J. Dairy Sci. 103:3994–4001
https://doi.org/10.3168/jds.2019-17541

Prevalence, molecular characterization, and antibiotic susceptibility of *Bacillus cereus* isolated from dairy products in China

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ABSTRACT

This study was conducted to reveal the prevalence, molecular characterization, and antibiotic susceptibility of *Bacillus cereus* isolated from dairy products including powdered infant formula, raw milk, pasteurized milk, ultra-high-temperature milk, and cheese. Five hundred samples collected from 5 provinces in China were analyzed in overall experiments. Multilocus sequence typing, distribution of toxin genes, and antibiotic susceptibility of the isolates were analyzed. Fifty-four *B. cereus* strains were detected; of these, 13 isolates (26%) were from raw milk, 12 isolates (12%) from pasteurized milk, 10 isolates (10%) from cheese, 12 isolates (8%) from ultra-high-temperature milk, and 7 isolates (7%) from powdered infant formula. These isolates were divided into 24 sequence types (ST); among them, ST24, ST26, ST82, ST142, ST377, ST857, and ST1046 were the main dominant ST. The results of detection of toxin genes (*hblA*, *hblC*, *hblD*, *nheA*, *nheB*, *nheC*, *cytK*, *entFM*, *bceT*, *hlyII*, and *cesB*) showed that 94.4% isolates carried *nheABC* genes, whereas only 11.1% of the isolates contained the *hblACD* gene cluster. In addition, detection rates of *cytK*, *bceT*, *entFM*, hlyII, and *cesB* genes were 75.9, 77.8, 85.2, 53.7, and 11.1%, respectively. The antibiotic susceptibility test indicated that most of *B. cereus* isolates were resistant to ampicillin, penicillin, cefepime, cephalothin, and oxacillin, and were susceptible to gentamicin, chloramphenicol, imipenem, tetracycline, ciprofloxacin, trimethoprim-sulfamethoxazole, erythromycin, kanamycin, and cefotetan. Therefore, this study revealed the prevalence and characteristics of *B. cereus* isolated from dairy products in China, indicating the potential risk and contributing to the effective prevention and control of this pathogen.
Key words: *Bacillus cereus*, characterization, dairy product

INTRODUCTION

Bacillus cereus is a gram-positive, endospore-forming, foodborne pathogenic bacteria, and causes outbreaks of foodborne illness worldwide (Ehling-Schulz et al., 2012; Bennett et al., 2013). This pathogen can also cause gastric disease, emesis, diarrhea, and even death, and has been reported to have a high contamination rate in dairy products (Rosenquist et al., 2005; Kumari and Sarkar, 2014; Zhang et al., 2016). The high heat resistance of *B. cereus* strains increases their survival rates after heat treatment; meanwhile, stronger antibiotic resistance is generally considered to be a barrier against the effectiveness of antibiotics and disinfectants, which are probably the most important reasons why *B. cereus* has a higher contamination rate than other foodborne pathogens in dairy foods (Johnson et al., 1982). In recent studies (Kumari and Sarkar, 2014, 2016; Fei et al., 2019), the main concern of researchers is the harm and control of *B. cereus* in dairy products, whereas the contamination of this pathogen in dairy products has been ignored; therefore, it was necessary to conduct a systematic study on the prevalence and characteristics of *B. cereus* in dairy products in China.

Analysis of genetic diversity could improve the understanding of population characteristics of *B. cereus*, and facilitate establishment of accurate and rapid detection methods, reliable tracking of *B. cereus*, and the designation of targeted prevention and control measures (Park et al., 2009; Malek et al., 2013; Moradi-Khatoonabadi et al., 2014). Furthermore, the pathogenicity of *B. cereus* is associated with several toxins including he-

Received September 4, 2019.
Accepted December 17, 2019.
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molysin BL (**Hbl**), nonhemolytic enterotoxin (**Nhe**), cytotoxin K (**CytK**), enterotoxin FM (**entFM**), potential enterotoxins hemolysin II (**HlyII**), enterotoxin T (**BceT**), and emetic toxin (cereulide) that are usually produced by this pathogen and regulated by the *ces* gene (Fagerlund et al., 2004; Ehling-Schulz et al., 2006; Gao et al., 2018). Therefore, it is essential to detect these toxin genes to prevent and reduce the acute or chronic harmfulness of *B. cereus* strains in the dairy industry.

The use of antibiotics is still considered the most effective way to treat *B. cereus* infection in humans (Pena-Miller et al., 2013). Antibiotic-resistant *B. cereus* strains have been found due to the long-term unwise use of antibiotics or the emergence of resistant genes, resulting in horizontal gene transfer (Agersø et al., 2002; Gao et al., 2018). In particular, the emergence of multidrug-resistant strains increases the chance of infection, resulting in a failure of antibiotic treatment (Ali et al., 2014). At present, the antibiotic resistance profile of *B. cereus* strains isolated from dairy products available in Chinese markets is not apparent. Hence, a relevant and fruitful investigation is necessary to increase the knowledge of the resistance status of *B. cereus* strains with a proposal of effective strategies to reduce the emergence of these strains.

The objective of this study was to investigate the contamination status of *B. cereus* isolates present in dairy products commonly available in Chinese markets and reveal the genotypes, toxin genes, antibiotic resistance, and biofilm formation of these isolates. A total of 54 *B. cereus* strains were isolated from 500 dairy products samples collected from locally available in Chinese markets from January 2018 to January 2019. Genotypes of the isolates were identified using multilocus sequence typing (**MLST**), and the toxin genes were detected using a PCR screening method. Antibiotic resistance of 54 *B. cereus* strains was also assessed. The data in this study would increase the ideas and knowledge about the pathogen *B. cereus* essentially present in dairy products and provide an important basis for the risk assessment of this pathogen.

MATERIALS AND METHODS

Sample Collection

A total of 500 dairy products samples were collected from January 2018 to January 2019, including 100 samples of powdered infant formula (**PIF**), 50 samples of raw milk (**RM**), 100 samples of pasteurized milk (**PM**), 150 samples of ultra-high-temperature milk (**UHTM**), and 100 samples of cheese. Among these samples, RM was collected from cattle farm and transferred to the sterile centrifuge tubes, then all samples were then stored in small coolers and quickly transported to the laboratory. Other samples were collected from commercially available dairy products and remained in their original commercial packaging, stored in small coolers before these samples arrived in the laboratory, and conserved at 4°C before isolation of *B. cereus*. These samples were collected from 5 provinces (Heilongjiang, Jilin, Hebei, Henan, and Guizhou) of northeast, central, and southwest China.

Isolation and Identification of Bacterial Strains

The isolation and identification of *B. cereus* were performed according to the method mentioned in National Food Safety Standard in China (GB4789. 14–2014; Ministry of Health of the People's Republic of China, 2014), with minor modifications. Twenty-five grams or 25 mL of sample was dissolved in 225 mL of brain-heart infusion broth followed by cultivation at 30 ± 1°C for 24 h. The cultures were streaked onto mannitol egg yolk polymyxin agar by adding mycosin B and yolk emulsion, and plates were incubated at 30°C for 24 h. Typical pink colonies were picked up and inoculated into brain-heart infusion broth followed by propagation at 30 ± 1°C for 24 h. After propagation DNA was extracted from the culture and targeted strains were identified through 16S rDNA sequencing.

MLST Analysis

The MSLT scheme was performed as described in a previous report (Zahner et al., 2013). Seven housekeeping genes (*glpF*, *gmk*, *ilvD*, *pta*, *pur*, *pycA*, and *tpi*) were amplified and sequenced by the Beijing Genomics Institute (Beijing, China). The type sequences (**ST**) of *B. cereus* isolates were determined by genetic sequence alignment in the *Bacillus cereus* MLST database (http://pubmlst.org/bcereus/). The phylogenetic relationship based on 2,829-bp sequences, concatenated sequences of 7 housekeeping genes, was analyzed using the maximum-likelihood algorithm in MEGA 6.0 (Arizona State University, Tempe), with 1,000 bootstrap replicates. *Bacillus cereus* ATCC 4342 and *B. cereus* 14579 were equally analyzed as reference strains.

Detection of Toxin Genes

Eleven toxin genes of *B. cereus* were detected using PCR screening, including 3 hemolytic enterotoxin genes (*hblA*, *hblC*, and *hblD*), 3 nonhemolytic enterotoxin genes (*nheA*, *nheB*, and *nheC*), 4 other enterotoxigenic

genes (*cytK*, *bceT*, *entFM*, and *hly*II), and 1 cereulide synthetase gene (*cesB*). The PCR primer design and amplification strategies were performed according to the procedures as reported in previous studies (Hansen and Hendriksen, 2001; Fagerlund et al., 2004; Seong, 2008).

Antibiotic Susceptibility Testing

In accordance with the guidelines of the Clinical Laboratory Standards Institute (CLSI, 2015), the antibiotic resistance of 54 strains of *B. cereus* isolated from dairy products was assessed using the Kirby–Bauer disk diffusion method. Ampicillin (10 μg), penicillin (10 U), cefepime (30 μg), cephalothin (30 μg), cefotetan (30 μg), kanamycin (30 μg), imipenem (10 μg), gentamicin (10 μg), erythromycin (15 μg), oxacillin (1 μg), ciprofloxacin (5 μg), chloramphenicol (30 μg), tetracycline (30 μg), clindamycin (2 μg), rifampin (5 μg), and trimethoprim-sulfamethoxazole (1.25 μg/23.75 μg) were used for the antibiotic susceptibility testing of *B. cereus* isolates. According to the diameter of inhibition zone, the antibiotic resistance of the isolates was interpreted as sensitive, intermediate, and resistant. *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923 were selected as the quality control organisms.

RESULTS

Prevalence of B. cereus Isolated from Dairy Products in China

As shown in Table 1, a total of 54 *B. cereus* strains were isolated from 500 dairy product samples with a contamination rate of 10.8%. Of these samples, the contamination rate by *B. cereus* was the highest (13 isolates, 26%) in RM samples followed by PM samples (12 isolates, 12%), cheese samples (10 isolates, 10%), UHTM samples (12 isolates, 8%), and PIF samples (7 isolates, 7%). In addition, among the 5 surveyed provinces, the highest contamination risk (12 isolates, 12%) was observed in Guizhou, whereas the lowest contamination rate (9 isolates, 9%) was in Hebei province.

Genotyping Based on MLST

Fifty-four strains of *B. cereus* isolates were divided into 24 ST; among them, ST24, ST26, ST82, ST142, ST377, ST857, and ST1046 were the main dominant ST (Table 2). *Bacillus cereus* ST82 was found in PIF, PM, and UHTM samples; meanwhile, *B. cereus* ST857 was detected in RM, PM, and UHTM samples. On the contrary, ST26, ST154, and ST392 were only found in cheese samples; ST43, ST205, and ST1341 were isolated from PM samples; and ST1336, and ST1968 appeared in only RM samples. An elaborated phylogenetic relationship of the 54 *B. cereus* strains (Figure 1) was clustered into 3 groups: group I (ST142, ST392, ST427, ST24, ST1968, ST154, ST66, ST857, ST1339, ST43, and ST1258), group II (ST82, ST125, ST377, and ST1098), and group III: (ST26, ST205, ST1046, ST1291, ST32, ST92, and ST1228). In addition, these results showed no obvious association between *B. cereus* genotype and collection regions.

Distributions of Toxin Genes Among B. cereus Isolates

The distributions of diarrheal enterotoxin genes (*nheABC*, *hblACD*, *cytK*, *bceT*, *hly II*, and *entFM*) and *cesB* associated with vomitus in 54 *B. cereus* isolates obtained from dairy products are shown in Table 3. Among diarrheal enterotoxin genes, detection rates of *hblA*, *hblC*, and *hblD* were 57.4, 68.5, and 16.7%, respectively, and the *hblACD* gene cluster was present in 11.1% of the *B. cereus* isolates. On the other hand, *nheABC* genes were present in almost all isolates (94.4%) and only 5.6% isolates did not have any of the *nheA* and *nheB* genes. In most of the isolates, *cytK*, *bceT*, *entFM*, and hlyII genes were found as 75.9, 77.8, 85.2, and 53.7%, respectively. In contrast, the *cesB* gene was detected in only 11.1% of the strains, which was significantly lower than any of the another 10 diarrheal enterotoxin genes.

Table 1. Prevalence and levels of *Bacillus cereus* isolated from dairy products in China

Bacterial strain[1]	Source[2]	No. of samples	No. of *B. cereus*	Contamination rate (%)
HN1, GZ1, GZ2, JL1, HB1, HLJ1, HLJ2	PIF	100	7	7
HN2, HN3, HN4, GZ3, GZ4, GZ5, JL2, JL3, HLJ3, HLJ4, HLJ5, HB2, HB3	RM	50	13	26
HN5, HN6, GZ6, GZ7, GZ8, JL4, JL5, JL6, HLJ6, HLJ7, HB4, HB5	PM	100	12	12
HN7, HN8, HN9, GZ9, GZ10, JL7, JL8, JL9, HLJ8, HLJ9, HB6, HB7	UHTM	150	12	8
HN10, HN11, GZ11, GZ12, JL10, JL11, HLJ10, HLJ11, HB8, HB9	Cheese	100	10	10
Total	Dairy products	500	54	10.8

[1]HN, GZ, JL, HB, and HLJ indicate that locations of isolation are Henan, Guizhou, Jilin, Hebei, and Heilongjiang provinces, respectively.
[2]PIF = powdered infant formula; RM = raw milk; PM = pasteurized milk; UHTM = ultra-high-temperature milk.

Antibiotic Resistance Profiles

The antibiotic susceptibility of the 54 *B. cereus* isolates to 17 antibiotics was tested and shown in Table 4. All isolates were susceptible to gentamicin and chloramphenicol, and most of the isolates were susceptible to imipenem (98.15%), tetracycline (98.15%), ciprofloxacin (94.44%), trimethoprim-sulfamethoxazole (85.18%), erythromycin (83.33%), kanamycin (83.33%), and cefotetan (72.22%). On the contrary, most the *B. cereus* isolates were resistant to ampicillin (96.30%), penicillin (96.30%), rifampin (96.30%), cefepime (96.30%), oxacillin (90.74%), and cephalothin (68.52%). In addition, 83.33% of isolates were intermediate to clindamycin.

DISCUSSION

The ability of *B. cereus* to survive in dairy products was increased because of its strong tolerance against adverse environmental factors. Heini et al. (2018) reported that contamination rates of PIF in Switzerland were 78%. The prevalence of *B. cereus* in the PIF Chinese retail market was also revealed by Zhang et al. (2017), who reported that 8.2% of PIF samples were contaminated with *B. cereus* strains. However, a more comprehensive understanding about the prevalence of *B. cereus* isolated from dairy products in China is still lacking. In this study, PIF, RM, PM, UHTM, and cheese were collected as research objects, and the contamination rates of the above-mentioned dairy products were 7, 26, 12, 8, and 10%, respectively, indicating the potential risk of *B. cereus* in dairy products of China.

In comparison to previous studies, genetic typing based on the MLST method is more conducive to revealing the molecular characteristics of the tested strains and contributing to the traceability of pathogenic bacteria (Fei et al., 2015). The 54 strains of *B. cereus* in this study were divided into 24 ST using MLST, indicating that *B. cereus* isolated from dairy products had a high genetic diversity. Among these, ST26 was found in rice flour, PIF, cooked rice, fried rice noodle, hot dog, and cold noodle samples (Yang et al., 2017a,b), which indicates that ST26 is likely to be the most predominant ST of *B. cereus* isolated from food samples. In the study of Zhang et al. (2017), *B. cereus* ST32 and ST142 strains were isolated from infant formula samples; similarly, our results showed that ST32 was found in PIF and UHTM samples, and ST142 was detected in cheese and RM samples. The strains of *B. cereus* ST92, ST205, ST427, and ST857 were detected in dairy products at varying degrees; interestingly, these ST were also found on the surfaces of equipment associated with processing of the UHT milk (Lin et al., 2017). Therefore, the processing equipment as well as environments might be the possible sources of *B. cereus* strains in dairy products.

The distribution and proportions of 10 toxin genes were revealed in this study. Toxins such as Hbl, Nhe, and CytK had been reported as the major etiology of diarrhea caused by food poisoning (Lindbäck et al., 2004; Ngamwongsatit et al., 2008). The presence of Hbl and Nhe toxins could be ascertained only with the detection of toxin genes *hblACD* and *nheABC*, respectively. In this study, the *hblACD* gene cluster was found in 11.1% of the *B. cereus* strains (Table 3) isolated from dairy products, which is significantly different from the previous studies (Cui et al., 2016; Yang et al., 2017a; Zhang et al., 2017). According to the reports of Yang et al. (2017b) and Zhang et al. (2017), no *hblACD* gene cluster was found in *B. cereus* isolated from infant formula in China, and *B. cereus* with *hblACD* gene cluster is not common in food from China. However, a higher detection rate (78.3%) of *hblACD* gene cluster was found in *B. cereus* strains of milk origin (Cui et al., 2016). The proportions of *nheABC* gene cluster and *CytK* in *B. cereus* in our study were 94.4 and 75.9%, respectively, which are approximately similar to the detection rates (93 and 73%, respectively) of the above-mentioned genes in *B. cereus* isolated from PM (Gao et al., 2018). Besides, *cesB* associated with vomitus was present in 11.1% of *B. cereus* strains (Table 3) isolated from dairy products, which is lower than that from food

Table 2. Multilocus sequence typing analysis of *Bacillus cereus* strains isolated from dairy products in China[1]

ST	Bacterial strain	No. (%) of isolates	Source
ST24	GZ11, GZ12, HN7	3/54 (5.56)	Cheese, UHTM
ST26	JL10, HLJ10, HLJ11	3/54 (5.56)	Cheese
ST32	GZ1, JL9	2/54 (3.70)	PIF, UHTM
ST43	HB5	1/54 (1.85)	PM
ST66	JL4, HB7	2/54 (3.70)	PM, UHTM
ST82	HB1, HLJ7, HN9	3/54 (5.56)	PIF, PM, UHTM
ST92	HN3, HLJ2	2/54 (3.70)	PIF, RM
ST125	GZ4, JL7	2/54 (3.70)	RM, UHTM
ST142	HN11, JL3, HLJ4	3/54 (5.56)	Cheese, RM
ST154	HB8, JL11	2/54 (3.70)	Cheese
ST205	GZ6, GZ8	2/54 (3.70)	PM
ST377	GZ2, HB3, GZ10	3/54 (5.56)	PIF, RM, UHTM
ST392	HN10, HB9	2/54 (3.70)	Cheese
ST427	HN1, HLJ8	2/54 (3.70)	PIF, UHTM
ST857	GZ5, JL5, HLJ9	3/54 (5.56)	RM, PM, UHTM
ST1046	HLJ1, JL1, JL2	3/54 (5.56)	PIF, RM
ST1098	HLJ3, GZ7	2/54 (3.70)	RM, PM
ST1228	HN5, HN8	2/54 (3.70)	PM, UHTM
ST1258	HN2, GZ9	2/54 (3.70)	RM, UHTM
ST1291	HN6, JL8	2/54 (3.70)	PM, UHTM
ST1336	GZ3, HB4	2/54 (3.70)	RM
ST1339	HLJ5, HB6	2/54 (3.70)	RM, UHTM
ST1341	JL6, HLJ6	2/54 (3.70)	PM
ST1968	HN4, HB2	2/54 (3.70)	RM

[1]ST = sequence type; PIF = powdered infant formula; RM = raw milk; PM = pasteurized milk; UHTM = ultra-high-temperature milk.

and clinical samples (100%) and UHT milk processing lines (48.15%; Lin et al., 2017; Yang et al., 2017a) but higher than that isolated from PM (5%) in China (Cui et al., 2016). Therefore, the outcomes of this study describe that 11.1% of the isolates, containing both diarrhea and vomit virulence, might induce a high risk in serious infections by *B. cereus* that would essentially be isolated from various dairy products available in Chinese markets.

The antibiotic susceptibility tests showed that most of *B. cereus* strains isolated from dairy products were resistant to β-lactam antibiotics including ampicillin, penicillin, cefepime, cephalothin, and oxacillin, which is consistent with previous studies on the antibiotic resis-

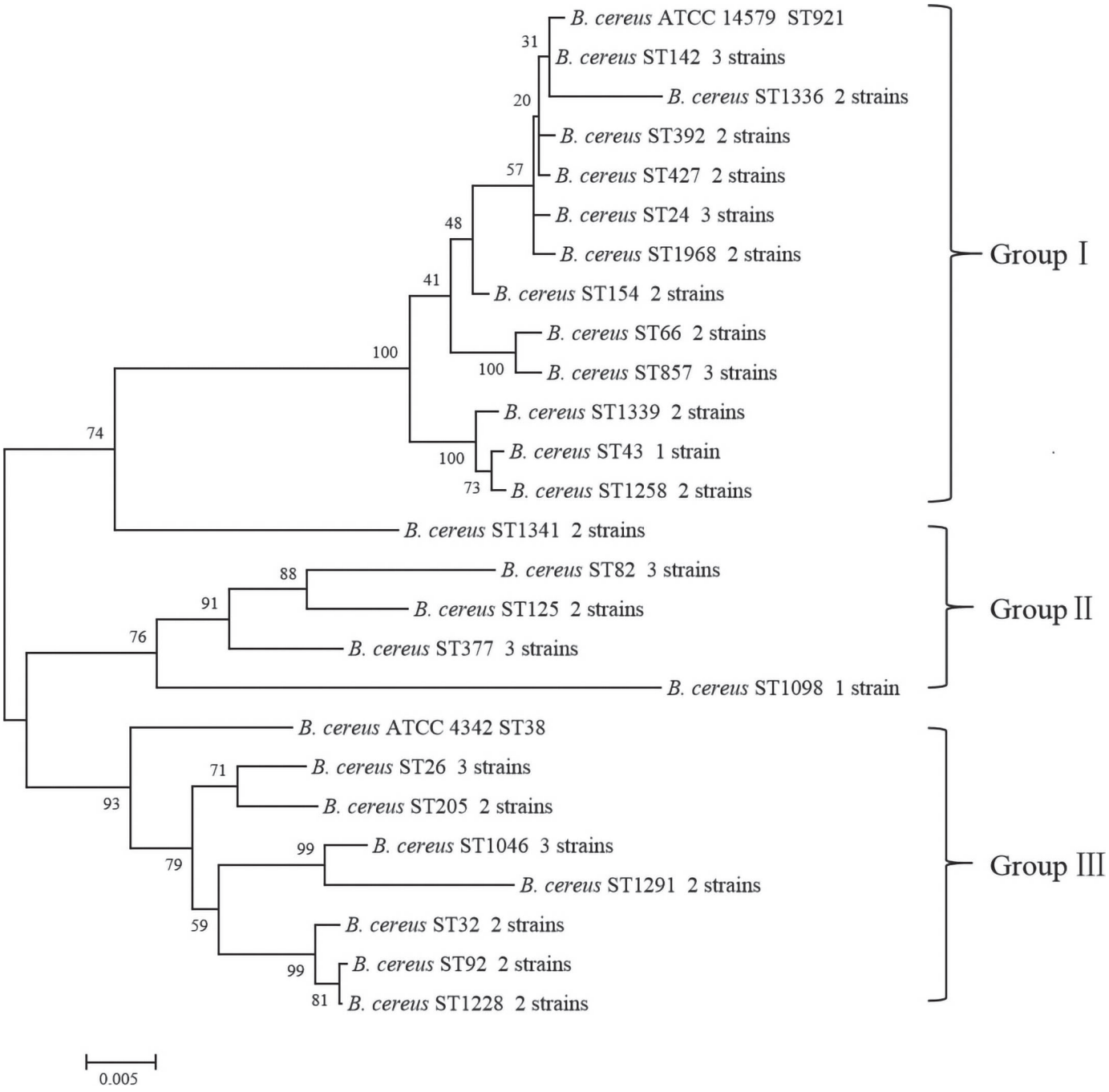

Figure 1. Maximum-likelihood tree of multilocus sequence typing 7 loci (*glpF*, *gmk*, *ilvD*, *pta*, *pur*, *pycA*, and *tpi*) of *Bacillus cereus* strains isolated from powdered food products in China. *Bacillus cereus* ATCC 4342 and *B. cereus* 14579 were equally analyzed as reference strains. The tree was generated using MEGA 6.0 (Arizona State University, Tempe) with 1,000 bootstrap replicates. ST = sequence type.

Table 3. Distributions of toxin genes among *Bacillus cereus* strains isolated from dairy products in China[1]

B. cereus strain	ST	Source	Toxin gene[2]										
			hblA	*hblC*	*hblD*	*nheA*	*nheB*	*nheC*	*cytK*	*bceT*	*entFM*	*hly*II	*cesB*
HN1	ST427	PIF	+	+	−	+	+	+	+	+	−	+	+
HN2	ST1258	RM	+	−	−	+	+	+	+	+	+	−	−
HN3	ST92	RM	+	+	−	+	+	+	−	+	−	+	−
HN4	ST1968	RM	−	+	+	+	+	+	+	+	+	+	−
HN5	ST1228	PM	+	+	−	+	+	+	+	−	+	−	−
HN6	ST1291	PM	+	+	−	+	+	+	+	+	+	+	+
HN7	ST24	UHTM	−	+	−	+	+	+	−	+	+	−	−
HN8	ST1228	UHTM	+	−	−	+	+	+	+	+	+	−	−
HN9	ST82	UHTM	−	+	−	+	+	+	+	+	+	+	−
HN10	ST392	Cheese	−	+	−	+	+	+	+	−	+	+	−
HN11	ST142	Cheese	+	+	+	+	+	+	+	+	+	−	−
HB1	ST82	PIF	−	+	−	+	+	+	+	+	+	+	−
HB2	ST1968	RM	−	−	+	+	+	+	+	+	+	−	−
HB3	ST377	RM	+	+	−	+	+	+	−	+	+	+	−
HB4	ST1336	PM	−	+	−	+	+	+	+	−	+	−	−
HB5	ST43	PM	+	+	−	+	+	+	+	+	+	+	−
HB6	ST1339	UHTM	−	−	−	+	+	+	−	+	+	−	−
HB7	ST66	UHTM	+	+	−	+	+	+	+	+	+	−	−
HB8	ST154	Cheese	+	−	−	+	+	+	+	+	+	+	−
HB9	ST392	Cheese	−	+	−	+	+	+	+	−	+	−	−
GZ1	ST32	PIF	+	+	+	+	+	+	−	+	+	+	−
GZ2	ST377	PIF	+	+	−	+	+	+	+	+	+	−	−
GZ3	ST1336	RM	−	+	−	+	+	+	+	+	+	−	−
GZ4	ST125	RM	+	+	−	+	+	+	+	+	+	+	−
GZ5	ST857	RM	−	−	−	−	−	+	−	−	−	−	−
GZ6	ST205	PM	+	+	−	+	+	+	+	+	−	+	−
GZ7	ST1098	PM	+	+	+	+	+	+	+	+	+	−	−
GZ8	ST205	PM	+	+	−	+	+	+	+	+	−	+	+
GZ9	ST1258	UHTM	−	−	−	+	+	+	+	+	+	−	−
GZ10	ST377	UHTM	+	+	−	+	+	+	−	−	+	+	−
GZ11	ST24	Cheese	−	+	−	+	+	+	+	+	+	+	−
GZ12	ST24	Cheese	−	+	+	+	+	+	+	+	+	−	−
JL1	ST1046	PIF	+	−	−	+	+	+	+	+	+	+	−
JL2	ST1046	RM	+	+	−	+	+	+	−	+	+	+	−
JL3	ST142	RM	−	+	−	+	+	+	+	+	+	−	−
JL4	ST66	PM	+	+	−	+	+	+	+	+	+	+	−
JL5	ST857	PM	−	−	−	−	−	+	−	−	−	−	−
JL6	ST1341	PM	+	+	+	+	+	+	+	+	+	+	−
JL7	ST125	UHTM	−	−	−	+	+	+	+	+	+	+	−
JL8	ST1291	UHTM	+	+	−	+	+	+	+	+	+	+	−
JL9	ST32	UHTM	+	−	+	+	+	+	+	+	+	−	−
JL10	ST26	Cheese	−	−	−	+	+	+	−	−	+	+	+
JL11	ST154	Cheese	+	+	−	+	+	+	+	+	+	−	−
HLJ1	ST1046	PIF	+	−	−	+	+	+	+	+	+	+	−
HLJ2	ST92	PIF	+	+	−	+	+	+	−	+	+	+	−
HLJ3	ST1098	RM	−	+	−	+	+	+	+	−	+	−	−
HLJ4	ST142	RM	+	+	−	+	+	+	+	+	+	+	−
HLJ5	ST1339	RM	+	+	+	+	+	+	−	+	+	−	−
HLJ6	ST1341	PM	+	−	−	+	+	+	+	−	+	−	−
HLJ7	ST82	PM	−	+	−	+	+	+	+	+	+	+	−
HLJ8	ST427	UHTM	+	+	−	+	+	+	+	+	−	+	−
HLJ9	ST857	UHTM	−	−	−	−	−	+	−	−	−	−	−
HLJ10	ST26	Cheese	−	−	−	+	+	+	+	+	+	+	+
HLJ11	ST26	Cheese	−	−	−	+	+	+	+	−	+	−	+
Rate (%)			57.4	68.5	16.7	94.4	94.4	100	75.9	77.8	85.2	53.7	11.1

[1]ST = sequence type; PIF = powdered infant formula; RM = raw milk; PM = pasteurized milk; UHTM = ultra-high-temperature milk.
[2]+ indicates a positive result, and − indicates a negative result.

tance of *B. cereus* in fermented soybean products, PM, and cheese (Yim et al., 2015; Yibar et al., 2017; Gao et al., 2018). Meanwhile, the antibiotic resistance profiles of 54 isolates to ciprofloxacin, kanamycin, gentamicin, erythromycin, chloramphenicol, clindamycin, rifampin, and trimethoprim-sulfamethoxazole were similar to that of this pathogen isolated from PM in China (Park et al., 2009; Gao et al., 2018). In addition, all of the 54

Table 4. Antibiotic susceptibility of 54 *Bacillus cereus* strains isolated from dairy products in China

	B. cereus (n = 54)[1]		
Antimicrobial agent	No. (%) of S	No. (%) of I	No. (%) of R
Ampicillin	1 (1.85)	1 (1.85)	52 (96.30)
Penicillin	2 (3.70)	0 (0)	52 (96.30)
Cefepime	2 (3.70)	0 (0)	52 (96.30)
Cephalothin	5 (9.25)	12 (22.22)	37 (68.52)
Cefotetan	39 (72.22)	7 (12.96)	8 (14.81)
Kanamycin	45 (83.33)	8 (14.81)	1 (1.85)
Imipenem	53 (98.15)	1 (1.85)	0 (0)
Gentamicin	54 (100)	0 (0)	0 (0)
Erythromycin	45 (83.33)	7 (12.96)	2 (3.70)
Oxacillin	2 (3.70)	3 (5.56)	49 (90.74)
Ciprofloxacin	51 (94.44)	3 (5.56)	0 (0)
Chloramphenicol	54 (100)	0 (0)	0 (0)
Tetracycline	53 (98.15)	1 (1.85)	0 (0)
Clindamycin	4 (7.41)	45 (83.33)	5 (9.26)
Rifampin	0 (0)	2 (3.70)	52 (96.30)
Trimethoprim-sulfamethoxazole	46 (85.18)	4 (7.41)	4 (7.41)

[1]After the treatments with antibiotics of recommended concentration, if the diameter of inhibition zones against *B. cereus* was greater than that of quality control organisms, the effect of antibiotics on *B. cereus* was defined as S; if the diameter of inhibition zones against *B. cereus* was less than that of quality control organisms, the effect of antibiotics on *B. cereus* was defined as R; and if the diameter was between R and S, the effect of antibiotics on *B. cereus* was defined as I.

strains of *B. cereus* were susceptible to gentamicin and chloramphenicol, which suggested that these 2 antibiotics could be used for treatment against the gastrointestinal diseases caused by *B. cereus* infections.

CONCLUSIONS

The present study revealed the contamination condition of *B. cereus* in dairy products available in China. The MLST analysis indicated that high genetic diversity is present in *B. cereus* isolated from dairy products in China, and ST24, ST26, ST82, ST142, ST377, ST857, and ST1046 were the main dominant ST. The *nheABC* genes were present in almost all isolates, whereas the *cesB* gene was detected in only 11.1% of the strains. In addition, compared with the previous monitoring, the antibiotic resistance of *B. cereus* isolated from dairy products in China has not increased significantly, and gentamicin and chloramphenicol are better used to prevent and control this pathogen. These results enhance the perception about contamination and characteristic outcomes of *B. cereus* strains isolated from different dairy products abundantly available in Chinese markets, and provide a theoretical basis for developing the effective measures to reduce the contamination of this pathogen.

ACKNOWLEDGMENTS

This study was supported by the Doctor Scientific Research Start-up Fund of Henan University of Science and Technology (13480068, Luoyang, China), Science and Technology Project of Henan Province (172102110019, Zhengzhou, China), and National Natural Science Foundation of China (31702218 and 31601450, Beijing, China). All authors have stated that there are no conflicts of interest.

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