Kafkas Univ Vet Fak Derg
23 (4): 635-642, 2017
DOI: 10.9775/kvfd.2017.17480

Kafkas Universitesi Veteriner Fakultesi Dergisi
Journal Home-Page: http://vetdergi.kafkas.edu.tr
Online Submission: http://vetdergikafkas.org

Research Article

Prevalence, Enterotoxin Production and Antibiotic Resistance of *Bacillus cereus* İsolated from Milk and Cheese [1] [2]

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[1] The study was supported by a grant of the Uludag University (grant number KUAP(V)-2013/12 & HDP(V)-2013/20)
[2] This study was presented at 6th National Veterinery Food Hygiene Congress with International Participation, 7-11 October 2015, Van, Turkey

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Article Code: KVFD-2017-17480 Received: 25.01.2017 Accepted: 29.03.2017 Published Online: 10.04.2017

Citation of This Article

Yıbar A, Çetinkaya F, Soyutemiz E, Yaman G: Prevalence, enterotoxin production and antibiotic resistance of *Bacillus cereus* isolated from milk and cheese. *Kafkas Univ Vet Fak Derg*, 23 (4): 635-642, 2017. DOI: 10.9775/kvfd.2017.17480

Abstract

Bacillus cereus is a type of bacteria that can cause severe food poisoning. The aim of this study was to determine the incidence of *B. cereus* in various full-fat milk and cheese samples and to assess the HBL (haemolysin BL) and NHE (nonhaemolytic enterotoxin) production and the resistance to several antimicrobial agents of the isolates. A total of 259 samples of full-fat milk (raw, pasteurized and UHT) and cheese obtained from different retail markets in Bursa province between July and December 2013 were analysed. Isolation of *B. cereus* was performed using Bacara agar according to the method suggested by FDA. Twenty six (10.04%) out of 259 samples were found to be contaminated with presumptive *B. cereus* based on their colony morphology and microscopic appearance, by counts that ranged from $1x10^1$ to $1.1x10^3$ CFU/mL in raw and pasteurized milk and from $4x10^1$ to $3.8x10^5$ CFU/g in cheese. Thirteen isolates of *B. cereus* were identified by API system. However, further analysis using MALDI-TOF-MS confirmed 19 isolates as *B. cereus*. Thirteen out of 19 (68.4%) isolates showed evidence of only NHE toxin production while six out of 19 (31.6%) isolates were positive for both NHE and HBL production. All isolates were resistant to penicillin G, although they were susceptible to oleondamycin, erythromycin and streptomycin. There were seven different patterns of multiple antibiotic resistance in this study. In our study, 84.2% (n = 16) of *B. cereus* isolates exhibited multiple antibiotic resistance.

Keywords: *Bacillus cereus, Milk, Cheese, Enterotoxin, Multiple antibiotic resistance*

Süt ve Peynirden İzole Edilen *Bacillus cereus*'un Prevalansı, Enterokoksin Üretimi ve Antibiyotik Direnci

Özet

Bacillus cereus ciddi gıda zehirlenmesine neden olabilen bir bakteri türüdür. Bu çalışmanın amacı, çeşitli süt ve peynir örneklerinde *B. cereus* insidansını belirlemek ve elde edilen izolatların HBL (hemolizin BL) ve NHE (nonhemolitik enterotoksin) üretimini ve çeşitli antimikrobiyal ajanlara direncini belirlemektir. Temmuz - Aralık 2013 tarihleri arasında Bursa ilinde farklı satış yerlerinden toplam 259 adet tam yağlı süt (çiğ, pastörize ve UHT) ve peynir örneği analiz edilmiştir. *B.cereus* izolasyonu FDA tarafından önerilen metoda göre Bacara agar kullanılarak yapıldı. Analize alınan çiğ ve pastörize süt örneklerinde $1x10^1$ ile $1.1x10^3$ kob/mL ve peynirde $4x10^1$ ile $3.8x10^5$ kob/g arasında değişen sayılarda olmak üzere, toplamda 259 örneğin 26 adetinin (%10.04) koloni morfolojileri ve mikroskobik görünüşlerine de dayalı olarak *B. cereus* ile kontamine olduğu tespit edilmiştir. Örneklerden elde edilen 13 adet *B. cereus* izolatı API sistemi ile identifiye edilmiştir. Bununla birlikte, MALDI-TOF-MS kullanılarak yapılan analizde, 19 adet izolat *B. cereus* olarak identifiye edilmiştir. Bu 19 izolatın 13'ünün (%68.4) sadece NHE toksini bakımından, altı izolatın da (%31.6) NHE ve HBL toksinleri bakımından pozitif olduğu gözlenmiştir. Tüm izolatlar oleondamisin, eritromisin ve streptomisine duyarlı olmalarına rağmen penisilin G'ye dirençlidir. Bu çalışmada, çoklu antibiyotik direnci gösteren yedi farklı model bulunmuştur. Çalışmamızda, *B. cereus* izolatlarının %84.2'si (n = 16) çoklu antibiyotik direnci göstermiştir.

Anahtar sözcükler: *Bacillus cereus, Süt, Peynir, Enterotoksin, Çoklu Antibiyotik Direnci*

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INTRODUCTION

B. cereus is a Gram-positive, aerobic or facultative anaerobic, rod-shaped, one of the most important endospore-forming spoilage microorganism in dairy environment and also responsible for foodborne outbreaks around the world [1]. Outbreaks caused by this pathogen have been reported in different parts of the world [2-5]. It has been associated with almost all categories of proteinaceous food products including raw milk, pasteurized milk and dairy products such as cheese, butter and cream [6]. *B. cereus* can contaminate the raw milk, pasteurized milk and dairy products via dirty teats, soil, feed and processing equipment and also post-pasteurization contamination of milk can occur [7,8].

There are two types of severe foodborne diseases caused by *B. cereus*, the emetic (heat-stable) and the diarrheal type (heat-labile) [9-12]. The emetic and the diarrheal syndromes can occur when the bacterial cell concentration reaches a level of 10^5 to 10^8 CFU/g and 10^5 to 10^7 CFU/g, respectively [13,14]. The emetic syndrome caused by an intoxication is characterised by an acute attack of nausea and emesis occurring within 1-5 hours after consumption, caused by cereulide, a heat stable, ring-structured dodecadepsipeptide toxin [15,16]. The diarrheal syndrome characterised by abdominal pain and diarrhea, with an incubation period of 4-16 h and symptoms that last for 12-24 h, can be caused by the enterotoxin-complexes nonhaemolytic enterotoxin (NHE), haemolysin BL (HBL) and enterotoxin FM (EntFM) and the single protein cytotoxin K (CytK) [17-19].

In recent years, antimicrobial resistance is one of most serious health threats worldwide and there have been a dramatic increase in the number of foodborne bacterial pathogens resistant to a variety of antibiotics. The wide-spried use of antibiotics in farming and through food chain contributes an important source of antimicrobial resistance [20,21]. Many previous reports have shown that *B. cereus* isolates obtained from different foods have resistance to several antibiotics [22-25].

Our study was planned to assess the prevalence and level of *B. cereus* contamination in full-fat milk (raw, pasteurized, UHT) and cheese; to determine the NHE and HBL enterotoxin production characteristics of the isolates and to examine antibiotic resistance and any possible multiple antibiotic resistance of the isolates.

MATERIAL and METHODS

Sample Collection

Between July and December 2013, a total of 259 full-fat milk and cheese samples including 53 raw milk, 50 pasteurized milk, 50 ultra-high temperature (UHT) milk and 106 cheese (kashar, white pickled, braided, stick, old, village, ricotta) were collected from Bursa province of Turkey. Raw milk samples were provided from several dairy farms while the other samples were purchased from different retail markets and neighbourhood bazaars. The samples were analysed on the day of arrival to laboratory under refrigerated conditions.

Sample Preperation

The detection of *B. cereus* in the samples was achieved according to the Standard Method of the U.S. Food and Drug Administration's (FDA) Bacteriological Analytical Manual (BAM) [26]. First, 10 mL or g portions of each sample were homogenized with 90 mL of sterile saline peptone water (0.1%, w/v) for 1 min in a Stomacher 400 (Seward, London, UK). Tenfold serial dilutions of homogenates were made in 0.1% peptone water as the diluents [27].

Isolation and Enumeration of B. cereus

Detection and enumeration of cultured bacteria were performed through plating on selective solid medium. For this purpose, 0.1 mL (and/or 0.5 mL) of each dilution was spread on (spread plate method) a plate of Bacara agar (bioMerieux, France) plates followed by incubation under aerobic conditions at 30°C for 24 h. After incubation, colonies with a pink/orange colour grown on Bacara agar were considered to have positive lecithinase activity and were subsequently enumerated. The results were expressed as colony forming units (CFU) per milliliter or gram of analysed sample. Three characteristic colonies were picked from each plate were isolated on Tryptone Soya Agar (Oxoid, UK) at 30°C for 24 h. The morphology of the cultures was also examined microscopically. The isolates which were rod-shaped and with central or subterminal spores were considered as presumptive *B. cereus* [28]. The purified isolates were transferred in cryo-tubes containing Nutrient Broth (Oxoid, UK) with 20% (v/v) glycerol and stored at -80°C for identification, further MALDI-TOF-MS analysis and the ability of toxin production.

Identification and Further Confirmation of Presumptive B. cereus Isolates

Identification of *Bacillus'* species were performed using a API 20E and API 50CHB test strips (bioMerieux, France) according to manufacturer's instructions. Confirmation of isolated bacteria was made using matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF-MS) (Microflex LT, Bruker Diagnostics, Germany), in collaboration with the Duzen Laboratories Group, Istanbul, Turkey.

Screening of Enterotoxic B. cereus

Haemolytic enterotoxin (HBL) and non-haemolytic enterotoxin (NHE) production was assessed using the Duopath® Cereus Enterotoxins Test Kit (Merck, Belgium) according to the manufacturer's instructions. The LODs (limit of detection) of this test were 6 ng/mL for NHE and 20 ng/mL for the HBL [29].

Antibiotic Resistance Testing

Antibiotic resistance of the isolates was tested by the Kirby-Bauer disc diffusion method [30]. Mueller-Hinton Agar (Oxoid, UK) was used for this test. The disks used (Oxoid, UK) and antibiotic concentrations were as follows: oleandomycin (15 µg), tetracycline (30 µg), polymyxin B (300 U), chloramphenicol (30 µg), erythromycin (15 µg), penicillin G (10 U), cephalothin (30 µg), ampicillin (10 µg), kanamycin (30 µg), vancomycin (30 µg), streptomycin (10 µg), and neomycin (30 µg). *B. cereus* ATCC 10876 was used as control strain. According to the inhibition zone measured, the isolates were classified as resistant, intermediate or susceptible as recommended by Bauer et al.[30].

RESULTS

In total, 259 samples consisting of 153 milk (raw milk, pasteurized milk, UHT milk) and 106 cheese were analysed. Of these, 26 (10.04%) were observed to be contaminated with *B. cereus* on the basis of the morphological and microscopically features. These isolates were obtained from raw milk, pasteurized milk and cheese samples while none of UHT milks contained bacteria. The bacterial counts varied from $1x10^1$ to $1.1x10^3$ CFU/mL in milk samples, and $4x10^1$ to $3.8x10^5$ CFU/g in cheese. The incidence and contamination levels of presumptive *B. cereus* in the samples are shown in *Table 1*.

Table 2 presents API (20E and 50CHB) and MALDI-TOF-MS identification results. Overall, 19 (73.1%) out of 26 presumptive isolates were confirmed as *B. cereus* by MALDI-TOF-MS whereas 13 (50%) of these isolates were initially identified by API test. Consequently, MALDI-TOF-MS identified 6 isolates as *B. cereus* that were not identified as such by API identification systems.

In the present survey, 19 isolates characterized as *B. cereus* were also analysed for enterotoxin production potential. Thirteen (68.4%) of the isolates tested showed the evidence of only NHE toxin production while six isolates (31.6%) were positive for both NHE and HBL production reaction *(Table 2)*.

The antibiotic resistance profiles of the tested isolates are presented in *Table 3* and *Table 4*. All isolates were resistant to penicillin G, 63.2% to ampicillin, 57.9% to polymixin B, 57.9% to cephalothin, 15.8% to kanamycin

***Table 2.** The results of testing for confirmation and toxin-producing capability of presumptive B. cereus isolates (n = 26)*

Sample	Identification		Toxin Production	
	API	MALDI-TOF-MS	NHE	HBL
Raw milk	-	+	+	+
Raw milk	-	-	NT	NT
Pasteurized milk	-	-	NT	NT
Pasteurized milk	+	+	+	-
Pasteurized milk	-	+	+	-
Pasteurized milk	-	-	NT	NT
Pasteurized milk	-	-	NT	NT
Pasteurized milk	+	+	+	-
Pasteurized milk	-	-	NT	NT
Pasteurized milk	+	+	+	-
Pasteurized milk	-	-	NT	NT
Pasteurized milk	+	+	+	-
Pasteurized milk	+	+	+	+
Pasteurized milk	-	-	NT	NT
Pasteurized milk	+	+	+	+
Kashar cheese	+	+	+	-
Kashar cheese	-	+	+	-
Kashar cheese	+	+	+	-
Kashar cheese	+	+	+	+
Kashar cheese	+	+	+	+
White pickled cheese	-	+	+	-
White pickled cheese	+	+	+	-
Stick cheese	-	+	+	-
Village cheese	+	+	+	-
Ricotta cheese	-	+	+	-
Old cheese	+	+	+	+

***NT:** not tested (MALDI-TOF-MS negative isolates (n = 7) were not tested for toxin production), **NHE:** nonhaemolytic enterotoxin, **HBL:** haemolysin BL, + positive, - negative*

***Table 1.** Incidence and the counts of presumptive B. cereus from milk and cheese*

Sample Type	No. of Samples Analysed	No. and Percentage (%) of Contaminated Samples by *B. cereus*	*B. cereus* Count (CFU/ml-g)		
			Minimum	Maximum	Mean±SD
Raw milk	53	2 (3.8)	$1x10^1$	$2.2x10^2$	$1.2x10^2 \pm 1.5x10^2$
Pasteurized milk	50	13 (26)	$1x10^1$	$1.1x10^3$	$2.1x10^2 \pm 3x10^2$
UHT milk	50	-	-	-	-
Cheese	106	11 (10.4)	$4x10^1$	$3.8x10^5$	$3.5x10^4 \pm 1.2x10^5$
All	259	26 (10.04)	$1x10^1$	$3.8x10^5$	$1.5x10^4 \pm 7.5x10^4$

***SD:** standard deviation*

***Table 3.** Antibiotic resistance profiles by disc diffusion method of B. cereus isolates (n = 19)*

Sample	Antibiotics											
	OL	**TE**	**PB**	**C**	**E**	**P**	**KF**	**AMP**	**K**	**VA**	**S**	**N**
Raw milk	S	S	R	S	S	R	R	R	S	R	S	S
Raw milk	-[1]	-	-	-	-	-	-	-	-	-	-	-
Pasteurized milk	-	-	-	-	-	-	-	-	-	-	-	-
Pasteurized milk	S	S	I	S	S	R	I	I	S	S	S	S
Pasteurized milk	S	S	I	S	S	R	I	S	S	S	S	S
Pasteurized milk	-	-	-	-	-	-	-	-	-	-	-	-
Pasteurized milk	-	-	-	-	-	-	-	-	-	-	-	-
Pasteurized milk	S	S	R	S	S	R	R	R	R	S	S	I
Pasteurized milk	-	-	-	-	-	-	-	-	-	-	-	-
Pasteurized milk	S	S	R	S	S	R	R	R	I	S	S	S
Pasteurized milk	-	-	-	-	-	-	-	-	-	-	-	-
Pasteurized milk	S	S	I	S	S	R	R	R	S	S	S	S
Pasteurized milk	S	I	R	I	S	R	R	R	R	S	S	S
Pasteurized milk	-	-	-	-	-	-	-	-	-	-	-	-
Pasteurized milk	S	S	I	S	S	R	R	R	S	S	S	S
Kashar cheese	S	S	R	S	S	R	I	I	I	S	S	S
Kashar cheese	S	S	R	S	S	R	I	I	I	S	S	S
Kashar cheese	S	S	I	S	S	R	S	R	S	S	S	S
Kashar cheese	S	S	R	I	S	R	R	R	R	S	S	S
Kashar cheese	S	S	I	S	S	R	I	S	I	S	S	S
White pickled cheese	S	S	R	S	S	R	R	I	S	S	S	S
White pickled cheese	S	S	R	S	S	R	R	R	I	S	S	S
Stick cheese	S	S	I	S	S	R	I	R	S	S	S	S
Village cheese	S	S	R	S	S	R	I	S	S	S	S	S
Ricotta cheese	S	S	I	S	S	R	R	R	I	S	S	S
Old cheese	S	I	R	S	S	R	R	R	I	I	S	I

*[1] MALDI-TOF-MS negative isolates (n= 7) were not tested for antibiotic resistance; **OL:** oleandomycin, **TE:** tetracycline, **PB:** polymixin B, **C:** chloramphenicol, **E:** erythromycin, **P:** penicillin G, **KF:** cephalothin, **AMP:** ampicillin, **K:** kanamycin, **VA:** vancomycin, **S:** streptomycin, **N:** neomycin, **R:** resistant, **IM:** intermediate resistant, **S:** susceptible*

and 5.3% to vancomycin. None of the isolates showed resistance to other antibiotics including oleondamycin, tetracycline, chloramphenicol, erythromycin, streptomycin and neomycin. Moreover, all of the isolates were found to be susceptible to oleondamycin, erythromycin and streptomycin. As shown in *Table 5*, seven different patterns of multiple antibiotic resistance has been observed in this study. Sixteen out of 19 *B. cereus* (84.2%) isolates exhibited resistance to multiple antibiotics.

DISCUSSION

Several studies have also demonstrated the occurrence of *B. cereus* (vegetative cells or spores) in milk from Turkey and other countries. The incidence of *B. cereus* in raw milk was recorded as 25% by Larsen and Jørgensen [31] and as 10.6% by Něměcková et al.[32]. In comparison to those studies, a lower incidence rate (3.8%) of *B. cereus* in raw milk was observed in the present study. It is likely to be due to improper hygienic conditions and poor farm management practices during feeding, milking and milk storage. A study conducted by Lin et al.[33] reported that the incidence of *B. cereus* in raw milk from holding tanks, raw milk from balance tanks, pasteurized milk from high-temperature short time pipes, pasteurized milk from holding tanks and the final product was 80%, 85%, 85%, 76% and 90%, respectively.

Twenty-six percentage (13/50) of pasteurized milk samples analysed in this study had *B. cereus* in counts ranging from $1x10^1$ to $1.1x10^3$ CFU/mL. The presence of *B. cereus* in pasteurized milk samples could perhaps be due to high initial load of spores in the milk used for production, inadequate pasteurization or post-contamination due to unsanitary conditions. On the other hand some other authors reported much higher *B. cereus* incidence levels. Te Giffel et al.[34] reported that 40% of pasteurized milk

***Table 4.** Number of resistant and susceptible B. cereus isolates to antibiotics*

Antibiotics	**No. of *B. cereus* Isolates *(n= 19)***		
	No. and Percentage (%) of Resistant Isolates	**No. and Percentage (%) of Intermediate Isolates**	**No. and Percentage (%) of Susceptible Isolates**
Oleandomycin	0	0	19 (100)
Tetracycline	0	2 (10.5)	17 (89.5)
Polymixin B	11 (57.9)	8 (42.1)	0
Chloramphenicol	0	2 (10.5)	17 (89.5)
Erythromycin	0	0	19 (100)
Penicillin G	19 (100)	0	0
Cephalothin	11 (57.9)	7 (36.8)	1 (5.3)
Ampicillin	12 (63.2)	4 (21.1)	3 (15.8)
Kanamycin	3 (15.8)	7 (36.8)	9 (47.4)
Vancomycin	1 (5.3)	1 (5.3)	17 (89.5)
Streptomycin	0	0	19 (100)
Neomycin	0	2 (10.5)	17 (89.5)

***Table 5.** Multiple antibiotic resistance patterns observed among B.cereus isolates (n = 19)*

Source	**No. (%) of Multiple Resistant Isolates**	**Resistance Patterns**
Raw milk	1 (5.3)	PB, P, KF, AMP, VA
Pasteurized milk (*n*= 2), kashar cheese (*n*= 1)	3 (15.8)	PB, P, KF, AMP, K
Pasteurized milk (*n*= 1), White pickled cheese (*n*= 1), old cheese (*n*= 1)	3 (15.8)	PB, P, KF, AMP
Pasteurized milk (*n*= 2), Ricotta cheese (*n*= 1)	3 (15.8)	P, KF, AMP
White pickled cheese	1 (5.3)	PB, P, KF
Kashar cheese (*n*= 2), Village cheese (*n*= 1)	3 (15.8)	PB, P
Kashar cheese (*n*= 1), stick cheese (*n*= 1)	2 (10.5)	P, AMP

***PB:** polymixin B, **P:** penicillin G, **KF:** cephalothin, **AMP:** ampicillin, **K:** kanamycin **VA:** vancomycin*

samples were contaminated with *B. cereus* and that the contamination levels were less than 5 CFU/mL in 77% of the samples. In a study conducted in India, in approximately 10% of the milk and milk samples the level of *B. cereus* contamination was more than 10^5 CFU/g [35]. Zhou et al.[36] informed that 92 isolates obtained from 54 samples of packaged pasteurized full-fat milk were identified as *B. cereus*. In Poland, milk collected from a farm have been found to contain *B. cereus*. Morever, the authors suggested that a considerable level of contamination by this bacteria of milk was also found in milk after pasteurization [37]. In another study [38] 12 isolates of *B. cereus* were identified from pasteurized milk.

Varying incidence rates of *B. cereus* in dairy products were also reported by different workers. Prevalence rates of 33.33% in raw pooled milk samples and 37.83% in pasteurized milk samples were reported by Rather et al.[39]. Absence of *B. cereus* in UHT milk in our study was similarly observed by Pacheco-Sanchez and Massaguer [40] on 6500 packed whole processed UHT milk samples. Merzouqui et al.[24] detected *B. cereus* contamination in 51.6% of milk and dairy products. The incidence of *B. cereus* in Port Salut Argentino cheese was notified as 50% [41]. A study performed by Cosentino et al.[42] on dairy products (pasteurized milk, UHT milk and cheese) indicated the presence of *Bacillus* spp. in 265 (70%) of 378 samples tested. By Khudor et al.[43], the incidence of *B. cereus* in milk, soft cheese, curls cheese and yogurt samples was reported as 32.7%, 16.6%, 18% and 26%, respectively. A previous study [22] demonstrated contamination with *B. cereus* in 31% of 215 dairy products included soft fresh cheese, soft ripening cheese, cottage cheese, cream cheese, butter and cream. Reyes et al.[44] recorded a 45.9% incidence of *B. cereus* in dried milk products. In India, Bedi et al.[35] reported an overall incidence of 53.8% of *B. cereus* in milk and various of dairy products. In Turkey, Gundogan and Avci [23] investigated the occurrence of *B. cereus* in raw milk, white cheese and ice cream samples from different dairy processing plants and determined that the contamination rates with *B. cereus* were 90% in raw milk, 70% in white cheese and 20% in ice cream samples. In the current study, the presence of *B. cereus* in raw milk, pasteurized milk and cheese samples could perhaps be due to high initial load of spores in the milk used for production, inadequate pasteurization or post-pasteurization contamination due to unsanitary conditions such as dirty teats, soil, feed and processing equipments. The spores also formed by this microorganism may resist pasteurization of milk.

In our study, initial identification of species level of *B. cereus* isolates was confirmed by API 20E and API 50CHB test systems and further with MALDI-TOF-MS analysis. The results of these tests did not always match. Performance of the MALDI-TOF-MS technique was higher for identification than API systems. Rapid and reliable identification of pathogenic microorganisms is important for the surveillance, prevention and control of foodborne illnesses [45]. In recent years, MALDI-TOF-MS has been demonstrated as a powerful method for identification of bacteria in routine laboratories [46]. Previous studies also described the performance of MALDI-TOF-MS for bacterial identification [47-51].

B. cereus causes two types of food poisoning, the emetic and diarrheal syndromes and produces one emetic toxin and four other enterotoxins: haemolysin BL (HBL), nonhaemolytic enterotoxin (NHE), cytotoxin K (CytK) and enterotoxin FM (EntFM). The HBL and NHE enterotoxins are considered as the primary virulence factors of diarrhea after infection by *B. cereus* [52,53]. Of 19 *B. cereus* isolates obtained from our study, 68.4% (13 isolates) were positive for NHE toxin production and 31.6% (6 isolates) for both NHE and HBL production. Enterotoxigenic characterization of *B. cereus* in milk and various dairy products has been examined by several authors. Te Giffel et al.[34] found that the 27% of the *B.cereus* isolates from pasteurised milk in household refrigerators produced haemolysin BL enterotoxin. Of the 37 *B. cereus* isolates 70-76%, were found to be enterotoxigenic (as determined by three different methods). Zhou et al.[36] determined that 33.7% of *B. cereus* isolates from pasteurized full-fat milk contained three enterotoxic HBL complex encoding genes *hbl*A, *hbl*C and *hbl*D. In another investigation, 28 of 94 (29.8%) isolates of *B. cereus* from dried milk products were positive for diarrheal enterotoxin production [44]. The results of the study performed by Cosentino et al.[42] exhibited the toxin-production ability of 72% of *B. cereus* isolates from dairy products by a reversed passive latex agglutination assay. Svensson et al.[54] suggested that mesophilic isolates compared with psychrotrophic *B. cereus* isolated from the farm, in silo tanks and pasteurized milk had higher enterotoxin (HBL and NHE) production potential. In Turkey and other countries, the detection of enterotoxigenic *B. cereus* isolates in milk and dairy products can pose a major public health threat because the toxins NHE and HBL could potentially be able to cause diarrhea.

In comparison to our results, studies conducted by Schlegelova et al.[22] demonstrated that 18 of 96 *B. cereus* isolates from dairy products exhibited resistance to streptomycin, four isolates to erythromycin, two isolates to neomycin and one isolate to tetracycline. Earlier reports also specified ampicillin and penicillin resistant *B. cereus* isolates from a variety of foods [23,24,55]. Our findings are in agreement with those reported by Khudor et al.[43] who observed that all *B. cereus* isolates from milk, cheese and yogurt had resistance to penicillin, but the susceptibility to neomycin. However, the same researchers found that 45.1% of the isolates were resistant to tetracycline, 6.4% to erythromycin and 3.2% to streptomycin. Susceptibility to oleondamycin, erythromycin and neomycin of all isolates, as observed in our study, is contrary to those reported by these authors. Merzouqui et al.[24] isolated *B. cereus* from milk, dairy products, spices and rice salads and screened the antibiotic susceptibility profiles of the isolates. They indicated susceptibility to chloramphenicol (67.2%) and erythromycin (84.4%), which are consistent with the outcome of our study. However, tetracycline resistance (90.6%) determined in their study is contrary to that found in our study which revealed susceptibility to this antibiotic (89.5%) of *B. cereus* isolates. A study performed in Turkey [55] showed that none of the 34 *B. cereus* isolates from ice cream samples was resistant to vancomycin, whereas one isolate of *B. cereus* from raw milk had resistance to vancomycin.

In conclusion, this study revealed that the presence of *B. cereus* in full-fat milk (raw and pasteurized) and cheese. In addition, our findings presented the potential of *B. cereus* isolates to produce the enterotoxins HBL and NHE as a health hazard. *B. cereus* counts of 3.8×10^5 CFU/g in cheese samples can be sufficient to cause illness by this bacterium. Although the numbers of *B. cereus* in the samples were relatively lower than the actual number of cells required to cause illness by this bacterium, it is important to consider that milk and milk products may be easily contaminated and bacterial counts may rapidly rise due to poor milking, equipment cleaning and sanitizing procedures and improper cooling. We also detected the occurrence of multiple antibiotic resistant *B. cereus* isolates. Therefore, antimicrobial agents should be responsibly and prudently used in veterinary medicine. As a result, to ensure the efficient pasteurization and cooling of milk, and to avoid the post-pasteurization contamination are of primary importance to prevent foodborne illnesses caused by consumption of milk and dairy products contaminated with *B. cereus*.

Conflict of Interest Statement

The authors declared that they have no conflict of interest.

Acknowledgment

This experiment was financially supported by the Unit of Scientific Research Projects, Uludag University (Project No: KUAP(V)-2013/12 and HDP(V)-2013/20).

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