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A study on toxin genes and cytotoxicity levels of *Bacillus cereus* in various ready-to-eat foods and pastry products in Turkey

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ABSTRACT

Bacillus cereus is a spore-forming and toxin-producing gram-positive bacteria widely isolated from soils, meat, milk, and vegetables. It is recognized as one of the pathogenic bacteria that can lead to food poisoning and food spoilage in food service systems due to its ease of contamination of foods and lack of guarantee of elimination by pasteurization and sanitation practices. *B. cereus* causes two types of diseases mainly characterized by diarrhea and vomiting type syndrome with the toxins it produces. Toxins produced by *B. cereus* are mainly heat-stable emetic toxin and three different heat-labile enterotoxins. Foodborne illnesses of the diarrheal type are caused by the single protein toxin; cytotoxin K (CytK), and both tripartite toxins; hemolysin BL (Hbl), and the non-hemolytic enterotoxin (Nhe), whereas the emetic type, is caused by an emetic toxin cereulide. In this study, 225 ready-to-eat foods and pastry products were analyzed for *B. cereus*, its toxin profiles, and cytotoxicity effects. Multiplex PCR is used to identify the presence of the Hbl, CytK, and emetic toxin encoding genes. Component-specific antibody-based ELISA tests were utilized to determine the Hbl-L_2 and NheB components. Cytotoxic activity of the *B. cereus* isolates on Vero cells was also identified. In total, *B. cereus* was detected in 37 out of 225 (16.4%) food samples. From the positive 37 *B. cereus* isolates, the *ces* gene was not identified, whereas 91.9% (34) Nhe, 56.8% (21) Hbl, and 8.1% (3) CytK encoding genes revealed positive results on PCR analysis. PCR results were also compatible with ELISA and Cytotoxicity tests. In a nutshell, 16.4% prevalence of *B. cereus* in foods is insufficient, and the presence or absence of toxin genes may not yield reliable results. It is critical to detect pathogenic *B. cereus* toxin gene profiles as well as toxin production ability at the same time. This study presents for the first time, data from a cell culture cytotoxicity test using specific monoclonal antibody-based sandwich ELISA and multiplex PCR for ready-to-eat foods and pastry products in Turkey

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Introduction

B. cereus is an aerobic or facultative anaerobic, gram-positive endospore-forming soil bacteria that grows at 28-35 °C optimum and is mainly isolated from meat, milk, and vegetable products. Its enterotoxins cause emetic or diarrheal characterized foodborne illness (Schoeni and Lee Wong, 2005; Wijnands et al., 2007; Stenfors Arnesen et al., 2008).

Single protein cytotoxin K (CytK) and three compounds Hemolysin BL (Hbl) and Non-hemolytic enterotoxins (Nhe) cause diarrheal-type foodborne illness. Hbl consists of B, L_1, and L_2 protein complexes, and Nhe consists of NheA, NheB, NheC protein complexes. In addition to these enterotoxins, there are recently published article claiming BceT, enterotoxin FM toxins, enzymes hemolysis II and III, InhA2 protease and phospholipase C enzymes are also associated with the formation of the diarrheal form (Lund and Granum 1996; Lund et al., 2000; Fagerlund et al., 2004; Lindbäck et al., 2010). More importantly, Hbl, Nhe, CytK and emetic toxins of *B. cereus* have been affiliated with food poisoning (Lindbäck et al., 2010).

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Contrasting to the single protein enterotoxin CytK and the three component enterotoxins Hbl/Nhe (Agata et al., 1995), the 17 different isoforms of the cyclic depsipeptide emetic toxin cereulide (Marxen et al., 2015) are responsible for emetic-type foodborne illness. Having said that, the diarrheal type of illness usually has mild symptoms for a longer period of time than the emetic form, such as mild abdominal pain and watery diarrhea. These symptoms start to appear 8 to 16 hours after consuming food and can last for 12 to 24 hours (Gaulin et al., 2002; Logan and Rodrigez-Diaz 2006; Murray et al., 2007). While produced in the small intestine during vegetative cell proliferation (Logan and Rodrigez-Diaz 2006) heat-labile diarrheal enterotoxins have an infectious dosage of 10^4–10^9 cell/g of food consumed. In contrast to the diarrheal form, the emetic form of the foodborne illness manifests its symptoms within a shorter period of 1 to 6 hours after food consumption. The infective dose of nausea and vomiting in the emetic form is 10^5-10^8 cells per gram, and it often goes away within 24 hours (Logan and Rodrigez-Diaz 2006).

Numerous studies have reported the detection of more than 90% Nhe and 43-83% in about 50% frequency of Hbl encoding toxin genes (Hansen and Hendriksen 2001; Yang et. al., 2005; Ehling-Schulz et al., 2006) diarrheal-type foodborne illnesses (Didier et al., 2016). Three components of the Nhe (A, B, C) encoded by the *nheA*, *nheB, nheC* genes; additionally the *hblC*, *hblD* and *hblA* genes encode the three partite toxins Hbl (L_2, L_1 and B) respectively (Granum et al., 1999).

For the highest level of biological activity, Nhe and Hbl require all three complex proteins (Clair et al., 2010; Sastalla et al., 2013; Dietrich et al., 2021), while the maximum toxic activity can be obtained with the optimum molar ratio between the three components A, B, C (10:10:1) and L_1, L_2, B (1:1:1) respectively (Lindbaeck 2004; Stenfors Arnesen et al., 2008; Didier et al., 2016).

Due to its proteolytic, lipolytic, and saccharolytic activities, the common contaminant *B. cereus* can cause food to spoil and become poisonous. It is considered one of the pathogenic bacteria that can endanger human life in food service systems (Ehling-Schulz et al., 2004; Tewari and Abdullah, 2015), as inactivation of *B. cereus* is not insured by pasteurization and sanitation techniques. In 413 epidemics between 2007 and 2014, the European Food Safety Authority (EFSA) reported that *B. cereus* was the source of 6657 cases. It has been noted that these cases most frequently occurred due to mixed-type foods and open buffet meals (EFSA Panel, 2016). The three main causes for *B. cereus*-related food poisoning are currently thought to be Hbl, Nhe, and emetic toxin (Schoeni and Wong, 2005; Stenfors Arnesen et al., 2008).

Foodborne outbreaks linked to *B. cereus* may become more frequent, necessitating quick, accurate test procedures. Currently, ELISA, PCR, rapid strip tests, various cell culture assays and LC-MC technics are used to detect *B. cereus* and its toxins. However, most rapid toxin kits have been used to qualitatively detect Hbl-L_2 or NheA components (Granum et al., 1993; Beecher and Wong, 1994), and the results of these rapid toxin kits are incompatible with molecular-based techniques (Ouoba et al., 2008; Ankolekar et al., 2009).

Dietrich et al. (1999, 2005) obtained monoclonal antibodies for the accurate immunochemical detection of each individual protein of Hbl and Nhe (mAbs). It's important to obtain consistent results when using molecular and immunological techniques. It is also crucial to determine the potency of cytotoxic capability and the detection of enterotoxins. Therefore, the objectives of this study were to investigate the consistency of 36 *B. cereus* foodborne isolates' molecular, immunological, cell culture, and cytotoxic test results.

Materials and Methods

Bacterial strains and isolates: Enterotoxigenic *B. cereus* reference strains; MHI163 (Hbl, Nhe), MHI165 (cereulide), MHI1307 (CytK1) and MHI 241 (Nhe) were obtained from the Milk Hygiene Institute (MHI). All other isolates were obtained from various ready-to-eat (RTE) foods and pastry products.

Microbial analyses: Enumeration of *B. cereus* in foods was carried out through ISO 7932:2004. Reference strains and 36 *B. cereus* isolates were cultured on supplemented with 5% blood sheep Columbia agar (Oxoid, PB 5039 A) and incubated for 24 h at 32 °C. After the first incubation, one colony was selected and inserted into a 5 mL Brain Heart Infusion (BHI) broth (Merck, 10493) for an additional 24 h at 32 °C incubation. DNA of *B. cereus* isolates were extracted from the 24 h incubated BHI cultures.

Conventional multiplex PCR: The Qiagen DNeasy blood and tissue kit was used in this study to extract the DNA of bacterial isolates in accordance with the manufacturer's instructions. The genes of the *B. cereus* toxin were identified using multiplex PCR. The samples were screened for the presence of *ces*, *cytK1*, *nheA*, *nheB*, *nheC* and *hblC* genes. A single primer was used to detect for the nheB and nheC target genes . Table 1 provides an overview of the PCR conditions and primers. Amplified PCR products with 5 µL loading buffer were run by 2% agarose gels in Tris acetate

Table 1. PCR conditions and primers

Target Gene	Primer	Primersequans (5'→3')	Amplification length (pb)	PCR conditions	References
hblC	L2aF	CGA AAA TTA GGT GCG CAA TC	411	94°C, 60 s; 51°C, 60 s; 72°C, 60 s (30 cycles)	(Moravek et al., 2004)
	L2aR	TAA TAT GCC TTG CGC AGT TG			
hblD	L1aF	AGG TCA ACA GGC AAC GAT TC	205	94°C, 60 s; 52°C, 60 s; 72°C, 60 s (30 cycles)	(Moravek et al., 2004)
	L1aR	CGA GAG TCC ACC AAC AAC AG			
hblA	HA-F1	ATT AAT ACA GGG GAT GGGA GAA ACT T	237	94°C, 60 s; 52°C, 60 s; 72°C, 60 s (30 cycles)	(Yang et al., 2005)
	HA-R1	TGA TCC TAA TAC TTC TTC TAG ACG CTT			
nheA	45c1	GAG GGG CAA ACA GAA GTG AA	186	94°C, 60 s; 52°C, 60 s; 72°C, 60 s (30 cycles)	(Moravek et al., 2004)
	45c2	TGC GAA CTT TTG ATG ATT CG			
nheBC	NhEBC1	ACA TTG CGA AAG ATA GCT GGA	300	94°C, 60 s; 48°C, 60 s; 72°C, 60 s (30 cycles)	(Dietrich et al., 2005)
	NhEBC2	TGT TCT GCT GCA AAA GGA TG			
ces	cesF1	GGT GAC ACA TTA TCA TAT AAG GTG	1271	94°C, 60 s; 52°C, 60 s; 72°C, 60 s (30 cycles)	(Ehling-Shulz et al., 2005 a,b)
	cesF2	GTA AGC GAA CCT GTC TGT AAC AAC A			
cytK1	F2 R7	AAC AGA TAT CGG TCA AAA TGC CGT GCA TCT GTT TCA TGA GC	623	94°C, 60 s; 52°C, 60 s; 72°C, 60 s (30 cycles)	(Lund et al., 2000)

EDTA (Sigma-Aldrich, T4038-5x1L) antigen retrieval buffer at 200 volts and 400 ma. for 45 minutes was used in nucleic acid electrophoresis.

Pre-enrichment of *B. cereus* isolates in CGY medium: Pre-enrichment in CGY (casein hydrolysate glucose yeast) broth (Merck, 101868) was used to stimulate enterotoxin production of *B. cereus* isolates. Pure cultures were added into supplemented CGY (18 mL CGY + 2mL 10% glucose) broth in the shaking water bath for 17 h at 32°C. The next day, 200µL of the enriched isolates were transferred into a new 20 mL of CGY broth. Liquid cultures were incubated for another 6 h with shaking at 32 °C. After the final incubation, the enriched culture was centrifuged at 3000 rpm at 4 °C for 20 minutes. At the end of the centrifugation, supernatants were transferred with a sterile filter (0,2 µm) into a new 2 mL tube containing 1mmol/L EDTA. Subsequently, aliquots were stored at -20 °C for use in the ELISA and cytotoxicity assays.

Enzyme immunoassay analyses (EIAs): Sandwich enzyme immunoassays (Sandwich EIAs) were carried out to determine NheB and Hbl-L_2 enterotoxin components. For this reason, specific mAbs; (5 µg/mL) 2B11 and 1E11; Horseradish peroxidase (HRP) 1:2000 against NheB, (10 µg/mL) 1A12 and 8B12 HRP 1:1000 against Hbl-L_2 were used as previously described (Dietrich et al., 1999, 2005). The same test procedures and chemicals were used as before (Jeßberger et al., 2017; Schwenk et al., 2020). The color-forming reaction was stopped after 20 minutes by adding 1 mol of H_2SO_4 (100L per well), and the plates were read at 450 nm in the final step of sandwich EIAs. Titer was defined as the reciprocal titer for the highest absorbance dilutions greater than or equal to 1.0. (Dietrich et al., 1999, 2005).

Cell cultures: African green monkey kidney (Vero) cell lines (Bio Whittaker, 76-108B) were studied for the cytotoxic effect of *B. cereus* isolates.

Cytotoxicity assays: As previously described, Vero cell lines were studied to associate the cytotoxic activity of *B. cereus* culture supernatants (Dietrich et al., 1999). Serial dilutions of the supernatants were added to 1 mL microplates containing 10^4 cells per well. Earle's salts containing 1% fetal bovine serum and 2 mmol L^1 glutamine were added to the growth medium and Eagle Minimum Essential Medium (EMEM). The plates were incubated at 37 °C in 5% CO_2 for 24 h. Finally, the absorbance of the tetrazolium salt WST-1 reagent at 450 nm was measured to determine the mitochondrial activity of viable cells. The 50% inhibitory concentration was calculated using the resultant dose-response curve and expressed as the reciprocal dilution giving 50% decrease in mitochondrial activity.

Results

***Bacillus cereus* results**: *B. cereus* was isolated from 37 contaminated RTE foods and pastry products from caterers, cafés, restaurants, bakeries, delicatessens, supermarkets, and mobile vendors in Istanbul. *B. cereus* was found at a level of $2x10^1$ to $2x10^4$ cfu/g in 26/150 (17.3%) RTE foods. Raw meatballs, minced meat pie and rice pilaf had the highest level of bacteria per gram. The amount of *B. cereus* were also enumerated between $2x10^1$ cfu/g and $7x10^2$ cfu/g in 11/75 (14.7%) pastry products. Results are presented in Table 2.

Table 2: *B. cereus* results

Food group	Total	*B. cereus* positiv (%)	*B. cereus* level (cfu/g)			
			>10-100	>100-1000	>1000-10000	>10000
Ready-to-eat foods	150	26 (17.3%)	10	12	3	1
Pastry products	75	11 (14.7%)	8	3	-	-

Multiplex PCR: For the *hblC*, *hblD*, *hblA*, *nheA*, *nheBC*, *ces*, and *cytK1* genes, agarose gel electrophoresis was used to amplify DNA fragments based on their size. In 26 RTE foods; 24/26 (92.3%) *nhe* (*ABC*), 16/26 (61.5%) *hbl* (*CDA*), 2/26 (7.7%) *cytK1* genes and a total of 11 pastry products; 10/11 (90.9%) *nhe* (*ABC*), 5/11 (45.5%) *hbl* (*CDA*) and 1/11 (9.1%) *cytK1* toxin-encoding genes were detected, whereas the *ces* gene was not determined in any samples. Figure 1(A), Figure 1(B) and Figure 1(C) represent different toxigenic patterns of certain *B. cereus* isolates after PCR assays.

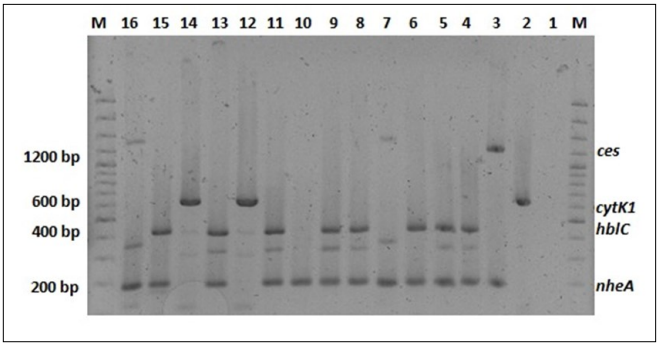

Figure 1(A). Toxigenic patterns of *B. cereus* isolates.
M: marker, 1: negative control, 2: MHI11307, 3: MHI165, 4: MHI163, 5-14: Food samples.

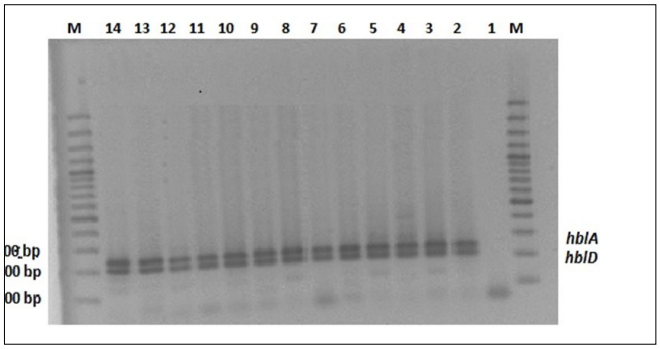

Figure 1(B). Toxigenic patterns of *B. cereus* isolates.
M: marker, 1: negative control, 2: MHI11307, 3: MHI165, 4: MHI163, 5-16: Food samples.

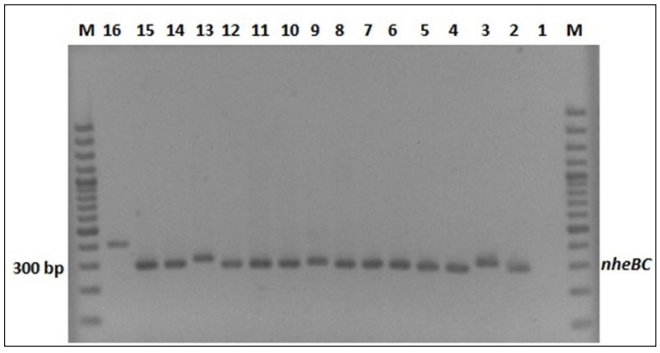

Figure 1(C). Toxigenic patterns of *B. cereus* isolates
M marker, 1 negative control, 2:MHI241, 3-16: Food samples

Enzyme immunoassay: Sandwich EIAs using monoclonal antibodies to detect Hbl-L_2 and NheB protein components were fully consistent with the multiplex PCR results. Nhe was detected in all Nhe positive isolates. Figures 2(A) and 2(B) show NheB and Hbl-L_2 titers .

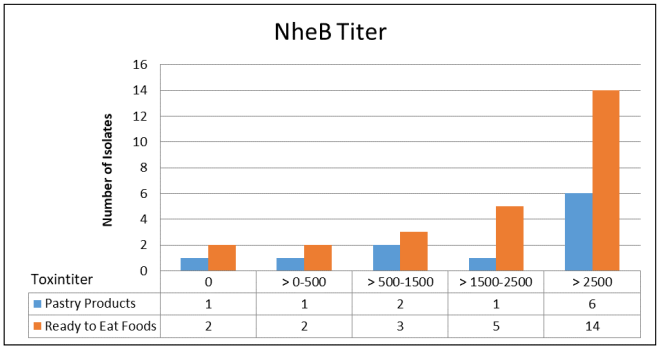

Figure 2(A). NheB titer

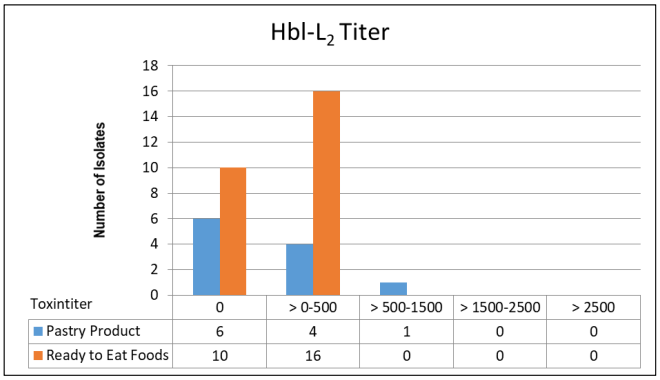

Figure 2 (B). Hbl-L_2 titer

Cytotoxicity assays: Vero cells were used to demonstrate the general cytotoxic activity of the isolates. The in-vitro cytotoxicity test yielded positive results in 24 of 26 RTE food samples and 11 pastry products. Cell culture test results show an agreement between immunoassay and PCR results. Table 3 shows the results of the PCR, EIAs and cytotoxicity tests.

Discussion

Foodborne infections are serious public health issues that affect millions of people worldwide. Because *B. cereus* is frequently found in RTE and reheated foods due to spore formation and its virulent nature. It is extremely difficult to prevent RTE food contamination by *B. cereus* that causes diarrheal or emetic type food poisonings (Agata et al., 1995).

Several studies have reported *B. cereus* levels in RTE foods. In the Netherlands, Van Netten et al. (1990) discovered B. cereus in 6% of 551 rice dishes, 3% of 35

Table 3. Results of PCR, ELISA and cytotoxicity tests

		PCR				ELISA		Cytotoxicity [&]
						NhE	Hbl	
Food Group	No	*nhe*	*hbl*	*cytK*	*ces*	B (+)	L_2 (+)	
Pastry products	11	10	5	1	0	10	5	1.2 -848
Ready-to-eat food	26	24	16	2	0	24	16	0-893

[&] Reciprocal cytotoxicity titre, Zero indicates negative ELISA result, Nhe (nonhaemolytic enterotoxin), Hbl (hemolysin BL)

lasagna dishes, and 1% of 72 RTE dishes (Van Netten et al., 1990). Ehling-Schulz et al. (2004) found *B. cereus* levels ranging from 10-100 cfu/g in 28% of RTE foods. Valero et al. (2007) discovered *B. cereus* in 109(48%) of 229 food samples, including bakery and meat products, milk, chocolate, spices, etc. *B. cereus* had a range of 10^2 to 10^6 cfu/g or ml. In our study, *B. cereus* levels ranged from $2x10^1$-$2x10^4$ cfu/g in 26 of the 150 RTE foods tested. *B. cereus* was isolated between $2x10^1$ cfu/g and $7x10^2$ cfu/g in 11 (14.7%) of the 75 pastry products collected from various production and sales points in Istanbul.

Aksu and Ergün (1996) isolated *B. cereus* from 5 (7.0%) of 71 pastry products, including milk-based desserts, in their study in Istanbul. Çadırcı et al. (2013) also determined *B. cereus* from 7 (7%) out of 100 milk pudding samples. Our result was higher than the rates stated in other studies mentioned above. Bonerba et al. (2010) isolated *B. cereus* from 34 (45%) of 74 patisserie products, which was higher than we found. In our study, eight of the eleven pasty products were milk-based desserts (rice pudding, panna cotta, creme brulee, and so on), with the remaining three being chocolate cake, fiora pandi, spagna, and pistachio dessert.

In the current study, it's found that positive enterotoxin gene PCR results (*hblC*, *hblD*, *hblA*, *nheA*, *nheBC*, *ces*, *cytK1*) confirmed component specific ELISA findings. NheB levels were higher in the isolates than in Hbl-L_2, and cytotoxicity titers ranged from 0 to 893. The majority of the isolates exhibited cytotoxic activity.

The findings from various studies (Hansen and Hendriksen, 2001; In't Veld et al., 2001; Guinebretiere et al., 2002) indicate that almost all *B. cereus* strains contain at least one diarrheal enterotoxins gene. Although the percentage of emetic strains in food isolates varies greatly depending on the food contents, studies have shown very few strains produce the *ces* gene in general, but the level of the percentage could be up to 20.2% (Jung et al., 2017). Our study found no evidence of the ces gene in any food isolates.

The results of PCR showed that 24 (92.3%) out of the 26 Nhe positive RTE food isolates produced complete Nhe consisting of the three components, whereas 16 (61.5%) out of 26 were Hbl-positive isolates for the B, L_1 and L_2 components. 2 (7.7%) out of 26 positive isolates contained the *cytK1* gene. In pastry products, 10 (90.9%) out of 11 isolates were Nhe positive for the 3 components, 45.5% (5) positive for the 3 components of Hbl isolates, 1 (9.1%) and out of 11 positive isolates contained the *cytK1* gene.

Wijnands et al. (2006) reported that 796 isolates and 182 different food samples contain approximately 95% Nhe encoding genes, with a further 66% and 50% determining positive for the genes encoded by Hbl and CytK, respectively. Except for the *cytK1* gene percentage, 50% of our study findings matched those of Wijnands et al. (2006). However, in our current study, we discovered Hbl levels lower than 95% of those found by Hwang and Park (2015) in RTE foods.

Çadırcı et al. (2013) obtained 20 isolates from 7 positive samples of 100 milk-based desserts. 70% (14/20) of isolates did not carry the Hbl encoding genes..In the Hbl encoding genes, 30% (6/20) of isolates contained three enterotoxic Hbl complex coding genes (*hblA*, *hblC* and *hblD)*. Additionally , 40% (8/20) of the isolates had three Nhe complex coding genes (*nheA*, *nheB* and *nheC*). 45% (9/20) contained two encoding genes (*nheA* and *nheB*) and 15% (3/20) had at least one Nhe gene (two *nheA* and one *nheB*) The *cytK1* gene was not detected in any sample.

The findings of our study were consistent with those of Çadırcı et al. (2013). While NheA, NheB and NheC components were found to be positive in 10 (90.9%) of 11 positive samples, 5 of the samples (45.5%) were found to have 3 components of Hbl. The number of samples containing both Nhe and Hbl genes was determined as 5/11 (45.5%). Cerelude was not found in any of the isolates, and only one sample (9.1%) tested positive for CytK1.

In RTE foods and pastry products isolates' cytotoxicity titers ranged from 0 to 893 and 1.2-848, respectively. In this study, the highest cytotoxicity levels were found in potato salad, creme brulee, lamb shank, and lentil meatballs. Two isolates containing *cytK1* (rice pilafs) genes had no cytotoxicity on Vero cells.

Moravek et al. (2006) demonstrated that the cytotoxicity titer of Hbl positive strains was lower than that of Nhe positive strains and that food poisoning strains produced more enterotoxins, particularly Nhe, showing that food poisoning strains produce more enterotoxins, particularly Nhe. Our findings revealed that the strain with the highest cytotoxicity titer was the Nhe positive, Hbl negative. Other strains containing only Nhe, on the other hand, had low cytotoxicity titers. This study's EIAs and cytotoxicity findings suggest that there was a good correlation between the level of NheB and the toxic activity of B. cereus isolates on Vero cells, as Moravek et al. (2006) discovered.

Based on the current information, the presence of only a small or large number of vegetative cells and spores is insufficient for food contaminated with B. cereus to be deemed dangerous to health if consumed. At the same time, the presence of all Hbl, Nhe, CytK encoding genes and cytotoxicity tests should determine the inhibition of the cells (Hep-2, Vero) mitochondrial metabolic activity of B. cereus toxins. Our results show that most of the B. cereus isolates have cytotoxic capability. When the B. cereus strains possess all 3 genes of the Hbl or Nhe enterotoxins at the same time, they have the potential to cause foodborne illness.

Conclusion

In Turkey, most research has focused on *B. cereus* enumeration, with only a few studies on *B. cereus* toxigenic gene profiles and commercial tests based on enterotoxin levels. Although PCR-based methods give fast results in detecting profiles of enterotoxigenic *B. cereus*, they are not a reliable way to predict toxin levels in food isolates (Dietrich et al., 2021). As a result, the presence of *B. cereus* at a 16.4% rate does not guarantee pathogenicity for *B. cereus*. Although potential toxin genes are present, they may not be expressed for the production of the related toxin. As a result, the production of toxins is an important factor in the risk of *B. cereus*-related foodborne illness. However, while much research has been conducted to date, there is little information about the incidence of *B. cereus* food poisoning cases in Turkey in terms of toxin production and genetic characterization. Çadırcı et al. (2013, 2018) used PCR analyses to detect enterotoxigenic structures of *B. cereus* isolated from dairy desserts and ice cream. Yibar et. al. (2017) used enterotoxin production in dairy products (milk and cheese) via using Duopath® Cereus Enterotoxins Test Kit (Merck) Overall, the majority of the studies are concerned with the enumeration of *B. cereus* and some with the detection of toxigenic profiles of *B. cereus* isolates. As a result, our current study contains data for the first time in Turkey containing *B. cereus* enterotoxigenic gene profiles, mAbs-based ELISA, and cytotoxicity assay using Vero cells in RTE foods and pastry products. As a result, future research on the cytotoxic potency of *B. cereus* in various food products is recommended in our country.

Compliance with ethical standards

Conflict of interest

The authors declare that there are no competing interests associated with the manuscript.

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References

Agata, N., Ohta, M., Mori, M., & Isobe, M. (1995). A novel dodecadepsipeptide, cereulide, is an emetic toxin of Bacillus cereus. *FEMS microbiology letters*, 129(1), 17-19.

Aksu, H. ve Ergün, Ö. (1996). Çeşitli pasta ürünlerinde ve sütlü tatlılarda *Bacillus Cereus*'un varlığı. *Gıda Sanayii, 46*, 25-27.

Ankolekar, C., Rahmati, T., & Labbé, R. G. (2009). Detection of toxigenic Bacillus cereus and Bacillus thuringiensis spores in US rice. *International journal of food microbiology, 128*(3), 460-466.

Beecher, D. J., & Wong, A. C. (1994). Identification of hemolysin BL-producing Bacillus cereus isolates by a discontinuous hemolytic pattern in blood agar. *Applied and environmental* microbiology, 60(5), 1646-1651.

Bonerba, E., Di Pinto, A., Novello, L., Montemurro, F., Terio, V., Colao, V., ... & Tantillo, G. (2010). Detection of potentially enterotoxigenic food-related Bacillus cereus by PCR analysis. *International journal of food science and technology, 45*(6), 1310-1315.

Çadırcı, Ö., Gücükoğlu, A., Terzi, G., Kevenk, T., & Alişarli, M. (2013). Determination of enterotoxigenic gene profiles of Bacillus cereus strains isolated from dairy desserts by multiplex PCR. *Kafkas Üniversitesi Veteriner Fakültesi Dergisi, 19*(5), 869-874.

Clair, G., Roussi, S., Armengaud, J., & Duport, C. (2010). Expanding the known repertoire of virulence factors produced by Bacillus cereus through early secretome profiling in three redox conditions. *Molecular and Cellular Proteomics, 9*(7), 1486-1498.

Didier, A., & Dietrich, R. (2016). Mä rtlbauer E. Antibody binding studies reveal conformational flexibility of the Bacillus cereus nonhemolytic enterotoxin (Nhe) A-component. *PLoS One, 11*(10), e0165135.

Dietrich, R., Fella, C., Strich, S., & Martlbauer, E. (1999). Production and characterization of monoclonal antibodies against the hemolysin BL enterotoxin complex produced by Bacillus cereus. *Applied and Environmental Microbiology, 65*(10), 4470-4474.

Dietrich, R., Moravek, M., Bürk, C., Granum, P. E., & Märtlbauer, E. (2005). Production and characterization of antibodies against each of the three subunits of the Bacillus cereus nonhemolytic enterotoxin complex. *Applied and Environmental Microbiology, 71* (12), 8214-8220.

Dietrich, R., Jessberger, N., Ehling-Schulz, M., Märtlbauer, E., & Granum, P. E. (2021). The food poisoning toxins of Bacillus cereus. *Toxins, 13*(2), 98.

EFSA Panel on Biological Hazards (BIOHAZ). (2016). Risks for public health related to the presence of Bacillus cereus and other Bacillus spp. including Bacillus thuringiensis in foodstuffs. *EFSA Journal, 14*(7), e04524.

Ehling-Schulz, M., Fricker, M., & Scherer, S. (2004). Identification of emetic toxin producing Bacillus cereus strains by a novel molecular assay. *FEMS Microbiology Letters, 232*(2), 189-195

Ehling-Schulz, M., Svensson, B., Guinebretiere, M. H., Lindbäck, T., Andersson, M., Schulz, A., ... & Scherer, S. (2005a). Emetic toxin formation of Bacillus cereus is restricted to a single evolutionary lineage of closely related strains. *Microbiology, 151*(1), 183-197.

Ehling-Schulz, M., Vukov, N., Schulz, A., Shaheen, R., Andersson, M., Märtlbauer, E., & Scherer, S. (2005b). Identification and partial characterization of the nonribosomal peptide synthetase gene responsible for cereulide production in emetic Bacillus cereus. *Applied and Environmental Microbiology, 71*(1), 105-113.

Ehling-Schulz, M., Guinebretiere, M. H., Monthán, A., Berge, O., Fricker, M., & Svensson, B. (2006). Toxin gene profiling of enterotoxic and emetic Bacillus cereus. *FEMS Microbiology Letters, 260*(2), 232-240.

Fagerlund, A., Ween, O., Lund, T., Hardy, S. P., & Granum, P. E. (2004). Genetic and functional analysis of the cytK family of genes in Bacillus cereus. *Microbiology, 150*(8), 2689-2697.

Gaulin, C., Viger, Y. B., & Fillion, L. (2002). An outbreak of Bacillus cereus implicating a part-time banquet caterer. *Canadian Journal of Public Health, 93*(5), 353-355.

Granum, P. E., Brynestad, S., & Kramer, J. M. (1993). Analysis of enterotoxin production by Bacillus cereus from dairy products, food poisoning incidents and non-gastrointestinal infections. *International Journal of Food Microbiology, 17*(4), 269-279.

Granum, P. E., O'sullivan, K., & Lund, T. (1999). The sequence of the non-haemolytic enterotoxin operon from Bacillus cereus. *FEMS Microbiology Letters, 177*(2), 225-229.

Guinebretière, M. H., Broussolle, V., & Nguyen-The, C. (2002). Enterotoxigenic profiles of food-poisoning and food-borne Bacillus cereus strains. *Journal of Clinical Microbiology, 40*(8), 3053-3056.

Hansen, B. M., & Hendriksen, N. B. (2001). Detection of enterotoxic Bacillus cereus and Bacillus thuringiensis strains by PCR analysis. *Applied and Environmental Microbiology, 67*(1), 185-189.

Hansen, B. M., Høiby, P. E., Jensen, G. B., & Hendriksen, N. B. (2003). The Bacillus cereus bceT enterotoxin sequence reappraised. *FEMS Microbiology Letters, 223*(1), 21-24.

Hwang, J. Y., & Park, J. H. (2015). Characteristics of enterotoxin distribution, hemolysis, lecithinase, and starch hydrolysis of Bacillus cereus isolated from infant formulas and ready-to-eat foods. *Journal of Dairy Science, 98*(3), 1652-1660.

In't Veld, P. H., W. S. Ritmeester, E. H. M. DelfgouvanAsch, J. B. Dufrenne, K. Wernars, E. Smit, and F. M. vanLeusden. (2001). Detection of genes encoding for enterotoxins and determination of the production of enterotoxins by HBL blood plates and immunoassays of psychrotrophic strains of Bacillus cereus isolated from pasteurised milk. *International Journal of Food Microbiology., 64*, 63-70

Jeßberger, N., Rademacher, C., Krey, V. M., Dietrich, R., Mohr, A. K., Böhm, M. E., ... & Märtlbauer, E. (2017). Simulating intestinal growth conditions enhances toxin production of enteropathogenic Bacillus cereus. *Frontiers in Microbiology, 8*, 627.

Jung, S. M., Kim, N. O., Cha, I., Na, H. Y., Chung, G. T., Kawk, H. S., & Hong, S. (2017). Surveillance of Bacillus cereus Isolates in Korea from 2012 to 2014. *Osong Public Health and Research Perspectives, 8*(1), 71–77.

Lindbäck, T., Fagerlund, A., Rødland, M. S., & Granum, P. E. (2004). Characterization of the *Bacillus cereus* Nhe enterotoxin. *Microbiology (Reading, England), 150(Pt 12)*, 3959–3967.

Lindbäck, T., Hardy, S. P., Dietrich, R., Sødring, M., Didier, A., Moravek, M., ... & Märtlbauer, E. (2010). Cytotoxicity of the Bacillus cereus Nhe enterotoxin requires specific binding order of its three exoprotein components. *Infection and Immunity, 78*(9), 3813-3821.

Logan, N. A., & Rodrigez-Diaz, M. (2006). Bacillus spp. and related genera. *Principles and Practice of Clinical Bacteriology, 2*, 139-158.

Lund, T., & Granum, P. E. (1996). Characterisation of a non-haemolytic enterotoxin complex from Bacillus cereus isolated after a foodborne outbreak. *FEMS Microbiology Letters, 141*(2-3), 151-156.

Lund, T., De Buyser, M. L., & Granum, P. E. (2000). A new cytotoxin from Bacillus cereus that may cause necrotic enteritis. *Molecular Microbiology, 38*(2), 254-261.

Marxen, S., Stark, T. D., Rütschle, A., Lücking, G., Frenzel, E., Scherer, S., ... & Hofmann, T. (2015). Multiparametric quantitation of the Bacillus cereus toxins cereulide and isocereulides A–G in foods. *Journal of Agricultural and Food Chemistry, 63*(37), 8307-8313.

Moravek, M., Wegscheider, M., Schulz, A., Dietrich, R., Bürk, C., & Märtlbauer, E. (2004). Colony immunoblot assay for the detection of hemolysin BL enterotoxin producing Bacillus cereus. FEMS *Microbiology Letters, 238*(1), 107–113.

Moravek, M., Wegscheider, M., Schulz, A., Dietrich, R., Bürk, C., & Märtlbauer, E. (2004). Colony immunoblot assay for the detection of hemolysin BL enterotoxin producing Bacillus cereus. *FEMS microbiology letters, 238*(1), 107–113.

Moravek, M., Dietrich, R., Buerk, C., Broussolle, V., Guinebretière, M. H., Granum, P. E., ... & Märtlbauer, E. (2006). Determination of the toxic potential of Bacillus cereus isolates by quantitative enterotoxin analyses. FEMS microbiology letters, 257(2), 293-298.

Murray, P.R., Baron, E.J., Jorgensen, J.H., Landry, M.L. and Pfaller, M.A. (2007) *Manual of Clinical Microbiology. 9th Edition,* American Society for Microbiology, Washington, DC.

Ouoba, L. I. I., Thorsen, L., & Varnam, A. H. (2008). Enterotoxins and emetic toxins production by Bacillus cereus and other species of Bacillus isolated from Soumbala and Bikalga, African alkaline fermented food condiments. International *journal of food microbiology, 124*(3), 224-230.

Sastalla, I., Fattah, R., Coppage, N., Nandy, P., Crown, D., Pomerantsev, A. P., & Leppla, S. H. (2013). The Bacillus cereus Hbl and Nhe tripartite enterotoxin components assemble sequentially on the surface of target cells and are not interchangeable. *PLoS One, 8*(10), e76955.

Schoeni, J. L., & Lee Wong, A. C. (2005). Bacillus cereus food poisoning and its toxins. *Journal of food protection, 68*(3), 636-648.

Schwenk, V., Riegg, J., Lacroix, M., Märtlbauer, E., & Jessberger, N. (2020). Enteropathogenic potential of Bacillus thuringiensis isolates from soil, animals, food and biopesticides. *Foods, 9*(10), 1484.

Stenfors Arnesen, L. P., Fagerlund, A., & Granum, P. E. (2008). From soil to gut: Bacillus cereus and its food poisoning toxins. *FEMS Microbiology Reviews, 32*(4), 579-606.

Tewari, A., & Abdullah, S. (2015). Bacillus cereus food poisoning: international and Indian perspective. *Journal of Food Science and Technology, 52*(5), 2500-2511.

Valero, M., Hernandez-Herrero, L. A., & Giner, M. J. (2007). Survival, isolation and characterization of a psychrotrophic Bacillus cereus strain from a mayonnaise-based ready-to-eat vegetable salad. *Food Microbiology, 24*(7-8), 671-677.

Van Netten, P., van De Moosdijk, A., Van Hoensel, P., Mossel, D. A. A., & Perales, I. (1990). Psychrotrophic strains of Bacillus cereus producing enterotoxin. *Journal of Applied Bacteriology, 69*(1), 73-79.

Wijnands, L. M., Dufrenne, J. B., & Rombouts, F. M. In't Veld, PH, Van Leusden, FM (2006). Prevalence of potentially pathogenic Bacillus cereus in food commodities in the Netherlands. *Journal of Food Protection, 69*, 2587-2594.

Wijnands, L. M., Dufrenne, J. B., Van Leusden, F. M., & Abee, T. (2007). Germination of Bacillus cereus spores is induced by germinants from differentiated Caco-2 cells, a human cell line mimicking the epithelial cells of the small intestine. *Applied and Environmental Microbiology, 73* (15), 5052-5054.

Yang, I. C., Shih, D. Y. C., Huang, T. P., Huang, Y. P., Wang, J. Y., & Pan, T. M. (2005). Establishment of a novel multiplex PCR assay and detection of toxigenic strains of the species in the Bacillus cereus group. Journal *of Food Protection, 68*(10), 2123-2130.

Yibar, A., Cetinkaya, F., Soyutemiz, E., & Yaman, G. (2017). Prevalence, enterotoxin production and antibiotic resistance of Bacillus cereus isolated from milk and cheese. *Kafkas Universitesi Veteriner Fakültesi Dergisi, 23,* 635-642.