Received: 19 May 2023, Accepted: 02 July 2023

Original Research

Journal of Advanced Veterinary Research
(2023) Volume 13, Issue 7, 1281-1287

Bacillus cereus in Raw Milk and its Virulence Genes

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Abstract

A frequent source of milk contamination is *Bacillus cereus*. The microorganism can contaminate raw milk at the time of milking since it is ubiquitous in the environment. In the current study, one hundred raw milk samples were obtained from farmers, supermarkets, street vendors and dairy shops in Egypt, collected samples were examined biochemically and by Vitek 2 compact system to isolate *Bacillus cereus*. Prevalence values of *B. cereus* were 0, 20,8 and 12% respectively. The Mean±SE of the pH values for milk samples were 6.54±0.04, 6.48±0.06, 6.44±0.05 and 6.53±0.06 respectively. On the other hand, Storch test for milk samples was positive in a percentage of 12, 8, 40 and, 16, respectively. Molecular characterization of *hblA* and *ces* showed positive reaction for 30 and 20% of samples. Growth, proteolytic and lipolytic activities of *Bacillus cereus* was 100, 100, 20 and 40, 40, 20 at 30◦C/48 h and 7°C/10 days, respectively. Outcomes of the in vitro testing for susceptibility indicated that the highest resistance to antibiotics was against macrolides and mono, B-lactamase, whereas moderate resistance was exhibited to gentamicin. The current study's findings exhibited evidence of *B. cereus* isolation from raw milk has a high potential for producing food poisoning, so it is necessary to employ the techniques to lessen bacterial contamination while processing dairy products.

KEYWORDS
Raw milk, *Bacillus cereus*, pH, Storch test, VITEK2 compact, *hblA*, *ces* genes.

INTRODUCTION

Bacillus cereus is ubiquitous microorganism isolated frequently from growing plants and soil. *B. cereus* has been developed to grow in the intestines of both animals and insects; It has been implicated in two types of food poisoning, diarrhea, and emesis (Thirkell *et al*., 2019). *B. cereus* is a facultative anaerobe, Gram-positive that forms rod-shaped spores. Peritrichous flagella, which are involved in movement and/or toxin production, are present in some *B. cereus* strains. Moreover, the cell wall of some strains is covered with a crystalline surface glycoprotein layer (S-layer), which protects them. *B. cereus* adheres to host cells through the S-proteins, layer's which also act as a barrier against γ-ray radiation. A variety of virulence factors, such as pore-forming toxins, cereulide, hemolysins, enterotoxins, proteases, and phospholipases are produced by *B. cereus*. (Jovanovic *et al*., 2021).

Two different gastrointestinal conditions are brought on by *B. cereus*. The toxin cereulide is responsible for emetic-type sickness. With temperature range of 12-37°C, this toxin is produced in food. It causes emetic disease with an average incubation time of 1-6 hours and symptom duration of 6-24 hours, and it is heat stable to 100°C for >2 hours.

A diarrhoeal-type illness is caused by one or several associated toxins, including enterotoxin FM, not pathogenic but contributing to severity; haemolysin BL (Hbl); and cytotoxin K (CytK), and non-haemolytic enterotoxin, Dormant spores are consumed, germinate, and multiply in the small intestine, and then generate the toxins, which is what causes the disease. A sickness of the diarrhoeal variety typically takes 8 to 16 hours to incubate and takes 12 to 24 hours to manifest. In the event that the proper heating and cooling procedures are followed, 6 spores should be removed after 3 minutes at 100°C.

The emetic and diarrheal symptoms, which manifest 0.5–6 hours and 8–16 hours, respectively, after consuming tainted food, are caused by *B. cereus*. An enterotoxigenic *B. cereus* foodborne infection is the cause of the diarrhoea. It is believed that heat labile enterotoxins produced in the small intestine by vegetative cells are what cause the disease once spores from infected food are ingested and spore outgrowth occurs in the intestine (Huang *et al*., 2020).

B. cereus spores exhibit high heat and desiccation resistance, as well as hydrophobicity (Rouzeau-Szynalski *et al*., 2020). Spores are firmly attached to surfaces and, after germinating, may produce biofilms that will make bacteria more resistant to cleaning and heat. The incubation times, symptom pattern, dietary laboratory data, and genomic sequencing of the *B. cereus* diarrheal gene, all point to a possible cause of *B. cereus* intoxication. Restaurant hygiene practices improvement, and heating/cooling methods were documented as part of public health measures (Candela *et al*., 2019; Dietrich *et al*., 2021).

In the dairy sector, antibiotics are used to treat common diseases including mastitis, as well as for prophylactic purposes such as dry cow therapy and foot bath disinfection (Schewe and Brock,

ISSN: 2090-6277/2090-6269/

2018). The World Health Organization has recently expressed serious concerns about milk and the antimicrobial residues that are associated with it, which can result in the development of resistance genes and the transmission of human and animal pathogens. As a result, the WHO has recommended that antibiotic use should be restricted to the treatment of infected animals only (Youssif *et al*., 2019).

Therefore, the current study's objective was to determine the frequency of *B. cereus* in raw milk using both traditional and molecular methods, as well as the detection of virulence genes, lipolytic and proteolytic activity and antibiotic resistance to choose the best antibiotics for controlling outbreaks.

MATERIALS AND METHODS

Collection of samples

One hundred samples of market milk (farmers, supermarkets, street vendors and, dairy shops), (25 of each), from a variety of randomly selected localities of Giza governorate, Egypt. The samples were gathered in sterile and dry containers, and then brought to the lab with minimum of delay according to Radostits *et al*. (2007).

Preparation of samples (APHA, 2004)

For bacterial examination, the samples were prepared in accordance with APHA (2004), each sample was thoroughly mixed and divided into two subsamples: the first for chemical examination and the second for bacteriological examination.

Chemical examination of samples

Determination of pH value

pH value was recorded by using the pH meter (Hanna Instrument, modelHI8471) Italy.

Storch's test for heat-treated milk detection (IDFA, 2006)

A test tube containing 5 ml of milk and tested using litmus paper. If the milk's action is not nearly neutral, the pH was corrected with 0.1N HCL or 1% NaOH, followed by the addition of one drop of 0.2% H_2O_2 and two drops of paraphenylene-diamine. Mixed firmly and inspected. Blue color was positive result.

Bacteriological evaluation of raw milk samples

Isolation of *B. cereus* (Fricker *et al*., 2008)

Previously prepared milk samples were streaked over agar made of polymyxin pyruvate, egg yolk mannitol, and bromothymol blue (PEMBA), a modified selective agar supplemented with polymyxin B (50.000 IU/500 ml medium) a selective antibiotic and egg yolk emulsion (25ml /500 ml medium) for detecting lecithinase activity of *B. cereus*. The Bromthymol blue is a pH indicator. The typical developed colonies were grey to turquoise blue, and the color turns to a peacock blue color after 48 h.

Identification of *B. cereus*

Morphological characteristics of Gram-stained suspected colonies were inspected for the presence of Gram-positive rod using an oil emersion microscope lens (Tallent *et al*., 2012). Pure cultures of the isolate were identified biochemically according to Roberts and Greenwood (2003).

Confirmation was done by using Vitek 2 compact system according to VITEK2 product information, document 510769-4EN1. bioMerieux. Inc., Durham, NC.

Molecular identification of toxigenic strains of B. cereus

DNA extraction

DNA extraction from samples was performed using the QIAamp DNA Mini kit (Qiagen, Germany, GmbH) with modifications from the manufacturer's recommendations. Briefly, 200 µl of the sample suspension was incubated with 10 µl of proteinase K and 200 µl of lysis buffer at 56°C for 10 min. After incubation, 200 µl of 100% ethanol was added to the lysate. The sample was then washed and centrifuged following the manufacturer's recommendations. Nucleic acid was eluted with 100 µl of elution buffer provided in the kit.

Oligonucleotide Primer

Primers used were supplied from Metabion (Germany) are listed in Table 1.

PCR amplification

Primers were utilized in a 25- µl reaction containing 12.5 µl of EmeraldAmp Max PCR Master Mix (Takara, Japan), 1 µl of each primer of 20 pmol concentrations, 5.5 µl of water, and 5 µl of DNA template. The reaction was performed in an applied biosystem 2720 thermal cycler.

Analysis of the PCR Products

The products of PCR were separated by electrophoresis on 1.5% agarose gel (Applichem, Germany, GmbH) in 1x TBE buffer at room temperature using gradients of 5V/cm. For gel analysis, 15 µl of the products was loaded in each gel slot. A gelpilot 100 bp plus ladder (Qiagen, Gmbh, Germany) was used to determine the fragment sizes. The gel was photographed by a gel documentation system (Alpha Innotech, Biometra) and the data was analyzed through computer software.

Table 1. Primers sequences, target genes, amplicon sizes and cycling conditions.

Target gene	Primers sequences	Amplified segment (bp)	Primary denaturation	Amplification (35 cycles)			Final extension	Reference
				Secondary denaturation	Annealing	Extension		
HblA	GTA AAT TAI GAT GAI CAA TTTC AGA ATA GGC ATT CAT AGA TT	1091	94˚C 5 min.	94˚C 30 sec.	49˚C 40 sec.	72˚C 1 min.	72˚C 10 min.	Ehling-Schulzet *al*. (2006)
Ces	GGTGACACATTATCATATAAGGTG GTAAGCGAACCTGTCTGTAACAACA	1271	94˚C 5 min.	94˚C 30 sec.	49˚C 40 sec.	72˚C 1 min.	72˚C 10 min.	

Determination of proteolytic activity of B. cereus using skim milk agar

Overnight cultures were spot inoculated onto skim milk agar plate count agar (Himedia Laboratories Ltd.) supplemented with 1% skimmed milk powder (Beerens and Luquet, 1990). The inoculated plates were incubated at 30°C for 72 h and 7°C for 10 days, respectively. The presence of transparent zones around the spots was recorded as positive that referring to protease production.

Determination of lipolytic activity of B. cereus using tributyrin agar

Lipolytic activities (LP) were determined using plate count agar (Himedia Laboratories Ltd.) supplemented with1% tributyrin (Sigma-Aldrich Co.) (Beerens and Luquet, 1990). Plates were incubated at 30 and 7°C for 72 h and 10 days, respectively. The presence of a clean zone around each colony served as an indicator for lipolytic activity.

Antimicrobial sensitivity testing

Antimicrobial sensitivity testing for isolated bacteria was performed using the disk diffusion method according to CLSI (2016).

RESULTS AND DISCUSSION

Raw milk is regarded as a favorable environment for growth and proliferation of algae, fungus, bacteria, and viruses as it contains the most essential nutrition components (Banaei and Mahdavi, 2020). It is generally recognized that pH is one of the most crucial criteria utilized to govern bacterial proliferation. The pH of fresh cow milk ranges from 6.5 to 6.7. Mastitic milk is indicated by values greater than 6.7, and presence of colostrum or bacterial degradation is indicated by values less than 6.5. Buffering capacity of milk making pH changes occurred due to development of considerable acids, bacterial activity usually causes this (O'Connor, 1995).

When the pH is between 6.0 and 6.5, luxuriant growth of bacteria can take place at +25°C. Hence, milk is an ideal environment for bacterial growth, and the population can double every 30 minutes. (Morandi *et al*., 2005). Table 2 shows the mean pH values of raw milk from different sources farmers, street vendors, and dairy shops and, supermarkets, which were recorded as 6.54±0.04, 6.48±0.06, 6.44±0.05, 6.53±0.06 and 6.49±0.03, respectively. Statistically, there were no significant difference in pH values in respect to the source of samples.

In this investigation, the average pH levels were all within the range of physiological variation as defined by Walstra *et al*. (2006). Many factors, including feeding and lactation stage, can be used to explain why milk from diverse sources had a pH value of 6.98, but it is also possible that this value is a sign of manually added water. Similar pH values were discovered by many authors, including Sraïri *et al*. (2006), who added that the pH of milk is not always increased by the addition of water. Minimum pH value was 5.8 in milk samples at the level of different sources in this study which referred to bacterial deterioration (O'Connor, 1995).

Table 2. Determination of pH value of examined raw milk samples.

Source of milk	No. of samples	pH of examined sample		
		Min.	Max.	Mean±SE
Farmers	25	6.1	6.8	6.54±0.04
Supermarkets	25	5.8	6.9	6.48±0.06
Street vendors	25	5.8	6.8	6.44±0.05
Dairy shops	25	5.9	6.8	6.53±0.06
Total	100	5.8	6.9	6.49±0.03

Table 3. Detection of heat treated milk samples by Storch's test

Source of milk	No. of samples	Positive samples	
		No.	%
Farmers	25	3	12
Supermarkets	25	2	8
Street vendors	25	10	40
Dairy shops	25	4	16
Total	100	19	19

Table 4. *Bacillus cereus* biochemical pattern by Vitek 2.

Biochemical pattern																
1	BXYL	-	3	LysA	-	4	AspA	-	5	LeuA	+	7	PheA	+	8	ProA	-
9	BGAL	-	10	PyrA	+	11	AGAL	-	12	AlaA	+	13	TyrA	-	14	BNAG	+
15	APPA	+	18	CDEX	-	19	dGAL	-	21	GLYG	-	22	INO	-	24	MdG	-
25	ELLM	-	26	MdX	-	27	AMAN	-	29	MTE	+	30	GlyA	-	31	dMAN	-
32	dMNE	-	34	dMLZ	-	36	NAG	+	37	PLE	-	39	IRHA	-	41	BGLU	-
43	BMAN	-	44	PHC	-	45	PVATE	-	46	AGLU	-	47	dTAG	-	48	dTRE	+
50	INU	-	53	dGLU	+	54	dRIB	+	56	PSCNa	-	58	NaCl 6.5%	+	59	KAN	-
60	OLD	-	61	ESC	+	62	TTZ	-	63	POLYB_R	+						

Regrettably, uncontrolled marketing techniques have made it difficult for consumers to preserve the quality of milk (Javaid *et al*., 2009). To lessen the financial losses caused by milk spoiling during transportation and sale, some prohibited procedures are used to increase the shelf life of milk. Table 3clarified that 3(12%) out of 25 samples collected from farmers, 2(8%) out of 25 samples collected from Supermarkets, 10 (40%) out of 25 samples collected from Street vendors and 4 (16%) out of 25 samples collected from Dairy shops, respectively were heat treated.

Results included in Table 3 almost identical to those described by Shaker *et al*. (2015) while WAFY (2019) and Shinawy *et al*. (2018) were unable to identify the thermal treatment in raw milk. Thermal treatment is a typical kind of adulteration since it hides the unhygienic circumstances in which milk is obtained (Draaiyer *et al*., 2009). Furthermore, undesirable smells and protein denaturation could be bad sequels. It was revealed that while milk from a healthy cow is of high hygiene, it is frequently contaminated after milking, making milk of poor keeping quality (Millogo *et al*., 2010). Conditions at which milk is critically contaminated are poor hygienic practices in udder preparation for milking, improper personal hygiene of milk handlers, and low hygienic practices associated with milking and storage equipment. Market milk is usually collected from small farmers so, it usually subjected to different ways of cross contamination as mixing fresh clean milk with mastitic milk, unclean utensils and unhygienic water supply used in milk production cycle (Megersa *et al*., 2019).

Bacillus cereus is one of the microorganisms that has generated issues for the food sector, either by product degradation or by posing a health risk to the public (Ghelardi *et al*., 2002). It produces two separate forms of food poisoning, the diarrheal form of which is linked to a number of different foods (Jessberger *et al*., 2020) identical to food poisoning caused by Clostridium perfringens type A and marked by watery diarrhoea and abdominal cramps; symptoms began after 8–18 h. and persistent for 12-24 h when the contaminated food was ingested such as proteinaceous food ,pudding, vegetables, sauces, milk and milk products (Stenfors Arnesen *et al*., 2008) with infective dose of 105-108 CFU/g or ml of food, vegetative cells secrete the enterotoxin(s): Hbl, Nhe and Cyt K in the small intestine in the late growth phase (Clavel *et al*., 2004) while the emetic form is characterized by rapid onset after 0.5-6 h and persist for 6-24 h,Vomition, nausea, and malaise with possibly of liver damage (fewer cases) occur due to intoxication from foods contaminated with emetic toxin (cereulide) (Logan, 2012).

Heavy contamination of dairy product can lead to the production of heat-stable toxins (emetic toxin) also, many isolates from cheese and milk are proteolytic and lipolytic and characterized by biofilm formation (Kohneshahri *et al*., 2016). Certain *B. cereus* strains are more dangerous due to their capacity to produce toxins as well as adapt to cold, chemical, and hot environments (Tirloni *et al*., 2022).

Based on the recognized biochemical techniques, the Vitek2 compact system (VITEK2 product information, document 510769-4EN1. bioMe'rieux. Inc., Durham, NC.) uses Gram negative cards to confirm the identification. where newly developed substrates measuring carbon source utilization resistance and enzymatic activities were employed. There are 47 separate biochemical tests on the identity card. Results only be available after almost 10 hours (Table 4).

Table 5 pointed out the incidence of *B. cereus* which was 10% of the total examined raw milk samples. The incidence of *Bacillus cereus* in raw milk collected from farmers, street vendors, dairy shops and supermarkets were 0(0%), 5(20%), 2(8%) and 3(12%), respectively. Statistically, P value was greater than 0.05 so, there was no significant difference in the incidence of *B. cereus* in relation to the source of raw milk samples.

The incidence of *B. cereus* was 10% of total examined raw milk samples (Table 5), this is in similarity with Yıbar *et al*. (2017) who reported 10.04% *B. cereus* incidence from raw milk meanwhile a lower result 8.2% was achieved by Tatsinkou *et al*. (2017). Moreover, higher incidence of 27%, 23.33%, 97.5% and 52% was detected by Yobouet *et al*. (2014); Elbassiony *et al*. (2021); Banaei

Table 5. Prevalence of *Bacillus cereus* in raw milk samples.

Source of milk	No. of examined samples	Positive samples	
		No.	%
Farmers	25	0	0
Supermarkets	25	5	20
Street vendors	25	2	8
Dairy shops	25	3	12
Total	100	10	10

Table 6. Molecular characterization of toxigenic genes of *B. cereus*

Total No. of isolates	Positive samples for *ces* gene*		Positive samples for *hblA* gene**		Negative for both genes	
	No.	%	No.	%	No.	%
10	2	20	3	30	6	60

Table 7. Assessment of growth, Proteolytic activity and Lipolytic activity of *Bacillus cereus* strains obtained from raw milk at different temperatures.

Total no. of isolates	Characteristics	30°C/48hrs		7°C/10d	
		No.	%	No.	%
10	Growth	10	100.0*	4	40
	Proteolytic activity	10	100	4	40
	Lipolytic activity	2	20	2	20
	Both enzymatic activities	2	20	2	20

and Mahdavi (2020) and Osama *et al.* (2020) from raw milk, respectively.

Bacillus is a contaminant of raw milk from external environment that related to milk production as soil, feed and bedding considered a potential source of contamination (Yobouet *et al.*, 2014). Our study revealed that 90% of examined samples showed negative growth for *Bacillus cereus*, this may be referred to the good practical hygiene and in general contamination of raw milk with *Bacillus cereus* could be avoided (Organji *et al.*, 2015). Noteworthy is that raw milk must be free of pathogenic microorganisms and the toxins which secreted by it, as stated by the Egyptian Standard (2014); therefore, none of the positive samples in present investigation met Egyptian standards.

B. cereus is nowadays the subject of growing public interest due to its capacity to create a variety of enterotoxins (Schoeni and Wong, 2005). Even if microorganism is found in low concentrations or the sample is infected with other microbes, PCR analysis is created as a quick and accurate way to identify the prevalence of organisms (Molva al., 2009). Table 6, and Fig.1 demonstrate that 10 *Bacillus cereus* isolates from raw milk had been checked for the presence of gene encoded for emetic toxin (*ces*), and molecular analysis revealed that 2 isolates (20%) were positive for emetic gene. Also, Table 6 and Fig. 2 show the positive strains for diarrhoea toxin (*hblA*) that were present in 3 (30%) isolates and only one gene (10%) was positive for both emetic and diarrhoeal toxins. Moreover, 6 (60%) isolates were negative for both genes.

Regarding results obtained in Table 6 and Figs. 1, 2, PCR analysis showed evidence of *B. cereus* DNA in 4 out of 10 isolates using *B. cereus* 1271 bp primers (Fig. 1) and 1091bp (Fig. 2). These outcomes closely resembled Aman *et al.* (2021) who discovered that 25% of *B. cereus* isolates had *ces* and 29.2% had *hblA* in *B. cereus* isolates from raw milk. Jovanovic *et al.* (2022) found *ces* gene in 23.2% in *B. cereus* isolates. On the other hand, our results didn't agree with Osama (2016) who found that all *B. cereus* strains were emetic and not harbored the diarrhoea gene. Hefny *et al.* (2020) found that all *B. cereus* strains did not harbor *ces* gene. Higher results obtained by Banaei and Mahdavi (2020) as the frequency of *hblA* gene found in *B. cereus* isolates was 89.7%.

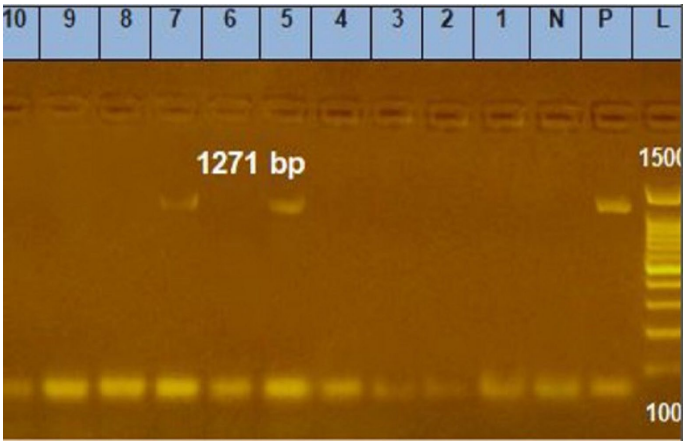

Fig. 1. Agarose gel electrophoresis of PCR of *ces* gene (176 bp) for characterization of *B. cereus*. Lane M: 100bp ladder as molecular size DNA marker. Lane C+: Control positive *B. cereus* for *ces* gene. Lane C-: Control negative. Lanes 5, 7: Positive *B. cereus* strains for *ces* gene. Lanes1, 2, 3, 4, 6, 8, 9 and 10: Negative *B. cereus* strains for *ces* gene.

In present study, isolates of *Bacillus cereus* strains that do not possess *hblA* gene may harbored other *hbl* genes (*hblb, hblc* and *hbld*), molecular characterization of *B. cereus* has revealed the presence of genes encoding at least one of the known diarrhoea toxins (Guinebretière *et al.*, 2010). *Bacillus cereus* hemolytic activity on blood agar was correlated with harboring hbl genes (diarrheal genes), this could be revealed by Merzougui *et al.* (2013) studies on *Bacillus cereus* isolates, 15 of these strains not showed hemolytic activity and didn't harbor any *hbl* genes. In the present study, all isolates were hemolytic. Therefore, negative isolates for *hblA* gene might not have any of other *hbl* types.

Typically, *Bacillus* spp. can create intracellular or extracellular enzymes that can withstand heat like lipases, proteases and phospholipases which can remain active in the milk even after the death of the microbes that generate them and induce a variety of problems in milk and dairy products, including off-flavor,

Table 8. findings of Antibiotic sensitivity test for *Bacillus cereus* isolates.

Antimicrobial agent (µg/ml)	*Bacillus cereus*n (n.= 10)		
	Sensitive	Resistant	Intermediate
Aztreonam (30 µg)		100%	
Ampicillin (10 µg)		100%	
Oxacillin (1µg)		100%	
Cephalothin (30 µg)		100%	
Erythromycin (15 µg)		100%	
Clarithromycin (30 µg)		100%	
Neomycin (30 µg)	100%		
Amikacin (30 µg)	100%		
Penicillin (10 IU)		100%	
Enrofloxacin (5 µg)	100%		
Amoxicillin+ clavulanic acid (30 µg)		100%	
Norfloxacin (10 µg)	100%		
Gentamycin (10 µg)			100%
Ofloxacin (30 µg)	100%		
Streptomycin (30 µg)	100%		

bitty or broken cream, flat sour, sweat curdling, bitterness, cheese blowing and ropiness (Samaržija *et al*., 2012). This contamination also causes economic loss through spoilage of the contaminated products.

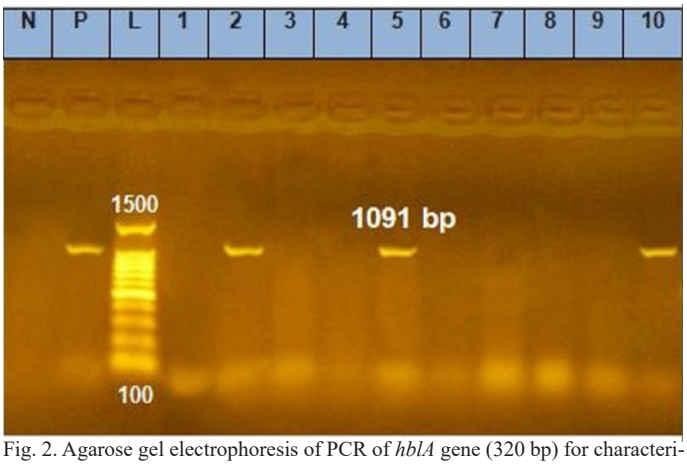

Fig. 2. Agarose gel electrophoresis of PCR of *hblA* gene (320 bp) for characterization of *B. cereus*. Lane M: 100 bp ladder as molecular size DNA marker. Lane C+: Control positive *B. cereus* for *hblA* gene. Lane C-: Control negative. Lanes 2, 5 and 10: Positive *B. cereus* strains for *hblA* gene. Lanes 1, 3, 4, 6, 7, 8 and 9: Negative *B. cereus* strains for *hblA* gene.

Table 7 demonstrates the growth of isolates at 30°C/48h and 7°C/10d and shows that all the 10 (100%) isolates were mesophilic, and 4(40%) isolates were psychrophilic. Also, lipolytic and proteolytic activities of *Bacillus cereus* strains (10 isolates) from raw milk at 30°C/48h was 100%, 20% and 20% for proteolytic, lipolytic and both enzymatic activities, respectively. At 7°C/10d was 40%, 20% and 20% for proteolytic, lipolytic and both enzymatic activities, respectively. Our outcomes nearly identical to Wijnands *et al*. (2006) who determined 4.4% of *B. cereus* isolates were psychrotrophic while most of them were mesophilic. And unlike to Montanhini *et al*. (2013) and Mohamed *et al*. (2016) who displayed that none of *Bacillus cereus* strains isolated during their studies were psychotropic. For proteolytic activity at 30°C/ 48 hours, our results were supported by those of Montanhini *et al*. (2013) who discovered that the entire isolates of *B. cereus* exhibited proteolytic activity at 30°C for 48 hours. And disagree with Montanhini *et al*. (2013) who found 5 strains (33%) also had lipolytic activity and Five strains of *B. cereus* (33%) had both lipolytic and proteolytic activities at 30°C for 48 hours. Tatsinkou *et al*. (2017) indicated that all the *B. cereus* strains were isolated from milk and milk products had lipolytic and proteolytic behavior, which suggests the possibility for spoiling. *B. cereus* is commonly regarded as a mesophilic bacterium, which has ideal temperature range for growth between 35 and 40°C . But increasingly, psychrotrophic *B. cereus* strains are being identified that can thrive in the refrigerator and are the cause of the microbial deterioration of foods that are kept there (Pretorius and Buys, 2021). Kohneshahri *et al*. (2016) reported that mesophilic strains of *B. cereus* have a high pathogenicity potential than psychrotrophic *B. cereus* strains as when psychrophilic strains of *B. cereus* contaminated refrigerated foods, only mild emesis caused, and their sanitary risk was low.

The emergence of antimicrobial resistance among foodborne bacteria has received considerable attention in recent years. Regarding the antibiotics resistance mentioned in Table 8, All the *B. cereus* isolates (10 isolates) were resistant to Aztreonam, ampicillin, Oxacillin, Cephalothin, Erythromycin, Clarithromycin, Penicillin and Amoxacillin+ clavulanic acid. On the other hand, *B. cereus* isolates were susceptible to Neomycin, Amikacin, Enrofloxacin, Norfloxacin, Ofloxacin and Streptomycin. Whereas moderate resistance was exhibited to gentamicin. These findings are agreed with Osama *et al*. (2020) and Kim *et al*. (2015) who stated that the entire *B. cereus* strains were β- lactam resistant. Which contains Penicillin, Amoxicillin and Ampicillin. Because of this, using β-lactam is useless for treating *B. cereus* infections, although Norfloxacin and Ciprofloxacin may be useful. The high resistance against cephalothin reported here agreed with Aman *et al*. (2021) and Yıbar *et al*. (2017). Furthermore, although *B. cereus* isolates detected in the current research are known to have a strong resistance against β-lactamase antibiotics including penicillin and oxacillin, in contrast Aman *et al*. (2021) reported only a moderate susceptibility to β-lactam antibiotics. It is possible that such alterations in antibiotic resistance are caused by differences in isolate sources, antibiotics used, and drug-resistance transmission.

CONCLUSION

The present study provided a comprehensive overview of *B. cereus* prevalence in raw milk, which is an obvious health hazard. Furthermore, enterotoxigenic genes identified in this study pose a significant threat to public health by increasing the risk of foodborne illnesses. Small-scale farmers need to be educated on good milk-processing practices. There is evidence that a thermal treatment lasting 100°C for 0.5–5 minutes is a sufficient combination between temperature and time for considerable lessening of *B. cereus* bacteria in raw milk. In order to minimize contamination of milk and dairy yield, it is advised to apply raw milk of high quality for manufacturing dairy products, to clean and disinfect equipment, to employ healthy individuals with valid health licenses. and to maintain proper sanitation in dairy industries. Furthermore, most isolates have variable resistance to most antibiotics according to antimicrobial susceptibility results. Hence, it is essential to justify the use of antimicrobials and regularly monitor the antibiotic susceptibilities of *Bacillus cereus* to minimize the risk of human exposure to antimicrobial resistant pathogens.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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