Benha Veterinary Medical Journal 45 (2023) 118-123

Official Journal Issued by Faculty of Veterinary Medicine

Benha Veterinary Medical Journal

Journal homepage: https://bvmj.journals.ekb.eg/

Since 1990

Original Paper

Prevalence of *Bacillus cereus* in milk and some milk products in Egypt

Ahmed M. Elgushi[1,2*], Hend A. Elbarbary[1], Hamdi A. Mohammed[1] and Dalia M.A. El-Masry[3]

[1]*Food Hygiene and Control Department, Faculty of Veterinary Medicine, Benha University, 13736, Egypt.*

[2]*Biotechnology Department, Animal Health Research Institute, Agriculture Research Center, Giza, 12618, Egypt.*

[3]*Nanomaterials Research and Synthesis Unit, Animal Health Research Institute, Agriculture Research Center, Giza, 12618, Egypt.*

ARTICLE INFO

Keywords

Bacillus cereus
Milk products
PCR.
Prevalence
Ras cheese
VITEK2

Received 08/08/2023
Accepted 30/08/2023
Available On-Line 01/10/2023

ABSTRACT

Bacillus cereus is a spore-forming bacterium that causes food spoilage and poisoning. This study aimed to evaluate the prevalence of enterotoxigenic *Bacillus cereus* in 180 dairy samples across three cites (Tanta, El-Santa, and El-Mahalla El-Kubra) in El-Gharbia Governorate, Egypt, including raw milk, milk powder and Ras cheese (60 of each). Samples were collected from December 2022 to April 2023 and analyzed using traditional isolation, biochemical identification with VITEK 2, and PCR targeting the *nhe*A gene as a virulence factor for *Bacillus cereus*. Traditional isolation on selective media indicated a prevalence of 1.66%, 24.99% and 4.99% in raw milk, milk powder and Ras cheese, respectively. However, VITEK 2 only confirmed that *Bacillus cereus* in milk powder isolates was 24.99%. PCR detection of the *nhe*A gene revealed that *Bacillus cereus* in the examined samples completely matched with traditional isolation. Overall, 10.5% of samples were positive for enterotoxigenic *Bacillus cereus* by PCR. Compared to PCR, traditional isolation showed 100% sensitivity and specificity, while VITEK 2 had 79% sensitivity and 100% specificity. Although biochemical methods like VITEK 2 enabled rapid automated identification, PCR detection of virulence genes provided superior sensitivity for identifying enterotoxigenic *Bacillus cereus* of clinical significance from dairy samples. Traditional culture techniques remain essential for isolating target bacteria prior to PCR. This study provided insights into the prevalence of enterotoxigenic *Bacillus cereus* contamination in some Egyptian dairy products.

1. INTRODUCTION

Bacillus cereus is a spore-forming bacterium that is a common cause of foodborne illness worldwide (Sadek *et al*., 2018). Raw milk appears a significant source of contamination, with *Bacillus cereus* introduced from the farm environment, bedding materials, and feces (Svensson *et al*., 2004; Magnusson *et al*., 2007). Powdered dairy products including milk powder, infant formula, and processed foods containing milk powder also frequently test positive for *Bacillus cereus* (Carp-Carare *et al*., 2000; Reyes *et al*., 2007; Kumari & Sarkar, 2014). High spore counts have been detected in both raw and pasteurized milk powder (Montanhini *et al*., 2013). *Bacillus cereus* is frequently detected in various cheeses (Molva *et al*., 2009; Mugadza & Buys, 2017), where it can introduce risk of toxin production during storage depending on temperature, pH, and background microflora (Tirloni *et al*., 2017).

The reported prevalence of raw milk contamination varied widely between studies. Yibar *et al*. (2017) found a prevalence of 3.8%, while Chang *et al*. (2021) and Mohamed *et al*. (2016) reported higher rates of 33.3% and 60%, respectively. The highest reported prevalence of 100% was documented by Osama *et al*. (2020). Studies investigating the prevalence of contamination in milk powder have reported varying rates. Zhang *et al*. (2017), Pei *et al*. (2018), and Mohamed *et al*. (2016) documented relatively lower prevalence of 6.8%, 7.5%, and 15%, respectively. Higher prevalence was noted by Reyes *et al*. (2007) and Kumari and Sarkar (2014), who reported contamination rates of 46% and 52%. The highest prevalence of 68% was reported in the study by Osama *et al*. (2020). Studies reporting the prevalence of contamination in cheese products have shown a wide range. Relatively lower rates were reported by Kumari and Sarkar (2014), Berthold-Pluta *et al*. (2019), and Yibar *et al*. (2017), who found prevalence of 4%, 8.6%, and 10.4% respectively. Higher contamination rates were documented by Montone *et al*. (2020) at 26.2%, Adame-Gómez *et al*. (2020) at 29.48%, Spanu *et al*. (2016) at 33.3%, and Owusu-Kwarteng *et al*. (2017) at 38.7%. Even higher prevalence was noted by Iurlina *et al*. (2006) at 50% and Osama *et al*. (2020) at 95%.

Milk and dairy products have frequently been implicated in *Bacillus cereus* outbreaks globally due to poor hygiene practices, improper food handling and storage temperatures permitting growth of vegetative cells and toxin production (Bennett *et al*., 2013; Osimani *et al*., 2018).

Traditional culture methods involving selective media and biochemical tests have been widely used for isolating and identifying *Bacillus cereus* from foods including dairy samples (Mohamed *et al*., 2016; Bursová *et al*., 2018). Multiple studies demonstrated that VITEK2 for

* Correspondence to: ahmedelgushi90@gmail.com

confirmation of *Bacillus cereus* isolates from milk and dairy products (Alanber *et al*., 2020; Jaber *et al*., 2021) and various food (Hashhash *et al*., 2023; Sidiq & Arif, 2023) with probability results indicate good confidence in identification of *Bacillus cereus* (Jaber *et al*., 2021; Sidiq & Arif, 2023). However, PCR-based molecular methods targeting virulence genes such as hemolysins (*hbl*), nonhemolytic enterotoxins (*nhe*), cytotoxins(*cyt*K) and cereulide toxins (*ces*) have become the gold standard for detecting and characterizing enterotoxigenic strains of *Bacillus cereus* with higher sensitivity and specificity compared to phenotypic methods (Molva *et al*., 2009, Zhang *et al*., 2016).

The *nhe* is comprised of three components encoded by an operonic gene cluster present in all *Bacillus cereus* strains. The key cytotoxicity factor is *nhe*A, while *nhe*B and *nhe*C function as binding components (Ankolekar *et al*., 2009). The *nhe* was first characterized following a foodborne outbreak in Norway caused by an isolate lacking the hemolytic BL (*hbl*) enterotoxin complex, and it is now believed to be the most dominant diarrheal toxin (Stenfors *et al*., 2008). This study aimed to determine and compare the prevalence of *Bacillus cereus* contamination in raw milk, milk powder and Ras cheese samples from three cities in Egypt. As well as comparing the methods of either traditional isolation, biochemical identification with VITEK 2, or PCR targeting the *nhe*A gene for detection of *Bacillus cereus* in the examined samples.

2. MATERIAL AND METHODS

2.1. Collection of samples:

A total of 180 dairy samples comprising raw cow's milk, milk powder and Ras cheese samples (60 of each) were obtained randomly from different supermarkets across three cities in the El-Gharbia Governorate of Egypt. Sampling was performed between December 2022 to April 2023, with aseptic collection of the samples and immediate transfer to the laboratory under refrigeration at 4°C.

2.2. Materials:

Peptone water, *Bacillus cereus* agar base (Hi MEDIA M833), Polymyxin B selective supplement (FD003), and Sterile egg yolk emulsion (FD045) (HiMedia, India). *nhe*A oligonucleotide primers and DNA polymerase were obtained from Willowfort, UK. Agarose, Tris base, boric acid, and EDTA were purchased from Sigma Aldrich, USA. RedSafe nucleic acid stain was purchased from Intron bio, USA. DNA ladder was purchased from GeneDirex, USA. A reference strain of *Bacillus cereus* (ATCC 11778) was obtained from the Food Hygiene Department, Animal Health Research Institute, Egypt. This served as a positive control.

2.3. Preparation and primary enrichment of the samples:

Primary enrichment of dairy samples was performed as described in previous studies. Briefly, 1 ml of raw milk sample was inoculated into 9 ml of peptone water and incubated overnight at 37°C (Mohamed *et al*., 2016). For cheese samples, 25 g of Ras cheese was added to 225 ml of peptone water, homogenized for 2 min using a stomacher, and incubated under the same conditions (Luiz *et al*., 2017). Similarly, 10 g of milk powder was enriched in 90 ml of peptone water, homogenized, and incubated at 37°C overnight (Heini *et al*., 2018). This primary enrichment allowed growth and recovery of cells from the various dairy samples.

2.4. Traditional isolation of Bacillus cereus:

To select for spores and inhibit vegetative cells, the enrichments were heat treated at 70°C for 10 minutes in a water bath. After heat shock, samples were inoculated onto *Bacillus cereus* selective agar base supplemented with polymyxin B and sterile egg yolk emulsion. *Bacillus cereus* agar base plates were streaked to obtain isolated colonies and incubated at 30°C for 48 hours. The selective conditions allowed growth and phenotypic confirmation of *Bacillus cereus* isolates based on colony morphology and lecithinase activity (Saleh-Lakha *et al.*, 2017).

2.5. Biochemical identification by VITEK 2 compact system:

Identification of bacterial isolates was performed using the VITEK2 compact system (BioMérieux) as per the manufacturer's instructions (BioMérieux Manual-414532, 2006). In brief, the isolated colonies were suspended in sterile saline and the turbidity adjusted to match a 1.8-2.2 McFarland standard using a densitometer. The standardized bacterial suspension was used to inoculate Bacillus (BCL) identification cards containing miniature biochemical substrates tailored to Bacillus species identification. The inoculated cards were introduced into the VITEK2 analyzer's vacuum filling station along with a cassette mechanism to transfer the suspension into the cards' microwells. After sealing, the loaded cards were incubated kinetically at 35.5°C in the carousel incubator, with frequent automated optical readings at 15-minute intervals to measure biochemical reactions. The kinetic data obtained was automatically analyzed by the VITEK2 software through comparisons to an onboard database, allowing rapid and standardized identification of Bacillus isolates based on species-specific metabolic patterns. This technique provides a highly reproducible inoculation, incubation and reading system for automated bacterial identification from culture plates.

2.6. Molecular identification for isolates:

2.6.1. DNA extraction.

Genomic DNA extraction from bacterial isolates was performed through a boiling method as described by Zhang *et al*. (2014). Fresh bacterial isolates were resuspended in 1 ml of sterile water, centrifuged at 8000 rpm for 5 minutes, and washed twice with an equal volume of sterile water. The cell pellets were then resuspended in 1 ml of sterile water, boiled for 15 minutes to lyse the cells, and release genomic DNA, and centrifuged again at 8000 rpm for 5 minutes to remove cell debris. The resulting supernatant, containing the extracted genomic DNA, was used as a template in subsequent procedures. This modified boiling protocol allows for the rapid isolation of genomic DNA from bacterial cultures in a simple and cost-effective manner.

2.6.2. Conventional PCR for detection of enterotoxigenic Bacillus cereus:

To amplify the 755 bp species-specific fragments for enterotoxigenic *Bacillus cereus*, a pairs of oligonucleotide primers specific for the *nhe*A gene (Table, 1) were used.

Table 1 Oligonucleotide primers specific for *nhe*A gene

gene	Primer sequence 5'→3'	Product size	Reference
*nhe*A	F-GTTAGGATCACAATCACCGC R-ACGAATGTAATTTGAGTCGC	755 bp	Guinebretière *et al.* (2002)

PCR amplification of target DNA was performed in 15 µl reactions containing 7.5 µl of DNA polymerase master mix, 1 µl of a 10 pmol primer mix, 2.5 µl of template DNA, and PCR-grade water for the remaining volume. The thermal cycling protocol consisted of an initial denaturation step at 95°C for 2 minutes followed by 30 cycles of denaturation at

95°C for 15 seconds, primer annealing at 56°C for 20 seconds, and extension at 72°C for 45 seconds. A final extension step at 72°C for 5 minutes was included to ensure complete amplification. To analyze PCR products, 7.5 µl of each amplified sample was separated by electrophoresis on 1.5% agarose gels with RedSafe dye at a constant 100V for 30 minutes. Gels were visualized using a Bio-Rad Gel Doc XR imaging system and amplicon sizes estimated by comparison against a 100 bp DNA ladder (Guinebretière *et al*., 2002).

2.7. Statistical analysis:
In order to report the prevalence, descriptive statistics were utilized. To compare the performance of traditional isolation, VITEK 2 biochemical testing, and PCR, diagnostic test evaluation statistics including sensitivity and specificity were calculated using SPSS software (version 20; spss Inc., Chicago, USA).

2.7.1. Method of calculation of sensitivity and specificity
sensitivity and specificity were calculated according to the following equations: -
Sensitivity: - TP / (TP + FN) *100
Specificity: -TN / (TN + FP) *100

3. RESULTS

3.1. Traditional isolation of *Bacillus cereus* from milk and some of its products:
The presumptive identification of *Bacillus cereus* was based on the observation of blue colonies surrounded by cloudy halos on *Bacillus cereus* agar plates. The cloudy halos are indicative of lecithinase production, a characteristic phenotype of *Bacillus cereus*. Traditional microbiological techniques were utilized for isolation and identification of *Bacillus cereus*. Prevalence data for *Bacillus cereus* in various dairy products was presented in Table (2). In raw milk samples, 1.66% tested positive for *Bacillus cereus*. A higher prevalence rate of 24.99% was observed in milk powder samples. Ras cheese samples exhibited an intermediate *Bacillus cereus* prevalence of 4.99%.

Table 2 Prevalence of *Bacillus cereus* using traditional isolation (n=60 each)

Products	Positive samples	Percentage %
Raw milk	1	1.66
Milk powder	15	24.99
Ras cheese	3	4.99
Total (180)	19	10.5

% According to No. of total samples (n=180)

3.2. Biochemical identification
The confirmation of presumptive isolates was achieved through biochemical profiling using the VITEK 2 automated system. The biochemical test results for all isolates were presented in table (3).

Table 3 Prevalence of *Bacillus cereus* using VITEK 2 biochemical approach (n=60 each)

Products	Traditional isolation Positive samples	VITEK 2 Positive samples	% VITEK 2 Positive samples
Raw milk	1	0	0
Milk powder	15	15	24.99
Ras cheese	3	0	0
Total (180)	19	15	8.33

% According to No. of total samples (n=180)

3.3. Molecular identification by PCR
Molecular identification of enterotoxigenic *Bacillus cereus* isolates was performed through PCR targeting of the *nhe*A gene, which encodes for the non-hemolytic enterotoxin. A 755bp amplicon indicates presence of the *nhe* A gene. As shown in Figure (1), PCR analysis demonstrated the expected 755bp band in a subset of the *Bacillus cereus* isolates, confirming enterotoxigenicity. The prevalence of enterotoxigenic *Bacillus cereus* amongst the dairy isolates is summarized in Table (4). PCR-based screening for virulence factors provides a rapid technique for assessing the pathogenic potential of bacterial isolates. The inclusion of *nhe*A screening expands the characterization of *Bacillus cereus* isolates beyond basic prevalence data, offering insight into the occurrence of enterotoxigenic strains across the dairy samples tested.

3.4. Sensitivity and specificity of different techniques in relation to PCR as a gold standard.
The diagnostic performance of traditional isolation, VITEK 2 biochemical identification, and PCR targeting the *nhe*A gene for detection of enterotoxigenic *Bacillus cereus* was compared, with PCR as a gold standard reference method. Calculated sensitivity and specificity values for each testing methodology are presented in Table (5). Traditional isolation technique exhibited 100% sensitivity and specificity relative to PCR, with complete agreement between the two methods. All 19 PCR-confirmed enterotoxigenic isolates were correctly identified through traditional culture-based analysis. The VITEK 2 automated system demonstrated slightly lower diagnostic sensitivity of 79% compared to PCR, failing to detect 4 of the 19 enterotoxigenic isolates. However, the VITEK 2 maintained perfect specificity, generating no false positives relative to PCR. Overall, while the VITEK 2 provides rapid biochemical confirmation of *Bacillus cereus*, traditional isolation paired with PCR molecular screening proved optimal for reliable detection of enterotoxigenic strains among the tested dairy isolates.

Table 4 Prevalence of *Bacillus cereus* using PCR molecular approach (n=60 each)

Products	Traditional isolation Positive samples	PCR Positive samples	% PCR Positive samples
Raw milk	1	1	1.66
Milk powder	15	15	24.99
Ras cheese	3	3	4.99
Total (180)	19	19	10.5

% According to No. of total samples (n=180)

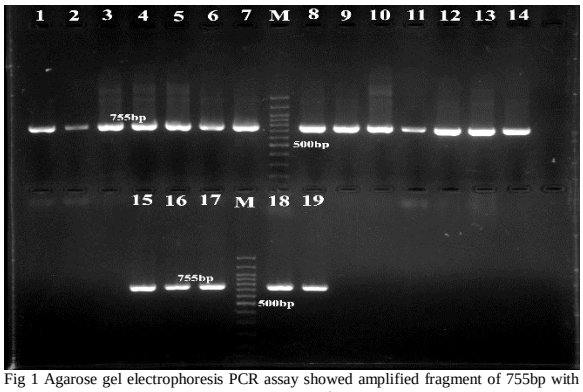

Fig 1 Agarose gel electrophoresis PCR assay showed amplified fragment of 755bp with enterotoxigenic *Bacillus cereus nhe*A gene specific primer. Lane M: molecular marker (100bp).

Table 5 Sensitivity and specificity of different techniques in relation to PCR as a gold standard (n=180)

Test	Traditional isolation		VITEK 2		PCR	
	TP-19	FP-0	TP-15	FP-0	TP-19	FP-0
	TN-161	FN-0	TN-165	FN-4	TN-161	FN-0
Sensitivity	100%		79%		100%	
Specificity	100%		100%		100%	
TP-True positive	FP-False Positive		TN-True Negative		FN-False Negative	

4. DISCUSSION

The prevalence of *Bacillus cereus* in dairy products is a relevant concern for quality assurance and food safety. Bacillus cereus is an established causative agent of foodborne illness outbreaks, attributable to its virulence factors and associated enterotoxins (Tirloni *et al*., 2022).

The prevalence of *Bacillus cereus* in raw milk was investigated using traditional isolation on selective media and PCR, resulting in a prevalence rate of 1.66%. Interestingly, VITEK2 did not detect any *Bacillus cereus* in the raw milk samples (Table 2,3,4). The 1.66% prevalence rate mirrors the low prevalence of ≤3.8% noted across studies in locales like Egypt and Turkey (Mohamed *et al*., 2016; Yibar *et al*., 2017). However, strikingly higher prevalence of 33.3% (50/150) from China (Chang *et al*., 2021) and up to 100% (25/25) has been documented in in raw milk from Egypt (Osama *et al*., 2020). This variation in prevalence could be attributed to seasonal fluctuations in spore counts, as past researchers have demonstrated heightened raw milk contamination during warmer spring and summer periods (Larsen & Jorgensen, 1997; Svensson *et al*., 2004; Bartoszewicz *et al*., 2008). The nature of *Bacillus cereus* permits multiplication during warm weather, which could promote seasonal spikes.

The prevalence of *Bacillus cereus* in milk powder was assessed using traditional isolation, VITEK2, and PCR, revealing a prevalence rate of 24.99% (Table 2,3,4). These results were in line with the prevalence rate of 27% (54/200) in Taiwan reported by Wong *et al*. (1988). Conversely, studies by Kumari and Sarkar (2014) and Osama *et al*. (2020) had reported exceedingly high prevalence rates of 52% (18/35) in India and 68% (17/25) in Egypt. On the other hand, Mohamed *et al*. (2016) reported a substantially lower prevalence of 15% (3/20) in milk powder from Egypt.

Evidence suggests ubiquity of implying cross-contamination during processing and packaging as a risk factor (Liu *et al*., 2018). Sporulation may also highly promote heat-resistant survival spores during the drying process (Sadiq *et al*., 2016). Large-scale factory production resulted in more obvious variation in the microbial content (Wang *et al*., 2018). Finally, the consumer becomes under risk from *Bacillus cereus* toxins resulting from extended storage of milk powder at improper temperatures (Bursová *et al*., 2018).

In Ras cheese, the prevalence of *Bacillus cereus* was found to be 4.99% using traditional isolation and PCR, while VITEK2 could not detect any contamination (Table 2,3,4). These results are consistent with the prevalence reported by Yibar *et al*. (2017) in Turkey, which was 10.4% (11/106). Additionally, Berthold-Pluta *et al*. (2019) reported a prevalence of 8.6% (3/35) in fresh acid cheese in Poland. In contrast, Iurlina *et al*. (2006) reported a remarkably higher prevalence of 50% (15/30) in Argentine cheese, and Berthold-Pluta *et al*. (2019) reported 52.5% (42/80) in mold cheese in Poland. These variations can be attributed to factors such as temperature, pH, and background microflora, which can influence toxin production during storage, as explained by Tirloni *et al*. (2017).

Comparing diagnostic performances provides valuable insights into the utility of various *Bacillus cereus* detection techniques. In the present study, traditional selective plating demonstrated 100% sensitivity and specificity relative to PCR targeting of enterotoxin genes (Fig.1) and (Table, 5). However, the labor and time intensive nature of selective culture may limit utility for high-throughput screening. Still, isolation provides critical strain availability for downstream characterization and toxin analysis. The automated VITEK 2 system offers rapid turnaround for presumptive identification (Alanber *et al*., 2020; Jaber *et al*., 2021).

However, the 79% sensitivity (Table, 5) found here echoes studies citing limitations in differentiating *Bacillus cereus* from closely related species via VITEK 2 biochemical panels (Navas *et al*., 2014; Hashhash *et al*., 2023). While 100% specificity aligns with past findings and affirms the utility of VITEK 2 BCL card as a reliable first-line screen (Halket *et al*., 2010; Alanber *et al*., 2020; Wen *et al*., 2022; Sidiq & Arif, 2023). PCR-based detection of toxin-encoding genes provides a sensitive and specific means of identifying enterotoxigenic strains capable of causing food poisoning. Thus, the 10.5% prevalence rate for potentially toxigenic *Bacillus cereus* may overestimate the true risk. Ultimately, PCR provides sensitive screening for potentially toxigenic strains (Guinebretière *et al*., 2002; Tirloni *et al*., 2022).

5. CONCLUSIONS

In conclusion, this study revealed concerning levels of contamination with enterotoxigenic *Bacillus cereus* in dairy products from El-Gharbia Governorate, Egypt. Traditional culture techniques, biochemical identification, and PCR targeting the *nhe*A gene were used to analyze raw milk, milk powder and Ras cheese samples collected from December 2022 to April 2023. Overall, 10.5% of the 180 dairy samples tested positive for enterotoxigenic *Bacillus cereus* by PCR, with the highest prevalence found in milk powder at 24.99%. Compared to PCR detection of the *nhe*A virulence gene, traditional isolation and biochemical identification with VITEK 2 showed reduced sensitivity, indicating these methods underestimate the levels of potentially enterotoxigenic *Bacillus cereus*. However, traditional culture remains essential to obtain bacterial isolates for further characterization.

6. REFERENCES

1. Adame-Gómez, R., Muñoz-Barrios, S., Castro-Alarcón, N., Leyva-Vázquez, M.A., Toribio-Jiménez, J., & Ramírez-Peralta, A. (2020). Prevalence of the strains of *Bacillus cereus* group in artisanal Mexican cheese. *Foodborne Pathogens and Disease, 17*, 8-14.
2. Alanber, M. N., Alharbi, N. S. & Khaled, J. M. (2020). Evaluation of multidrug-resistant Bacillus strains causing public health risks in powdered infant milk formulas. *Journal of Infection and Public Health, 13*, 1462-1468.
3. Ankolekar, C., Rahmati, T. & Labbe, R. G. (2009). Detection of toxigenic Bacillus cereus and Bacillus thuringiensis spores in U.S. rice. *International Journal of Food Microbiology, 128*, 460-466.
4. Bartoszewicz, M., Hansen, B. M. & Swiecicka, I. (2008). The members of the Bacillus cereus group are commonly present contaminants of fresh and heat-treated milk. *Food Microbiology, 25*, 588-596.
5. Bennett, D. S., Walsh, K. A. & Gould, L. H. (2013). Foodborne disease outbreaks caused by Bacillus cereus, Clostridium perfringens, and Staphylococcus aureus-United States, 1998-2008. *Clinical Infectious Diseases, 57*, 425-433.
6. Berthold-Pluta, A., Pluta, A., Garbowska, M. & Stefa`nska, I. (2019). Prevalence and toxicity characterization of Bacillus cereus in food products from Poland. *Foods, 8*, 269. https://doi.org/10.3390/foods8070269.
7. BioMérieux VITEK-2 Compact ref Manual – Ref-414532 2006. National Committee for Clinical Laboratory Standards. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved Standard M7 A5.
8. Bursová, Š., Necidová, L. & Haruštiaková, D. (2018). Growth and toxin production of Bacillus cereus strains in reconstituted initial infant milk formula. *Food Control, 93*, 334-343.

9. Carp-Carare, M., Floristean, V. & Chidon, A. (2000). Observations concerning the incidence of B. cereus in powder milk. *Lucrai-Stiinifice-Medicina-Veterinara Universitatea-de-Stiinte-Agricole-si-Medicina-Veterinara-Ion-Ionescu-de-la-Brad-Iasi, 43*, 171-175.
10. Chang, Y., Xie, Q., Yang, J., Ma, L. & Feng, H. (2021). The prevalence and characterization of Bacillus cereus isolated from raw and pasteurized buffalo milk in southwestern China. *Journal of Dairy Science,104*, 3980-3989.
11. 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*, 3053-3056.
12. Halket, G., Dinsdale, A. E. & Logan, N. A. (2010). Evaluation of the VITEK2 BCL card for identification of Bacillus species and other aerobic endospore formers. *Letters in Applied Microbiology, 50*, 120-126.
13. Hashhash, A. A., Abo EL-Roos, N. A., Abd El-Aziz, M. A. & Elsabagh, R. (2023). Risk assessment of Bacillus cereus in cooked meat products by using VITEK 2 and PCR. *Benha Veterinary Medical Journal, 44*, 9-12.
14. Heini, N., Stephan, R., Ehling-Schulz, M., & Johler, S. (2018). Characterization of Bacillus cereus group isolates from powdered food products. *International Journal of Food Microbiology, 283*, 59-64.
15. Iurlina, M. O., Saiz, A. I., Fuselli, S. R. & Fritz, R. (2006). Prevalence of Bacillus spp. in different food products collected in Argentina. *LWT, 39*, 105-110.
16. Jaber, N. N., Hadi, N. S., Mohammed, A. L., Idan, M. & Niama, A. (2021). Vitik Detection of Aerobic Spore-Forming Bacteria Isolated from Raw Milk, Skim Milk Powder, and UHT Milk. *Medico-legal Update, 21*, 678-683.
17. Kumari, S. & Sarkar, P.K (2014). Prevalence and characterization of Bacillus cereus group from various marketed dairy products in India. *Journal of Dairy Science and Technology, 94*, 483-497.
18. Larsen, H. D. & Jorgensen, K. (1997). The occurrence of Bacillus cereus in Danish pasteurized milk. *International Journal of Food Microbiology, 34*, 179-186.
19. Liu, Y., Ge, W., Zhang, J., Li, X., Wu, X., Li, T., Zhang, X. & Wang, X. (2018). Detection of Bacillus cereus sensu lato from environments associated with goat milk powdered infant formula production facilities. *International Dairy Journal, 83*, 10-16.
20. Luiz, L. M. P., Castro, R. D., Sandes, S. H. C., Silva, J. G., Oliveira, L. G., Sales, G. A., Nune, A.C. & Souza, M. R. (2017). Isolation and identification of lactic acid bacteria from Brazilian Minas artisanal cheese. *CyTA-Journal of Food*, *15*, 125-128.
21. Magnusson, M., Christiansson, A. & Svensson, B. (2007). Bacillus cereus Spores During Housing of Dairy Cows: Factors Affecting Contamination of Raw Milk. *Journal of Dairy Science, 90*, 2745-2754.
22. Mohamed, A. S., Alnakip, M. E. A. & Abd-El Aal, S.F. (2016). Occurrence of Bacillus cereus in raw milk and some dairy products in Egypt. *Japanese Journal of Veterinary Research, 64*, 95-103.
23. Molva, C., Sudagidan, M. & Okuklu, B. (2009). Extracellular enzyme production and enterotoxigenic gene profiles of Bacillus cereus and Bacillus thuringiensis strains isolated from cheese in Turkey. *Food Control, 20*, 829-834.
24. Montanhini, M. T. M., Colombo, M., Nero, L. A. & Bersot, L. S. (2013). Short communication: Presence of neutral metallopeptidase (npr) gene and proteolytic activity of Bacillus cereus isolated from dairy products. *Journal of Dairy Science, 96*, 5641-5643.
25. Montone, A.M.I., Capuano, F., Mancusi, A., Di Maro, O., Peruzy, M.F., Proroga, Y.T.R., & Cristiano, D. (2020). Exposure to *Bacillus cereus* in water buffalo mozzarella cheese. *Foods, 9*, 1899. https://doi.org/10.3390/foods9121899.
26. Mugadza, D. T. & Buys, E. M. (2017). Diversity of Bacillus cereus strains in extended shelf life. *International Dairy Journal, 73*, 144-150.
27. Navas, M., Pincus, D. H., Wilkey, K., Sercia, L., LaSalvia, M., Wilson, D., Procop, G. W. & Richter, S. S. (2014). Identification of Aerobic Gram-Positive Bacilli by Use of Vitek MS. *Journal of Clinical Microbiology, 52*, 1274-1277.
28. Osama, R., Ahmed, M., Abdulmawjood, M. & Al-Ashmawy, M. (2020). Prevalence and antimicrobial resistance of Bacillus cereus in milk and dairy products. *Mansoura Veterinary Medical Journal, 21*, 11-18.
29. Osimani, A., Aquilanti, L. & Clementi, F. (2018). Bacillus cereus foodborne outbreaks in mass catering. *International Journal of Hospitality Management, 72*, 145-153.
30. Owusu-Kwarteng, J., Wuni, A., Akabanda, F., Tano-Debrah, K., & Jespersen, L. (2017). Prevalence, virulence factor genes and antibiotic resistance of *Bacillus cereus* sensu lato isolated from dairy farms and traditional dairy products. *BMC Microbiology, 17*, 65.
31. Pei, X., Yang, S., Zhu, J., Song, X., Hu, X., Liu, G., Ma, G., Li, N., & Yang, D. (2018). Prevalence of *Bacillus cereus* in powdered infant and powdered follow-up formula in China. *Food Control, 93*, 101-105.
32. Reyes, J. E., Bastías, J. M., Gutiérrez, M. R. & Rodríguez, M. D. O. (2007). Prevalence of Bacillus cereus in dried milk products used by the Chilean School Feeding Program. *Food Microbiology, 24*, 1-6.
33. Sadek, Z. I., Abdel-Rahman, M. A., Azab, M. S., Darwesh, O. M. & Hassan, M. S. (2018). Microbiological evaluation of infant foods quality and molecular detection of Bacillus cereus toxins relating genes. *Toxicology Reports, 5*, 871-877.
34. Sadiq, F. A., Li, Y., Liu, T., Flint, S., Zhang, G., Yuan, L., Pei, Z. & He, G. (2016). The heat resistance and spoilage potential of aerobic mesophilic and thermophilic spore-forming bacteria isolated from Chinese milk powders. *International Journal of Food Microbiology, 238*, 193-201.
35. Saleh-Lakha, S., leon-velarde, G.C., chen, S., lee, S., shannon, K., fabri, M., downing, G. & keown, B. (2017). A Study to Assess the Numbers and Prevalence of Bacillus cereus and Its Toxins in Pasteurized Fluid Milk. *Journal of Food Protection, 80*, 1085-1089.
36. Sidiq, R. A. & Arif, H. H. (2023). Investigation of toxigenic Bacillus cereus isolated from raw and cooked rice in Sulaimani city, KRG. *Euphrates Journal of Agriculture Science, 15*, 60-70.
37. Spanu, C., Scarano, C., Spanu, V., Pala, C., Casti, D., Lamon, S., Cossu, F., Ibba, M., Nieddu, G., & De Santis, E.P.L. (2016). Occurrence and behavior of *Bacillus cereus* in naturally contaminated ricotta salata cheese during refrigerated storage. *Food Microbiology, 58*, 135-138.
38. Stenfors, A.L.P., Fagerlund, A. & Granum, P.E. (2008). From soil to gut: B. cereus and its food poisoning toxins. *FEMS Microbiology Reviews, 32*, 579-606.
39. Svensson, B., Ekelund, K., Ogura, H. &Christiansson, A. (2004). Characterization of B. cereus isolated from milk silo tanks at eight different dairy plants. *International Dairy Journal, 14*, 17–27.
40. Tirloni, E., Ghelardi, E., Celandroni, F., Bernardi, C. & Stella, S. (2017). Effect of dairy product environment on the growth of Bacillus cereus. *Journal of Dairy Science, 100*, 7026-7034.
41. Tirloni, E., Stella, S., Celandroni, F., Mazzantini, D., Bernardi, C., & Ghelardi, E. (2022). Bacillus cereus in dairy products and production plants. *Foods, 11*, 2572. https://doi.org/10.3390/foods11172572.
42. Wang, J., Zheng, Y., Xi, X., Hou, Q., Xu, H., Zhao, J., Li, J., Bian, Y., Ma, H., Wang, Y., Kwok, L.-Y., Zhang, H. & Sun, Z. (2018). Application of PacBio Single Molecule Real-Time (SMRT) sequencing in bacterial source tracking analysis during milk powder production. *Food Control, 93*, 226-234.
43. Wen, H., Xie, S., Liang, Y., Liu, Y., Wei, H., Sun, Q., Wang, W., Wen, B., & Zhao, J. (2022). Direct Identification, Antimicrobial Susceptibility Testing, and Extended-Spectrum β-Lactamase and Carbapenemase Detection in Gram-Negative Bacteria Isolated from Blood Cultures. *Infection and Drug Resistance, 15*, 1587-1599.
44. Wong, H. C., Chang, M. H. & Fan, J. Y. (1988). Incidence and characterization of Bacillus cereus isolates contaminating dairy products. *Applied and Environmental Microbiology, 54*, 699-702.
45. Yibar, A., Çetinkaya, F., Soyutemiz, E. & Yaman, G. (2017). Prevalence, enterotoxin production, and antibiotic resistance of Bacillus cereus isolated from milk and cheese. *Kafkas Üniversitesi Veteriner Fakültesi Dergisi, 23*, 635-642.

46. Zhang, Y., Chen, J., Feng, C., Zhan, L., Zhang, J., Li, Y., Yang, Y., Chen, H., Zhang, Z., & Zhang, Y., et al. (2017). Quantitative prevalence, phenotypic and genotypic characteristics of *Bacillus cereus* isolated from retail infant foods in China. *Foodborne Pathogens and Disease, 14*, 564-572.
47. Zhang, Z., Feng, L., Xu, H., Liu, C., Shah, P. N. & Wei, H. (2016). Detection of viable enterotoxin-producing Bacillus cereus and analysis of toxigenicity from ready-to-eat foods and infant formula milk powder by multiplex PCR. *Journal of Dairy Science, 99*, 1047-1055.
48. Zhang, Z., Wang, L., Xu, H., Aguilar, Z. P., Liu, C., Gan, B., Xiong, Y., Lai, W., Xu, F., & Wei, H. (2014). Detection of non-emetic and emetic Bacillus cereus by propidium monoazide multiplex PCR (PMA-mPCR) with internal amplification control. *Food Control, 35*, 401-406.