC			
	ces	cesF1	GGTGACACATTATCATATAAGGTG		1271	[41]
		cesR2	GTAAGCGAACCTGTCTGTAACAACA			

PCR, polymerase chain reaction; F, forward; R, reverse

Statistical analysis

The data were statistically analyzed using Statistical Package for Social Sciences (SPSS), version 26.0 (IBM Corp., Armonk, NY, USA). Pearson's chi-square and Kruskal Wallis tests were used to determine the statistical differences in number of positive isolates on PEMBA between different sources (i.e., meat and chicken) as well as different meat (minced meat, luncheon, sausage, shawarma, burger and kofta) and chicken (chicken meat, luncheon, sausage and shawarma) products. *P* values of < 0.05 were considered statistically significant.

Results

As shown in Table 2, the bacteriological examination of the collected samples revealed that *B. cereus* group species were found in 43 out of 200 different meat and chicken products with a percentage of 21.5%. The incidence of *B. cereus* group isolates was high in chicken samples with a total percentage of 25.71% distributed as 30% in chicken meat and 25% in both chicken sausage and luncheon. While in meat samples, the isolates were recovered with a percentage of 19.23% being presented as 25% in beef burger and 20% in each of beef kofta, shawarma and luncheon. Statistical analysis revealed non-significant differences ($P > 0.05$) in the levels of contamination of *B. cereus* isolated either from meat or chicken products.

The occurrence of *B. cereus* group isolates and their CFU/g of samples are shown in Table (3). Interestingly, 40 samples (25 of meat products and 15 of chicken products) were contaminated with *B. cereus* group isolates with total colony counts of $\geq 10^3$ CFU/g (range= 1×10^3 - 4×10^5 CFU/g).Out of the 40 *B. cereus* positive samples, 31 (15.3%) were contaminated with $>10^3$ -10^4 CFU/g, 4 (2%) samples only had $>10^4$ CFU/ g, those were higher reported in meat products (3/130, 2.3%) than chicken products (1/70; 1.4%) and the remaining five samples had 10^3 CFU/g.

Table (2): Incidence of *B. cereus* group isolates in different meat and chicken products at Zagazig, Sharkia, Egypt.

Sample type	Number of samples	No. of positive isolates on PEMBA (%)	*P* value*
Meat products	**130**	**25 (19.23)**	
Minced meat	35	6 (17.14)	
Luncheon	25	5 (20.00)	
Sausage	20	3 (15.00)	> 0.05
Shawarma	10	2 (20.00)	
Burger	20	5 (25.00)	
Kofta	20	4 (20.00)	
Chicken products	**70**	**18 (25.71)**	
Chicken meat	20	6 (30.00)	
Luncheon	20	5 (25.00)	> 0.05
Sausage	20	5 (25.00)	
Shawarma	10	2 (20.00)	
Total	**200**	**43(21.50)**	

PEMBA; Polymyxin egg yolk mannitol bromothymol blue agar
P values indicates non-significant differences

Table (3): Colony forming units of recovered *B. cereus* group isolates from different meat and chicken products at Zagazig, Sharkia, Egypt.

Sample type (No)	No (%) of recovered isolates *	No (%) of positive samples with *B. cereus* group within the range of	
		$>10^3$-10^4 CFU/g	$>10^4$ CFU/g
Meat products (130)			
Minced meat (35)	6 (17.14)	4 (66.67)	1 (16.67)
Luncheon (25)	5 (20.00)	5 (100.00)	0 (0.00)
Sausage (20)	3 (15.00)	2 (66.67)	1 (33.33)
Shawarma (10)	2 (20.00)	2 (100.00)	0 (0.00)
Burger (20)	5 (25.00)	2 (40.00)	1 (20.00)
Kofta (20)	4 (20.00)	4 (100.00)	0 (0.00)
Chicken products (70)			
chicken meat (20)	6 (30.00)	3 (50.00)	1 (16.67)
Luncheon (20)	5 (25.00)	5 (100.00)	0 (0.00)
Sausage (20)	2(10.00)	2 (100.00)	0 (0.00)
Shawarma (10)	2 (20.00)	2 (100.00)	0 (0.00)
Total (200)	40 (20.00)	31(15.50)	4 (2.00)

CFU, colony forming units

* isolates of total colony count $\geq 10^3$ CFU/g

Conventional isolation and identification of *B. cereus* group isolates revealed that only one species (*B. cereus*) was currently identified. Twenty one of the recovered isolates were selected for molecular confirmation and toxin genes identification representing analyzed samples from all sources. These isolates (n= 21) were confirmed by the cPCR for detection of *groEL* gene with a product size of 533 bp as shown in Table (4) and Figure (1A).

B. cereus isolates (n= 21), were then subjected for the molecular detection of toxin genes by multiplex PCR. As shown in Table 4 and Figure 1B, the toxin gene profiling results showed that both *ces* and *nhe* genes (corresponding to cereulide synthetase and non-hemolytic toxins, respectively) were presented in all tested isolates with amplicons of 1271and 766 bp, respectively. Meanwhile, *hbl* gene encoding haemolysin toxin complex was detected in five isolates (5/21, 23.8%) with a product size of 1091 bp. The *cytk* gene encoding cytotoxin K toxin was detected only in two isolates (2/21, 9.52%) with an amplicon of 421bp.

Table 4: Enterotoxin gene profile of *B. cereus* isolated from meat and chicken products at Zagazig, Sharkia,

Isolate code No.	Isolates source	*hbl*	*ces*	*cytK*	*Nhe*
1	Meat sausage	-	+	+	+
2	Chicken meat	-	+	-	+
3	Meat sausage	-	+	-	+
4	Chicken sausage	-	+	-	+
5	Minced meat	+	+	-	+
6	Meat luncheon	-	+	-	+
7	Meat kofta	-	+	-	+
8	Meat shawarma	-	+	-	+
9	Meat shawarma	+	+	-	+
10	Meat kofta	+	+	-	+
11	Chicken meat	+	+	-	+
12	Chicken meat	-	+	-	+
13	Meat kofta	-	+	-	+
14	Meat kofta	-	+	-	+
15	Chicken meat	-	+	-	+
16	Meat burger	-	+	-	+
17	Chicken meat	-	+	-	+
18	Meat luncheon	-	+	-	+
19	Chicken luncheon	+	+	-	+
20	Chicken luncheon	-	+	-	+
21	Meat burger	-	+	+	+

All isolates were positive for *groEl* gene

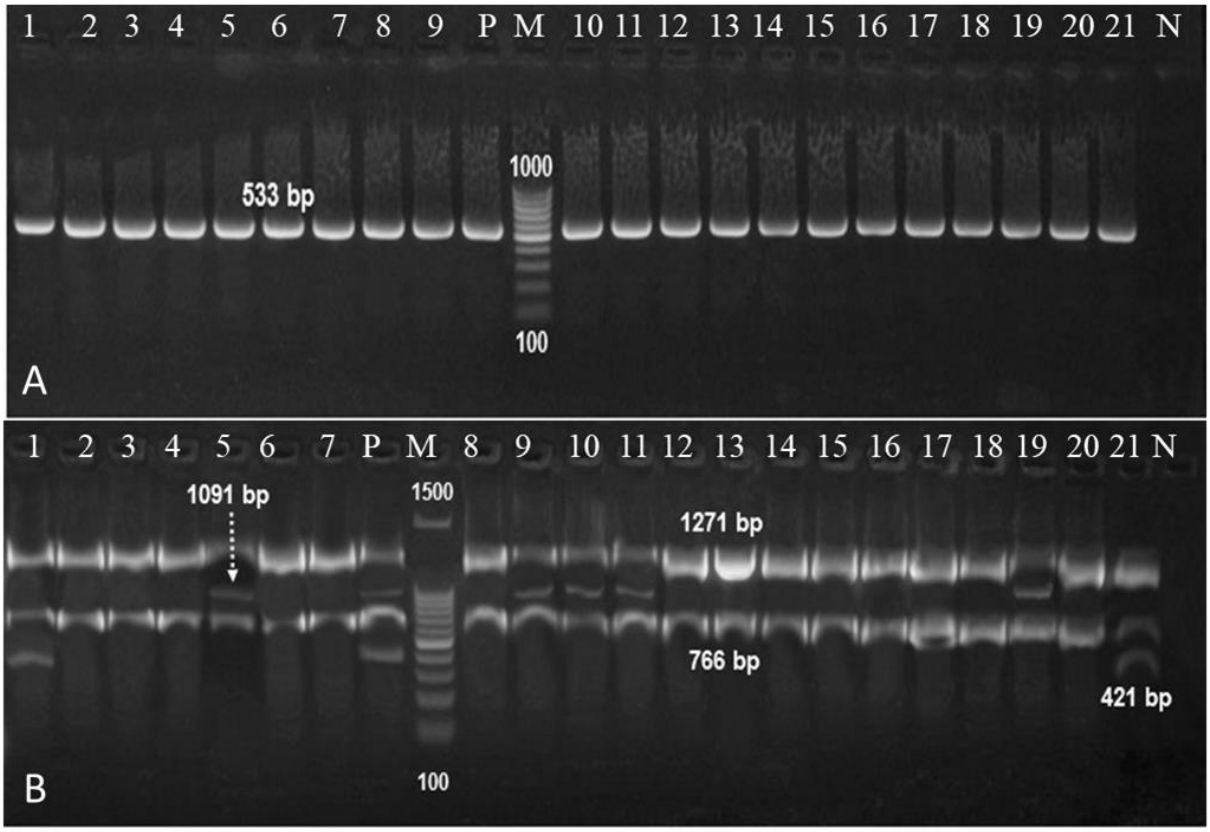

Figure 1: Agrose gel electrophoresis showing PCR products of 21 amplified B. cereus group isolates. A: lanes 1-21: B. cereus positive isolates for groEL gene (533 bp) by uniplex PCR. B: Toxin gene profiling targeting the genes amplicons of ces (1271bp), hbl (1091bp), nhe (766bp) and cytK (421bp) by multiplex PCR. M: 100 bp DNA Marker (QIAGEN, USA), P: positive control, N: negative control

Double or triple combinations of detected toxin genes were surprisingly found among tested isolates without any single occurrence while using a multiplex PCR assay. The results showed that, 23.8% (7/21) of tested *B. cereus* isolates originated mostly from meat products contained three various toxin genes; including isolates Nos. 1 and 21 (*ces*, *nhe* and *cytk*) and Nos. 5, 9, 10, 11 and 19 (*ces*, *nhe* and *hbl*). The seven *B. cereus* isolates were recovered from meat samples of four different food companies of famous origin, which has public health hazard.

Discussion

Food safety represents a public health concern. Ingestion of contaminated food by pathogenic bacteria and their toxins could result in severe diseases [43-45]. This study shed the light on the incidence and contamination level of meat and chicken products with *B. cereus* group isolates along with the simultaneous detection of *B. cereus* enterotoxin genes using multiplex PCR. Our results revealed that *B. cereus* group isolates were detected and identified as one species (*B. cereus*) in different meat and chicken products with a percentage of 21.5%. Higher percentages of *B. cereus*; (30.9%) [46], **(**29.33%) [47] in India and (27.8%) in Tunisia [48]**,** were previously reported in raw meat and meat products. This variation may be due to advanced and better hygienic practices tracked in restaurants and meat shops in recent times. Herein, *B. cereus* recovery was high in chicken meat with a total percentage of 25.71%, which is higher than the previously recorded results; (20%) in India [49] and (22.4%) in Turkey [50]. In contrast, our results were lower than the recorded percentages of 27.3% in India [46] and 26.6% in Pakistan [51].

In the current study, *B. cereus* meat isolates were recovered with a percentage of 19.23%. In Egypt, higher percentages of 43%

[52] and 38.33% [53] were previously reported. The recovery percent of *B. cereus* isolates was 25% in beef burger, which is lower when compared with the reported percentages of 43.3% [52] and 36.67 [53] in Egypt, 34% [54] in China**,** 31.25% [55] In Iran and 30% [56] in beef products in Egypt. The incidence of *B. cereus* was 20% in the examined meat luncheon samples. In the same way, previous studies in Egypt [53, 57] are consistent with our reported results (20%). Despite, much higher percentages (26.7, 44 and 35%) of *B. cereus* which were previously reported in Egypt [52, 58, 59, respectively**]**, lower *B. cereus* incidence (16%**)** was recorded in South Egypt by Hamouda [60]. Moreover, in meat sausage, *B. cereus* incidence was relatively low (15%). However, higher percentage was recorded by Abd El-Wahaab [61] (40%) and a recent study of Tharwart *et al*. [58] (32%) in Egypt. In our study, minced meat showed 17.14% *B. cereus* incidence. Conversely, it is lower than the recorded results of Abd-Elaziz *et al.* [57] (20%) in Egypt. The existence of *B. cereus* in uncooked meat products and/or chicken meat may be attributed to the contamination during slaughtering as well as processing, transportation, delivery or meat storage. The insufficient storage temperatures of the uncooked meat and poultry samples may also allow the bacterial growth [62].

B. cereus considered dangerous for human consumption at concentrations of $>10^5$ CFU/mL or g food sample according to United Kingdom standard guidelines [63]. However, it should be $\geq 10^4$ CFU/mL or g of food as documented by Food Standards of Australia-New Zealand [64]. *B. cereus* concentration of less than 10^3 CFU/mL or g of food is considered acceptable [63]. However, low *B. cereus* contamination level (10^3 CFU/g or mL of food sample) may be enough to induce food poisoning thereafter [12, 65].

It is significant to denote that food products may be simply contaminated due to storage and handling conditions or insufficient cleaning and sanitation of the tools and utensils. These causes rapidly increase in bacterial count to reach the unsafe level of up to 10^4 CFU/mL or g. Our data showed that *B. cereus* was recovered from diverse foodstuffs of beef and chicken sources and their products. In all, 31 (15.3%) analyzed samples were contaminated by *B. cereus* group isolates with CFU value of $>10^3$ - 10^4 CFU/g, while only 4 (2%) samples had $>10^4$ CFU/g. Near count ranging from 10^3 - 10^4 CFU/mL or g sample was obtained in a Turkish study on spices [66], and different food samples in a Tunisian study [48]. The latter study showed that *B. cereus* count of 10^3 - 10^4 CFU/mL or g was proved in 15.7% of samples and conversely, they showed that count of $>10^4$ was occurred in a percentage of 6.8% of food samples.

The current high counts of $> 10^4$ CFU/g were depicted in three (3/130; 2.3%) meat product samples (including minced meat, sausage and burger) and one (1/70; 1.4%) chicken meat sample. High bacterial load in chicken and meat products was consistent with a previous result [46] of meat products in India. Additionally, it was similar to those reported in different foodstuffs other than meat including fresh-cut vegetables, cereals, pastry products and cooked foods in Tunisia [48]. In Denmark, Rosenquist and his colleagues [67] reported high *B. cereus* count in fresh tomatoes and cucumbers, heat-treated products, cake custard and dessert. However, an Indian study proved much higher *B. cereus* counts ($>10^5$ CFU/g) in 10% of dairy product samples [68]. The detected high counts in meat product samples may result from using of food additives such as contaminated spices that may increase the number of bacterial spores and consider a danger in the case of insufficient heat treatment, which was documented previously [69] (10^5CFU/g of spices).

In the current study, multiplex PCR was done for the simultaneous detection of heat-labile, chromosomally mediated enterotoxins as hemolysin BL, non-hemolytic enterotoxin and cytotoxin K, which are responsible for the diarrheal form of infection, in addition to the cereulide which is related to emetic form of *B. cereus*. Our results revealed that *nhe* and *ces* gene (100%, each) were the most frequently detected enterotoxin genes of *B. cereus*. In

relation to *nhe* gene, similar studies have reported this gene in all analyzed raw meat and beef luncheon [53, 70, 71]. But slightly lower percentages of *nhe* gene was recorded previously [46] (89.7%) and [57] (50%) in minced meat samples. On the other hand, a lower percentage of *ces* gene was reported in many previous studies; [54] (7%), and [33] (0%). Regarding the *hbl* gene, it was reported here with a percentage of 23.8%. A higher percent of *hbl* gene (55.2%) was obtained previously [46]. However, in another study, examined meat samples were negative for this gene [72]. Likewise, the *cytK* gene was detected in the current study by a lower percentage (9.52%). On contrary, a higher percent of *cytK* gene was recorded by previous investigations; [56] (100%), [73] (52.6%), and [46] (41.4%). These findings, involved with a higher incidence of *B. cereus* and their toxin genes in meat and chicken products mark the isolated strains as a significant foodborne pathogens and a risk for consumers.

Conclusion

Herewith, we reported a relatively high incidence of *B. cereus* in chicken and meat products, which poses a potential public health threat. Inclusion of enterotoxigenic genes in the recovered isolates potentiates the hazard of food poisoning. Consequently, strict maintenance of good practices during processing, strengthened by maintaining the cold chain during transport, distribution and carcass commercialization is of central importance to ensure both public health and food quality.

Conflict of interest

The authors declare not conflict of interest.

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الملخص العربي

تفاعل البلمرة المتسلسل المتعدد لتحديد جينات السموم لمجموعة الباسيليس سيريس المعزولة من منتجات اللحوم والدواجن

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تعد الباسيليس سيرس سنسولاتو سبب هام من اسباب فساد الغذاء والذى يرجع الى عمل العديد من انزيمات التحلل الموجودة بها. تهدف هذه الدراسة الى تحديد مدى حدوث ومستوى التلوث ببكتريا مجموعة الباسيليس سيريس فى منتجات اللحوم والدواجن فى محافظة الشرقية، مصر، إلى جانب دراسة الصورة الجينية للسم المعوي. اجمالا، 43 من 200 (21.5٪) عينة تم فحصها كانت موجبة لمجموعة الباسيليس سيريس وقد تم تصنيفها جميعا لنوع واحد فقط وهو الباسيليس سيريس. كانت الباسيليس سيرس اكثر تواجدا فى عينات الدواجن بنسبة 25.71٪ بأعلى نسبة تواجد فى لحوم الدجاج (30٪) يليها السوسيس واللانشون (25% لكل منهما). في حين تم عزل مجموعة الباسيليس سيريس من 19.23٪ من منتجات اللحوم، والتي كانت سائدة في برجر اللحم (25٪) يليها كل من كفتة اللحم ، الشاورما واللانشون (20٪) واللحوم المفرومة (17.14٪) وسوسيس اللحم (15٪). من الجديد بالذكر ان اعلى عدد للباسيليس سيريس (اكبر من 10^4 لكل جرام من العينة) تواجد فقط فى 2% فى العينات الايجابية، بينما 15.5 ٪ من العينات كان عدد البكتريا بها 10^4-10^3 لكل جرام من العينه. أظهر التحليل الجزيئى لجينات سم الباسيليس سيرس باستخدام تفاعل البلمرة المتسلسل المتعدد تواجد كلا من الجينين *ces* و *nhe* في 100٪ من العزلات بينما جينات *hbl* و *cytk* كانت موجودة فقط في 23.8٪ و 9.52٪ من العزلات على التوالي. نستخلص من هذه النتائج و المتضمنة ارتفاعاً نسبياً لمعدل حدوث بكتيريا الباسيليس سيريس في منتجات اللحوم و الدجاج انها من مسببات الأمراض الغذائية الهامة في مصر. تواجد جينات السموم فى البكتريا المعزولة يزيد من خطر التسمم الغذائى. وبالتالى, فإن اتباع الإجراءات الجيدة أثناء المعاملات والتى يتم تعزيزها من خلال التبريد أثناء النقل والتوزيع وتسويق المنتجات يكون لها اهمية كبيرة لضمان الصحة العامة وجودة الغذاء.