

## Characterization of *Bacillus cereus* Isolates from Local Dairy Products in Egypt (Pencirian Pencilan *Bacillus cereus* daripada Produk Tenusu Tempatan di Mesir)

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### ABSTRACT

*Bacillus cereus* is an important, opportunistic, foodborne pathogen found in various dairy products. In this study, the prevalence, physiological characteristics, antimicrobial resistance profile, and enterotoxigenic genes (*ces* and *hblA*) of *B. cereus* were investigated in isolates from Egyptian dairy products. A total of 150 samples, including soft white cheese, milk powder, and ultra-high temperature (UHT) milk (50 of each), were collected from local dairy stores in EL-Gharbia governorate, Egypt from April 2019 to October 2019. Of these, 29 samples were contaminated with *B. cereus* (an overall prevalence of 19.3%). Based on cultural, morphological, and biochemical characteristics, 48 isolates were detected including 27 (56.25%) from soft white cheese, 9 (18.75%) from milk powder, and 12 (25%) from UHT milk. Antibiotic susceptibility assessment showed that all isolates exhibited high sensitivity to amikacin, doxycyclin, gentamycin, ciprofloxacin, while significant resistance to kanamycin, clindamycin, nalidixic acid, cephalothin, and sulphamethoxazole was also observed. All isolates were examined for the presence of emetic (*ces*) and diarrheal (*hblA*) genes using the PCR method; *ces* was detected in 12 (25%) isolates, *hblA* in 14 (29.2%) isolates, while 22 (45.8%) isolates did not harbor either gene. These findings indicate the need for the application of adequate preventive measures and personnel hygiene in dairy processing lines to minimize *B. cereus* load in final products.

**Keywords:** Antibiotic resistance; *Bacillus cereus*; dairy products; enterotoxigenic genes

### ABSTRAK

*Bacillus cereus* adalah patogen bawaan makanan yang oportunistik dan penting yang terdapat dalam pelbagai produk tenusu. Dalam kajian ini, kelaziman, ciri fisiologi, profil rintangan antimikrob serta gen enterobertoksin (*ces* dan *hblA*) daripada pencilan *B. cereus* dikaji daripada produk tenusu Mesir. Sebanyak 150 sampel, termasuk keju putih lembut, susu tepung dan susu bersuhu tinggi (UHT) (masing-masing 50 sampel), dikumpulkan dari kedai susu tempatan di EL-Gharbia, Mesir dari April 2019 hingga Oktober 2019. Daripada jumlah tersebut, 29 sampel dicemari dengan *B. cereus* (kelaziman keseluruhan 19.3%). Berdasarkan ciri kultur, morfologi dan biokimia, 48 pencilan dikesan termasuk 27 (56.25%) daripada keju putih lembut, 9 (18.75%) daripada susu tepung dan 12 (25%) daripada susu UHT. Penilaian kerentanan antibiotik menunjukkan bahawa kesemua pencilan menunjukkan kepekaan yang sangat tinggi terhadap amikasin, doksisaiklin, gentamisin, siprofloksasin, sementara ketahanan yang signifikan juga dilihat terhadap kanamaisin, klindamaisin, asid nalidiksik, sefalotin dan sulfametoksazol. Semua pencilan diperiksa untuk mengenal pasti kehadiran gen emetik (*ces*) dan diareal (*hblA*) menggunakan kaedah PCR; *ces* dikesan pada 12 (25%) pencilan, *hblA* di dalam 14 (29.2%) pencilan, sementara itu, 22 (45.8%) pencilan tidak mempunyai gen. Penemuan ini menunjukkan perlunya ada penerapan langkah pencegahan dan kebersihan peribadi yang mencukupi dalam pemprosesan tenusu untuk meminimumkan kehadiran *B. cereus* dalam produk akhir.

**Kata kunci:** *Bacillus cereus*; gen enterotoksigenik; kerentanan antibiotik; produk tenusu

### INTRODUCTION

*Bacillus cereus* are ubiquitous, Gram-positive, motile, spore-forming, rod-shaped bacteria; they are widely distributed in the environment and commonly isolated

from soil, water, air, and plants. Different types of food and foodstuffs can be contaminated by *B. cereus* including rice, potatoes, dairy products, pasta, meat products, coffee, and vegetable sprouts (Aman et al. 2016; Samapundo et al. 2011). Unfortunately, pasteurization does not destroy all

foodborne pathogens: *B. cereus* can survive pasteurization and various species have been isolated from a variety of pasteurized dairy products (Haughton et al. 2010).

Contamination of foods with *B. cereus* is typically associated with two types of food poisoning: emetic and diarrheal types. The emetic disease is associated with the production of a heat stable cereulide synthesized by a nonribosomal peptide synthetase encoded by *ces* genes (Ehling-Schulz et al. 2005; Rajkovic et al. 2008); such disease is characterized by nausea and vomiting, which occurs a few hours after ingestion of contaminated food. The emetic toxin, usually produced by cells that grow in the contaminated food (Kramer & Gilbert 1989), is not inactivated during food processing or gastrointestinal passage because it is highly resistant to heat treatments, extreme pH conditions, and protease activity (Rajkovic et al. 2008). The diarrheal type, on the other hand, is typically caused by heat labile enterotoxins, such as hemolysin BL, non-hemolytic enterotoxin, cytotoxin K, and enterotoxin FM (Fagerlund et al. 2004; Hansen & Hendriksen 2001; Sergeev et al. 2006), which are generated by vegetative cells growing in the small intestine following ingestion of spore-contaminated food and/or vegetative cells (Kramer & Gilbert 1989); the diarrheal disease is marked by abdominal pain and watery diarrhea within 8-16 h of ingesting contaminated food (Schoeni & Wong 2005).

Different strains of *B. cereus* have the ability to produce several potentially pathogenic substances including proteases, lipases, phospholipase C, collagenases, amylases, and  $\beta$ -lactamases (Turnbull et al. 2002); consequently, when *B. cereus* contaminates milk products, it reduces their organoleptic qualities (Kumari & Sarker 2014). Such changes can include production of a bitter flavor, clotting, or gelation of milk by proteases (Chen et al. 2003). In addition, lipase enzyme production in milk and milk products can cause 'bitty' cream and unpleasant flavors such as rancid, butyric, buttery, unclean, or soapy flavors (Furtado 2005).

Antibiotic therapy is still commonly used to treat *B. cereus* infections. However, misuse of antibiotics has led to the emergence and dissemination of multi drug-resistant *B. cereus* strains (Ageroso et al. 2002; Brown et al. 2003) to the extent that they now pose a potential threat to public health. Previous studies have reported the phenotypic resistance of *B. cereus*, isolated from a variety of foods, to several antimicrobial agents (da Silva Fernandes et al. 2014; Merzougui et al. 2014).

Soft white cheese is one of the most popular types of cheese in Egypt. During production, it is ripened for a few weeks in whey and then packed into metal cans; therefore, it has high moisture content, making it an ideal environment in which microorganisms can grow (El-Baradei et al. 2007). Milk powder, a processed product that is formulated and marketed for children and adults, is used in varying amounts during the production of manufactured fluid milk, dairy products, and bakery products (Robinson & Itsaranuwat 2002). UHT milk is a type of processed milk that is heat-treated at a temperature of 138 °C for a short period (~1-2 s) and aseptically packaged (Neumann et al. 2010). This production method is sufficient to kill all microbes present in the milk, as well inactivating all enzymes, thereby giving UHT milk an improved shelf-life and appropriate sensory appeal (Bylund 1995).

To date, few studies have focused on the prevalence, toxin-producing genes, and antibiotic resistance of *B. cereus* in Egyptian dairy products, especially at the consumer level. Therefore, the specific objectives of this study were to determine the prevalence of *B. cereus* in soft white cheese, milk powder, and UHT milk samples, and to assess the isolated *B. cereus* for virulent enterotoxigenic genes and phenotypic resistance to commercially available antimicrobials.

## MATERIALS AND METHODS

### SAMPLE COLLECTION

In total, 150 samples of soft white cheese, milk powder, and UHT milk (50 of each) were collected from various markets in EL-Gharbia Governorate, Egypt during the period from April 2019 to October 2019. Cheese samples were collected from various shops in clean, sterile containers; milk powder and UHT milk samples with various production dates on their container (across 5 months of production) were purchased from various retailers. All samples were placed in clean and sterile containers, initially stored at 4 °C in an icebox, and then immediately transferred to the laboratory for further investigation.

### ENUMERATION, ISOLATION, AND IDENTIFICATION OF *B. cereus* (ISO 21528-2:2004)

For isolation of *B. cereus* from soft white cheese, 10 g of cheese was first mixed with 90 mL of sterile 2% sodium

citrate in a sterile stomacher and then homogenized for 2 min using a BagMixer Stomacher (Seward, London, UK). For isolation from milk powder, ~25 g was added to 225 mL of 0.1% sterile worm distilled water (40 °C) and mixed well. For isolation from UHT milk, packaging was cleaned well at the site of opening, 11 mL of each sample was added to 99 mL of sterile peptone water 0.1%, and the solution was mixed well. All mixtures were first heated in a water bath at 80 °C for 10 min to kill all vegetative bacteria and allow spores to outgrow before being cooled rapidly to room temperature (Rahimi et al. 2013). Ten-fold serial dilutions were produced in sterile phosphate buffer saline; 0.1 mL of each dilution was seeded evenly onto duplicated plates of Mannitol Egg Yolk Polymyxin Agar (Oxoid, Hampshire, England, UK). Plates from each dilution were incubated at 37 °C for 48 h and observed for characteristic colonies of *B. cereus*. Finally, the number of CFU/mL was calculated for each sample using the standard equation of Nicolaou and Goodacre (2008). The presumed colonies, characterized by their pink color and surrounding white halo, were collected, purified, sub-cultured on the surface of nutrient agar slant (Oxoid), and then incubated at 37 °C for 24 h. Subsequently, the slopes were maintained in a refrigerator according to the method recommended by Nabradalik and Grata (2011). All isolates were identified following previously described methods (Macfaddin 2000). Briefly, the purified isolates were examined for Gram staining and cell morphology, and then identified biochemically with a set of biochemical tests for catalase, oxidase, lecithinase, and urease, as well as fermentation of D-glucose, D-xylose maltose, D-mannitol, and lactose.

#### STARCH HYDROLYSIS, AND PROTEOLYTIC AND LIPOLYTIC CHARACTERISTICS OF ISOLATED *B. cereus*

The proteolytic activity of *B. cereus* isolates was detected using skim milk agar medium (Oxoid) according to the method recommended by Nabradalik and Grata (2011). A clear zone of hydrolysis around colonies was observed after incubation at 37 °C for 24 h. The lipolytic activity of *B. cereus* was observed using nutrient medium (Oxoid) based on tributyrin (glycerol tributyrate). Specifically, production of the enzyme lipase split tributyrin resulting in lipolytic colonies surrounded by a clear zone in the opaque medium. The ability of the *B. cereus* isolates to hydrolyze starch was tested by first streaking the cultures onto starch agar and then incubating them at 37 °C for 3 days. Lugol's iodine solution was subsequently added to the surface of the agar, which turned yellow in the presence of starch (MacFaddin 2000).

#### ANTIBIOGRAM FOR ANTIBIOTIC SENSITIVITY OF *B. cereus* ISOLATES

*Bacillus cereus* isolates were tested for their susceptibility against 14 commercially available antimicrobial agents (Oxoid) using a Kirby-Bauer disc diffusion assay according to the CLSI standards and interpretive criteria (CLSI 2012). The following antimicrobial agents were tested: tetracycline, 30 µg; ampicillin, 10 µg; cefotaxim, 30 µg; clindamycin, 10 µg; gentamicin, 10 µg; nalidixic acid, 30 µg; doxycycline, 30 µg; ciprofloxacin, 5 µg; erythromycin, 15 µg; amikacin, 30 µg; penicillin-G, 10 IU; cephalothin, 30 µg; kanamycin, 30 µg, and sulphamethoxazole, 25 µg. The susceptibility of the isolates to most antimicrobial agents was categorized (susceptible, intermediate, or resistant) by measuring the inhibition zone according to interpretive criteria adhering to the CLSI guidelines (CLSI 2012).

#### MOLECULAR DETECTION OF CES AND HBLA GENES IN *B. cereus* ISOLATES

Genomic DNA was extracted using a Gene JET Genomic DNA Purification Kit (Fermentas) according to manufacturer's instructions. All *B. cereus* isolates were tested more than once for the presence of both *ces* and *hblA*. PCR with primers *ces*-F (5'-CACGCCGAAAGTGATTATACCAA-3') and *ces*-R (5'-CACGATAAAACCACTGAGATAGTG-3') was performed to amplify the 176 bp of *ces* as previously described by Fricker et al. (2007). The PCR reaction mixture contained 200 µg of genomic DNA, 2 mM of each deoxynucleoside triphosphate, 2.5 mM of MgCl<sub>2</sub>, 20 pmol of primer, and 2.5 U of Taq polymerase (ATGC Biotechnologie, Noisy-le-Grand, France). Sterile distilled water was added to bring the final volume to 20 µL. The amplification was performed on a Thermal Cycler (Master Cycler, Eppendorf, Hamburg, Germany), with the following PCR cycling protocol applied: initial denaturation at 95 °C for 30 s, followed by 30 cycles of denaturation at 95 °C for 60 s; annealing at 60 °C for 30 s; and initial extension at 72 °C for 45 s, followed by a final extension at 72 °C for 7 min.

For screening *hblA*, the primers *hbl*-F (5'-GTGCAGATGTTGATGCCGAT-3') and *hbl*-R (5'-ATGCCACTGCGTGGACATAT-3') were used to amplify 320 bp (Banerjee et al. 2011). The PCR reaction was conducted as previously described (Hansen & Hendriksen 2001). The reaction mixture (25 µL) invariably consisted of 5 µL of the bacterial lysate, 200 µM of dNTP mix solution, 0.5 µL of each of the forward and reverse

primer (10 pmol), and 1.0 U of Taq DNA polymerase. Sterile distilled water was added to bring the final volume to 50  $\mu$ L. Amplification conditions consisted of initial denaturation at 94 °C for 2 min followed by 30 cycles of denaturation at 94 °C for 15 s, annealing at 55 °C for 45 s, and initial extension at 72 °C for 30 s followed by a final extension at 72 °C for 4 min. Amplified DNA fragments were analyzed using 1.5% agarose gel electrophoresis in 0.5 X Tris boric ethylene-diamine tetra-acetic acid sodium salt containing 0.5 mg of ethidium bromide buffer. Finally, 5  $\mu$ L of each amplicon was electrophoresed at 90 V for 35 min in 1.5% agarose gel stained with ethidium bromide, and then visualized and captured on a UV transilluminator. To determine the fragment sizes, 100 bp plus DNA ladder were used. DNA from the reference strain, *B. cereus* NCIM 2106, was used as a standard positive control, while an isolate of *Escherichia coli* was used as negative control.

#### RESULTS AND DISCUSSION

Among the 150 examined dairy product samples, 29 were positive for *B. cereus* with an overall prevalence of 19.3% (Table 1). Prevalence of *B. cereus* varies across previous studies. For example, a recent study in India found *B. cereus* in milk products with a prevalence of 28.37% (Yusuf et al. 2018), while another Indian study reported *B. cereus* prevalence of 53.8% in milk and various dairy products (Bedi et al. 2005). However, substantially lower *B. cereus* prevalence rates in dairy products have been reported in Turkey (7%) (Çadirci et al. 2013) and Italy (8%) (Cosentino et al. 1997). According to studies of *B. cereus* contamination in milk and dairy products, evidence suggests that the adulteration of milk by water and rice flour (Lin et al. 1998) and the use of contaminated utensils (Te Giffel et al. 1996) are likely routes by which bacterial contamination occurs.

In Egypt, soft white cheese is consumed in large amounts, accounting for around 75% of the cheese produced and consumed in the country (El-Baradei et al. 2007). In the current study, *B. cereus* was detected in 17 of 50 soft white cheese samples, with a prevalence of 34% and counts from  $1 \times 10^2$  to  $25 \times 10^3$  CFU/g (Table 1). This frequency is similar to that previously reported in India (33%) (Kumari & Sarker 2014), but much lower than that reported in Turkey (70%) (Gundogan & Avci 2004). However, other studies in Egypt (Abdeen et al. 2020; Khater & Abdella 2017; Khudor et al. 2012) and Turkey (Molva et al. 2009) have reported lower *B. cereus* contamination rates in cheese. This discrepancy could be due to the type of cheese samples analyzed or potential postproduction contamination.

In the 50 milk powder samples analyzed here, *B. cereus* was detected in 16% with counts from  $1 \times 10^2$  to  $19.3 \times 10^3$  CFU/mL. One previous study from India reported a much higher prevalence of *B. cereus* in milk powder (52%) (Kumari & Sarker 2014), whereas other investigations from Egypt reported *B. cereus* prevalence rates of 6.9% (Abdeen et al. 2020) and 7.5% (Elbagory et al. 2015) in similar samples. In contrast to all of these studies, an Australian study failed to detect any *B. cereus* isolates in tested milk powder samples (Eglezos et al. 2010).

In 50 UHT milk samples, *B. cereus* was detected in 8% with counts from  $3 \times 10$  to  $4.3 \times 10$  CFU/mL. This finding is in contrast to those of De Rezendo (1998) and Lesley et al. (2017), who detected *B. cereus* in UHT milk samples with prevalence rates of 34.14 and 30%, respectively. Furthermore, a higher (but more similar) *B. cereus* prevalence rate (15%) was detected in a previous study from Egypt (Abou Zeid & Yassin 2017), whereas other studies have failed to detect *B. cereus* in any UHT milk sample examined (Pacheco-Sanchez & Massaguer 2007; Yiber et al. 2017).

The Egyptian Standard (Egyptian Standard 2014) stipulates that *B. cereus* should be absent in milk products; therefore, none of the positive samples in the present investigation met Egyptian standards. Moreover, the presence of *B. cereus* in dried milk products suggests carelessness during the manufacturing process. Food contamination with *B. cereus* even at low levels is considered as a potential vehicle for foodborne disease because dried milk products contain high levels of carbohydrates (starch, sucrose, or lactose) and minerals that enhance the proliferation and enterotoxin production of *B. cereus* when the products are reconstituted and held at ambient temperature for long periods (Jaquette & Beuchat 1998).

In recent years, considerable attention has been paid to the emergence of antimicrobial resistance among foodborne bacterial pathogens. In the present study, irrespective of the origin (e.g. soft cheese, milk powder, or UHT milk) of the isolates, the highest antimicrobial resistance of *B. cereus* was observed for kanamycin and clindamycin (100% each), followed by nalidixic acid, cephalothin, and sulphamethoxazole. In contrast, the highest antimicrobial susceptibility determined for the isolated bacteria was against amikacin, doxycyclin, gentamycin, ciprofloxacin, ampicillin, and erythromycin (Table 2). These observations support previously reported findings (Cui et al. 2016; Owusu-Kwarteng et al. 2017; Yusuf et al. 2018). However, in the present study, *B. cereus*

isolates showed higher resistance to clindamycin, which is in contrast to findings reported by Owusu-Kwarteng et al. (2017). Nevertheless, the high resistance against cephalothin reported here was in agreement with findings from Turkey (Yiber et al. 2017). Furthermore, although the *B. cereus* isolates detected in the present study are known to produce  $\beta$ -lactamase (Bottone 2010), they showed only a moderate susceptibility to  $\beta$ -lactam antibiotics; in contrast, several previous studies on the antimicrobial susceptibility of *B. cereus* have reported higher resistance to  $\beta$ -lactam antibiotics such as oxacillin, penicillin, and amoxicillin (Abraha et al. 2017; Gao et al. 2018; Kumari & Sarker 2014; Owusu-Kwarteng et al. 2017; Yiber et al. 2017; Yusuf et al. 2018). For tetracycline, 24 (50%) of the examined *B. cereus* isolates showed resistance, similar to isolates studied by Khudor et al. (2012); however, much higher resistance to tetracycline (90.6%) was reported by Merzouqui et al. (2013), whereas much higher susceptibility to tetracycline (97%) was reported by Owusu-Kwarteng et al. (2017). Such variation in resistance to antibiotics may be attributable to differences in isolate sources, the antibiotics used, and drug-resistance transfer.

*Bacillus cereus* causes self-limiting food-poisoning syndromes that have two distinct forms, namely emetic and diarrheal. In the present study, 48 isolates of *B. cereus* were screened for the presence of emetic (*ces*) and diarrheal (*hblA*) genes. Irrespective of their origin (soft cheese, milk powder, or UHT milk), 12 (25%) *B. cereus* isolates carried *ces*, 14 (29.2%) carried *hblA*, while 22 (45.8%) isolates did not harbor either of the tested genes (Table 3). The emetic toxin-producing gene *ces* was detected in *B. cereus* isolates recovered from soft white cheese, milk powder, and UHT milk samples with prevalence rates of 29.6, 22.2, and 16.7%, respectively (Figure 1). Previous studies have reported different prevalence rates for *ces*, typically between 1.5 and 17.2%, in *B. cereus* strains isolated from various food sources (Hoton et al. 2009; Wijnands et al. 2006; Yim et al. 2015). Even lower prevalence rates of

*ces* have been found in *B. cereus* in other studies (Gao et al. 2018; Owusu-Kwarteng et al. 2017; Yu et al. 2019).

Moreover, Kim et al. (2015) failed to detect emetic *ces* in either reference or commercial strains of *B. cereus*. A recent study in Egypt reported that *ces* was detected in 22.2 and 50% of isolates from milk powder and Ras cheese, respectively (Abdeen et al. 2020). Such variation in *ces* prevalence rates could be due to differences in sample sources, sample size, or the hygienic standards applied in the respective dairy product industries.

Hemolysin BL is considered to be one of the most important virulence factors for the diarrheal form of *B. cereus*-related food poisoning (Labbe & Garcia 2013). In the current study, *hblA* was found in 33.3, 11.1 and 33.3% of soft white cheese, milk powder, and UHT milk samples, respectively (Figure 2). Similar findings were previously reported in Turkey (Çadirici et al. 2013). In Brazil, two studies have reported the prevalence of *hblA* at ~40% in *B. cereus* isolated from dairy products including pasteurized milk, milk powder, and UHT milk (Aragon-Alegro et al. 2008; Reis et al. 2013). However, in India (Rather et al. 2011) and Thailand (Chitov et al. 2008), higher detection rates of 60-70% were reported for *hblA*, *hblC*, and *hblD* in *B. cereus* recovered from dairy products.

In addition to the public health importance of *B. cereus*, it affects the quality of milk by altering the odor and taste. This occurs due to production of extracellular enzymes such as proteases, lipases, and phospholipases, which can remain active in the milk even after the death of the microbes that generate them (Meer et al. 1991). Such contamination also causes economic loss through spoilage of the contaminated products, which can include sweet curdling, 'bitty' cream, and off-flavors (Barkley & Delaney 1980). Of the 48 isolates examined in the present study, 22 (45.8%) produced protease, 10 (20.8%) produced lipase, and 16 (33.3%) produced both enzymes. However, hydrolysis of starch was observed for all *B. cereus* isolates.

TABLE 1. Prevalence of *Bacillus cereus* in soft white cheese, milk powder, and UHT milk samples

Sample types	No. of samples (150)	Positive samples (29)		<i>B. cereus</i> isolates (48)		Count (CFU/g or mL)			
		No.	%	No.	%	Minimum	Maximum	Mean	SEM $\pm$
Soft cheese	50	17	34	27	56.25	$1 \times 10^2$	$25 \times 10^3$	$1.2 \times 10^3$	$5.7 \times 10^2$
Milk powder	50	8	16	9	18.75	$1 \times 10^2$	$19.3 \times 10^3$	$6.6 \times 10^2$	$0.43 \times 10$
UHT milk	50	4	8	12	25	$3 \times 10$	$4.3 \times 10$	$3.72 \times 10$	$0.08 \times 10$

UHT, ultra-high temperature; CFU, colony forming unit

TABLE 2. Antimicrobial susceptibility of *Bacillus cereus* isolates

Antimicrobial agents	Soft cheese (n = 27)						Milk powder (n = 9)						UHT milk (n = 12)					
	S		I		R		S		I		R		S		I		R	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Kanamycin	0	0	0	0	27	100	0	0	0	0	9	100	0	0	0	0	12	100
Clindamycin	0	0	2	7.4	25	92.6	0	0	1	11.1	8	88.9	0	0	3	25.0	9	75.0
Nalidixic	1	3.7	4	14.8	22	81.5	0	0	3	33.3	6	66.7	1	8.3	3	25.0	8	66.7
Cephalothin	2	7.4	3	11.1	22	81.5	2	22.2	1	11.1	6	66.7	2	16.7	2	16.7	8	66.7
Sulphamethoxazole	5	18.5	2	7.4	20	74.1	0	0	4	44.4	5	55.6	4	33.3	0	0	8	66.7
Penicillin G	8	29.6	0	0	19	70.4	1	11.1	3	33.3	5	55.6	3	25.0	2	16.7	7	58.3
Cefotaxim	10	37.0	1	3.7	16	59.3	2	22.2	2	22.2	5	55.6	5	41.7	1	8.3	6	50.0
Tetracycline	11	40.7	2	7.4	14	51.9	4	44.4	0	0	5	55.6	5	41.7	2	16.7	5	41.7
Erythromycin	13	48.1	1	3.7	13	48.1	4	44.4	1	11.1	4	44.4	6	50.0	1	8.3	5	41.7
Ampicillin	17	63.0	1	3.7	9	33.3	5	55.6	1	11.1	3	33.3	7	58.3	1	8.3	4	33.3
Ciprofloxacin	18	66.7	2	7.4	7	25.9	6	66.7	0	0	3	33.3	8	66.7	0	0	4	33.3
Gentamicin	21	77.8	2	7.4	4	14.8	5	55.6	2	22.2	2	22.2	8	66.7	1	8.3	3	25.0
Doxycycline	24	88.9	1	3.7	2	7.4	6	66.7	1	11.1	2	22.2	10	83.3	1	8.3	1	8.3
Amikacin	26	96.3	0	0	1	3.7	8	88.9	0	0	1	11.1	11	91.7	0	0	1	8.3

UHT, ultra-high temperature; S, susceptible; I, intermediate; R, resistant; No, number of isolates

TABLE 3. Incidence of emetic (*ces*) and diarrheal (*hblA*) genes in *Bacillus cereus* isolates

Sample types	No. of isolates	<i>ces</i> gene		<i>hblA</i> gene	
		No.	%	No.	%
Soft cheese	27	8	29.6	9	33.3
Milk powder	9	2	22.2	1	11.1
UHT milk	12	2	16.7	4	33.3
Total	48	12	25.0	14	29.2

UHT, ultra-high temperature; No, number of isolates

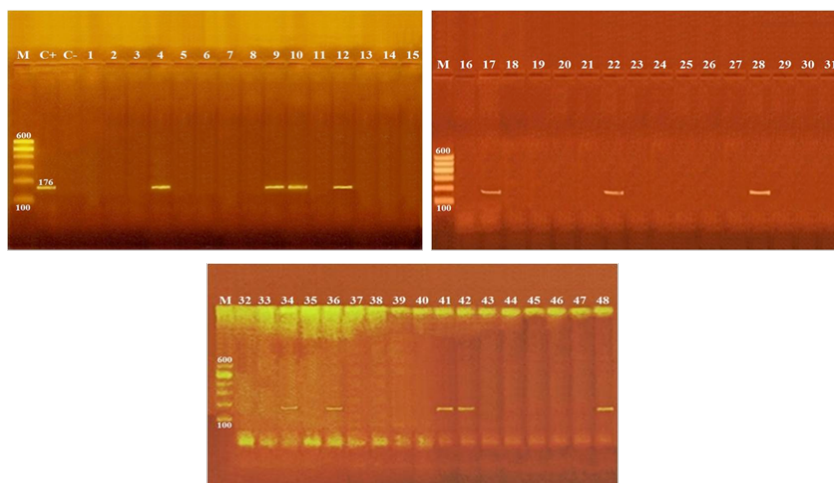


FIGURE 1. Agarose gel (1.5%) electrophoresis patterns of the PCR product (176 bp) for detection of the *ces* gene in *Bacillus cereus* isolates. Lane M: 100 bp ladder as a molecular size standard; lane C+: positive control; lane C-: negative control; lanes 9, 10, 22, 34, 36, 41, 42, and 48: positive isolates from soft white cheese; lanes 4 and 17: positive isolates from milk powder; lanes 12 and 28: positive isolates from UHT (ultra-high temperature) milk; lanes 1-3, 5-8, 11, 13-16, 18-21, 23-27, 29-33, 35, 37-40, and 43-47: negative isolates

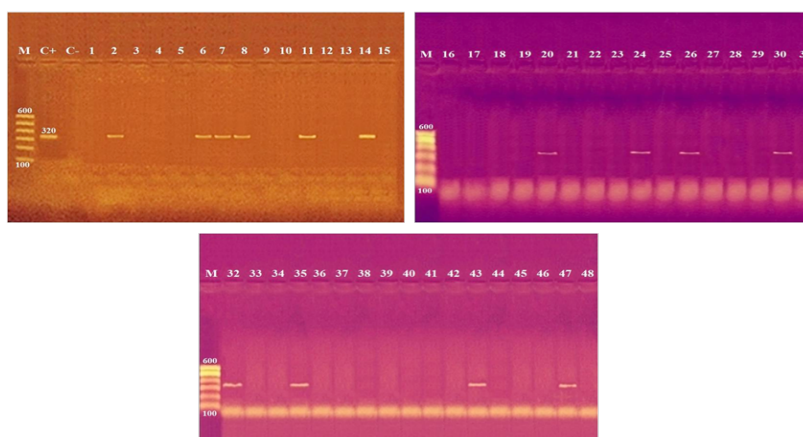


FIGURE 2. Agarose gel (1.5%) electrophoresis patterns of the PCR product (320 bp) for detection of the *hblA* gene in *Bacillus cereus* isolates. Lane M: 100 bp ladder as a molecular size standard; lane C+: positive control; lane C-: negative control; lane 2: positive isolate from milk powder; lanes 6-8, 20, 24, 32, 35, 43, and 47: positive isolates from soft white cheese; lanes 11, 14, 26, and 30: positive isolates from UHT (ultra-high temperature) milk; lanes 1, 3-5, 9-10, 12, 13, 15-19, 21-23, 25, 27-29, 31, 33, 34, 36-42, 44-46, and 48: negative isolates

## CONCLUSION

In conclusion, our findings provide a comprehensive overview of *B. cereus* prevalence in various dairy products marketed in Egypt. *Bacillus cereus* was found to be most prevalent in soft white cheese followed by milk powder and UHT milk; the overall prevalence was 19.3%. Thus, the high level of contamination found in these products is an obvious health concern. The relatively high percentage of enterotoxigenic genes identified in this study is also alarming as these pose a significant threat to public health by increasing the risk of foodborne infections. Additionally, our results for antimicrobial susceptibility showed that most isolates had variable resistance against most antibiotics. This indicates the importance of justifying the use of antimicrobials and regularly monitoring the antibiotic susceptibilities of *B. cereus* to minimize the risk of human exposure to antimicrobial resistant pathogens.

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