Journal of Medical Bacteriology

Molecular Detection of *bla*$_{TEM}$, *bla*$_{CTX-M}$ and *bla*$_{SHV}$ Beta-Lactamase Genes in *Bacillus cereus* Strains Isolated from Infant Dry Milk Samples

Ciamak Ghazaei

Department of Microbiology, University of Mohaghegh Ardabili, Ardabil, Iran.

ARTICLE INFO

Article type:
Research Article

Article history:
Received: 20 Sep 2020
Revised: 13 Dec 2020
Accepted: 27 Apr 2021
Published: 23 Jun 2021

Keywords: *Antibiotic susceptibility, B. cereus, Metallo-beta-lactamase, Milk, Infants.*

ABSTRACT

Background: *Bacillus cereus* is one of the important pathogen, which can be found in food samples like milk and principally responsible for food poisoning. Metallo-beta-lactamase (MBL) genes present in the *Bacillus cereus* bacterium, which provide resistance to the bacteria against the extreme condition. The objective of this study is to carry out molecular detection of *bla*TEM, *bla*$_{CTX-M}$ and *bla*$_{SHV}$ MBL genes in *B. cereus* strains, isolated from infant dry milk samples.

Methods: Total 50 samples of infant dry milk were collected from the drug store, and 19 samples were selected for this investigation. After morphological and biochemical characterization of suspected colonies which are obtained from infant dry milk samples, these isolates were confirmed for *B. cereus*. Antibiotic susceptibility tests were done as per criteria of Clinical and Laboratory Standards Institute (CLSI). The phenotypic confirmatory analysis was done in Mueller Hinton agar (MHA) plates with clavulanic acid. If inhibition diameter is ≥5 mm increases in the clavulanic acid (CA) containing plate than to a plate without CA, it confirmed the presence of MBL genes. PCR used for detection of MBL genes in the isolated strains.

Results: PCR detected the *bla*$_{CTX-M}$ (100%), *bla*$_{SHV}$ (4%) and *bla*$_{TEM}$ (84.2%) gene of *B. cereus* in the infant dry milk.

Conclusion: The study confirms that the infant dry milk is a good source of *B. cereus*, if dry milk has absorbed water content from the air and hence providing a perfect condition for the growth of the bacterium, so It should be kept airtight the dry milk and stored in the cold condition.

- ***Please cite this paper as:***. Ghazaei C. Molecular Detection of *bla*$_{TEM}$, *bla*$_{CTX-M}$ and *bla*-$_{SHV}$ Beta-Lactamase Genes in *Bacillus cereus* Strains Isolated from Infant Dry Milk Samples. *J Med Bacteriol.* 2021; **10** (1, 2): pp.1-10.

*Corresponding Authors: Ghazaei Ciamak, Department of Microbiology, University of Mohaghegh Ardabili, Ardabil, Iran, P.O. Box 179.

Tel: +98 045-33512081-9, *E-mail:* ciamakghazaei@yahoo.com

Introduction

Infant foods are the primary source of nutrition for infants before they can digest other types of food (1). Today, millions of infants of first two years get their nourishment from powdered milk (2). As per the World Health Organization (WHO), *B. cereus* is the most common foodborne bacterium found in the pasteurized food products (1). *B. cereus* frequently contaminated dried milk products or baby food (3). Moreover, powdered forms of milk can be used for a long time, because of low water content that prevents the growth of bacteria. But sometimes impaired packaging permit to absorb water and facilitate the growth of bacteria that remain even after heat treatment and pasteurization.

B. cereus is a Gram-positive, rod-shaped, motile, facultative anaerobe, spore-forming bacterium, commonly present in the soil (4). *B. cereus* forms endospores that make it resistant to extreme high condition and responsible to cause diarrhoea, emesis and other serious complications (1). It belongs to the Bacillaceae family that makes extended-spectrum β-lactamase (ESBL), which play an essential role in developing the infection (5,6). Resistance in the *Bacillus* is directly proportional to the numbers of ESBLs created by bacteria during infection.

The *B. cereus* shows resistance against the extreme condition, mainly due to the expression of *bla*-$_{TEM}$, *bla*-$_{SHV}$, and *bla* $_{CTX-M}$ genes. These genes are responsible for the production of β-lactamases. The BLA genes also encode antibiotic resistance. Therefore, bacteria belonging to the Enterobacteriaceae family mainly show increasing resistance against antibiotics due to the presence of these genes (7). Various studies investigated the *B. cereus* genes in the baby food products, but no study detected the Metallo-beta-lactamase genes in the infant dry milk. Since, infants have less developed immune system and hence they are prone to foodborne microbial infections, which are caused due to pathogenic antibiotic-resistant strains that are present in infant formula products. Thus, it is important to scrutinize the safety of infant powdered milk products (6). Therefore, the objective of this investigation study focused on molecular detection of *bla*-$_{TEM}$, *bla* $_{CTX-M}$ and *bla*-$_{SHV}$ Metallo-beta-lactamase genes of *B. cereus* strains from infant dry milk samples.

Materials and Methods

Preparation of the Samples

Total 50 infant dry milk samples were collected from drugstores in Ardabil city, Iran. Each stock sample was prepared by using 90 ml of 0.1% peptone water to dilute 10g of dry milk sample. Then the sample was homogenized through vigorous vortexing for three minutes at room temperature.

Isolation and Identification of B. cereus

Morphological Characterization

0.1 ml of diluted milk samples were spread with the help of spreader on the Mannitol egg Yolk Polymyxin Agar medium (MYP Agar; HiMedia, Laboratories) plates. Then, these plates were incubated for 24-48 hours, at the temperature of 30 °C. Large pink colonies with a sedimentary halo were observed, which were considered as suspicious colonies of *B. cereus* since the zone of precipitation indicates the production of lecithinase (8). After identification of colony morphology, these presumptive colonies of *B. cereus* were tested for bacterial cell morphology by using Gram's staining technique and further biochemical characterization were performed.

Biochemical characterization

Biochemical tests such as catalase test, anaerobic conditions for the growth of bacteria, bacterial growth under 45 °C, lecithinase C test, methyl red Voges-Proskauer (MR-VP) test, haemolysis appeared in agar with 5% sheep blood

(9,10), sensitivity to penicillin 10 IU and nitrate reduction were used for identification of *B. cereus* strains in isolated colonies (10,11). The catalase test can help in the detection of the catalase enzyme producing bacteria (12,13). *Bacillus cereus* bacteria are capable of growing in anaerobic conditions and under the temperature of 45 °C (5, 14-16). For lecithinase test bacteria were allowed to grow on *B. cereus*-selective MYP agar and incubated for 24 h at 30 °C to observe the growth on MYP medium (10).

With the positive Voges–Proskauer reaction by carrying out MR-VP test, *B. cereus* can be confirmed (10,16). For hemolysin activity testing, incubation of *B. cereus* isolates was done on Tryptic Soy Agar (TSA) containing 5% of sheep blood, for 24 hrs at 36 °C. After incubation, the formation of hemolytic zones was checked (17). To test nitrate to nitrite reduction reaction by suspicious *B. cereus* isolates, 3mm loopful from each culture was inoculated in 5 ml of nitrate broths and further incubated for 48 hrs at 35 °C. Later, to assess the nitrite production in the culture inoculated medium, 0.25 ml of nitrite test reagents A and C were added to each culture (10).

Antibiotic susceptibility testing

Antibacterial susceptibility testing was performed using the agar disk diffusion method as per recommendations of the Clinical and Laboratory Standards Institute (CLSI) (18). The antibacterial disks (HiMedia, India) were tested for antibiotics: imipenem (IMP: 10µg), ciprofloxacin (CIPRO: 5µg), gentamicin (GM: 10µg), cefotaxime (CTX: 30µg), ceftazidime (CAZ: 30µg), ceftizoxime (ZOX: 30µg), piperacillin (PIP: 100µg), clindamycin (CLI: 10µg), vancomycin (VAN: 30µg), oxacillin (OX: 5µg), and ampicillin (AMP: 10µg; Table 1).

Phenotypic detection of Extended-spectrum ß-lactamase (ESBLs)

Prescreening test for ESBLs

Prescreening test was the part of antibiotic susceptibility testing, which was done according to CLSI criteria (18). Two Mueller Hinton agar (MHA) plates were used with CAZ: 30µg and cefotaxime (CTX: 30µg). An inhibition zone was ≤18mm observed for both ceftazidime and cefotaxime, indicated that the isolated strain produced beta-lactamase genes.

Confirmatory test for phenotypic detection of ESBLs

The ESBL production was tested for phenotypically screening of β-lactamases from the isolated colonies as per the CLSI criteria (18). For doing this, we inoculated standard inoculum (0.5 McFarland) of the test isolate into the plates containing Mueller Hinton agar (MHA). It was tested for CAZ: 30µg and CAZ: 30µg with clavulanic acid (10µg). ESBL producer was confirmed if the inhibition zone diameter ≥5 mm increases in the clavulanic acid containing plate than to a plate without clavulanic acid.

DNA Extraction

In this study, we have used boiled method for deoxyribonucleic acid (DNA) extraction from the *B. cereus* colonies, which was used as templates for Polymerase chain reaction (PCR). A sterile toothpick was used to pick the isolated colony, inoculated into the 100 µl sterilized water and boiled it for 15 minutes at 95 °C to lyse the cells completely. Then, it centrifuged (11000 g, 10 s) for removing the cell debris. Transparent supernatant contained genomic DNA, which was stored at −20 °C (1,19).

PCR detection for Beta-lactamases genes

In this study, we used the primer sets for testing the presence of β-lactamases genes in the isolated strains. The detail of the primer sets is presented

in Table 2 (2). PCR amplification was performed in a 50 µl reaction mixture. Reaction mixtures had 1µM each of the primers, 200µM deoxynucleoside triphosphate (dNTP), 1x reaction buffer containing 1.5mM $MgCl_2$, 2.5 U Taq DNA polymerase, and 25ng of genomic DNA as a template. Denaturation was performed as an initial step for 2 minutes at 94 °C, followed the 30 cycles of amplification which were performed as again denaturation for 1 min at 94 °C, annealing for 1 min at 55 °C, and DNA extension for 1.5 min at 72 °C. PCR products were analyzed in agarose gel electrophoresis (AGG), stained with the ethidium bromide for visualization under ultraviolet (UV) light (20).

Results

Isolation and identification of Bacillus cereus

Total 50 infant dry milk samples were collected from the drugstore and samples were assessed for *B. cereus* culture. Out of them, 19 samples (38%) were positive for *B. cereus*. A total of 7 (14%) dry milk samples had more than 100 CFU/g.

Morphological characterization

After incubation of diluted samples on Manitolegg Yolk Polymyxin Agar (MYP Agar; HiMedia, Laboratories) plates for 24-48 hrs at 30 °C, the large, circular, pink, and uniform colonies were observed with surrounding precipitation zones. Zones of precipitation indicated lecithinase enzyme production by the colonies and such colonies were suspected for *B. cereus*. These isolates were further tested for the confirmation of *B. cereus* (8). After Gram's staining, cellular morphology was observed microscopically and observed bacteria were Gram-positive, rod shaped, and observed in the chain as well as scattered forms.

Biochemical characterization

Further biochemical characterization all 19 presumptive isolates were confirmed for *B. cereus* bacteria. Results for all biochemical tests are explained in (Table 3).

Antibiotic susceptibility testing

The antibiotic susceptibility test was done by disc diffusion method. As observed, the highest rate of resistance was found 100% for ceftazidime and oxacillin and the lowest for ciprofloxacin (26.3%) and gentamicin (36.7%). The rest antibiotics included 89.5% resistance to cefotaxime, 84.2% to ceftizoxime, 94.7% to piperacillin, 78.9% to clindamycin, 36.8% to vancomycin, and 94.7% to ampicillin (Figure 1).

Inhibition zone diameter was ≤18mm observed for all antibiotics. The isolates were classified as resistant, sensitive or semi-sensitive based on the inhibition zone. As per confirmatory test inhibition zone diameter, ≥5 mm increased in the clavulanic acid-containing plates than to plates without clavulanic acid. It was found that a total of 19 (38%) samples were positive for MBL production.

Above Figure 2 shows the PCR amplification products of β-lactamase genes. The PCR procedure was done with 19 samples. The PCR successfully detected the $bla_{\text{CTX-M}}$, $bla_{\text{-SHV}}$ and bla_{TEM} gene of *B. cereus* in the infant dry milk. The result showed that 100% of samples carried the bla_{TEM} gene, 84.2% had the $bla_{\text{CTX-M}}$ gene and 4% harbored the $bla_{\text{-SHV}}$ genes (Figure 2).

Table 1. Antibiotics used in the study.

Antibiotics	Antibotics code	Quantity
Imipenem	IMP	10µg
Ciprofloxacin	CIPRO	5µg
Gentamicin	GM	10µg
Cefotaxime	CTX	30µg
Ceftazidime	CAZ	30µg
Ceftizoxime	ZOX	30µg
Piperacillin	PIP	100µg
Clindamycin	CLI	10µg
Vancomycin	VAN	30µg
Oxacillin	OX	5µg
Ampicillin	AMP	10µg

Table 2. Primer sequences used in this study to detect β-lactamases genes.

Primer Target	Primer Name	Sequence (5'-3')	Product size (bp)
TEM (bla_{TEM})	TEM-F	AGTGCTGCCATAACCATGAGTG	431
	TEM-R	CTGACTCCCC GTCGTGTAGATA	
SHV (bla_{SHV})	SHV-F	GATGAACGCTTTCCCATGATG	214
	SHV-R	CGCTGTTATCGCTCATGGTAA	
CTX-M (bla_{CTX-M})	CTX-M-F	ATGTGCAGYACCAGTAARGTKATGGC	593
	CTX-M-R	TGGGTRAARTARGTSACCAGAAYCAGCGG	

Table 3. Biochemical characterization of colonies for *B. cereus*.

Sr. No.	Biochemical test	Observations	Results
1)	Catalase test	Immediate effervescence due to breakdown of H_2O_2	+
2)	Lecithinase C test	Opaque Halo formation on media	+
3)	Methyl Red Voges-Proskauer	Pink colour indicated presence of acetylmethylcarbinol,	+
4)	Hemolysin	Hemolytic zone formation	+
5)	Nitrate reduction	Orange colour indicated the presence of nitrite	+
6)	Sensitivity to penicillin10(IU)	Resistant	
7)	Growth under anaerobic conditions	Growth was observed	+
8)	Growth below temp of 45^0 C	Growth was observed	+

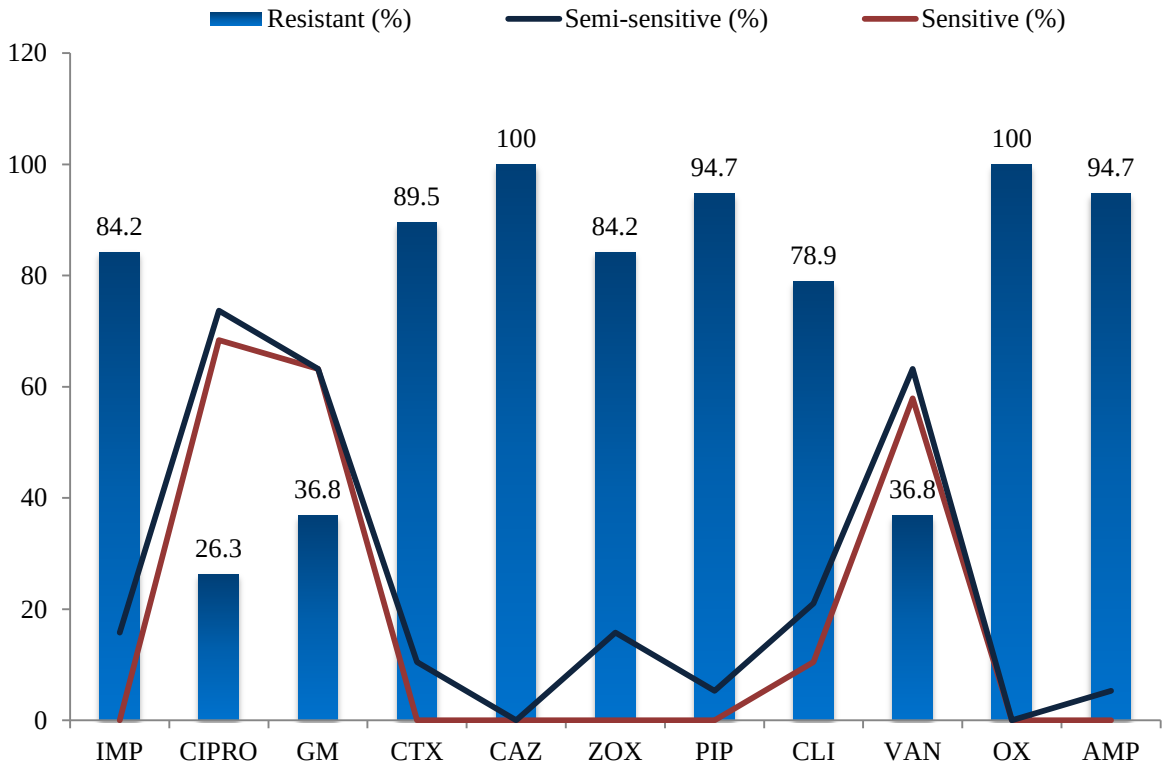

Figure 1. Antibiotic susceptibility results against *B. cereus* isolated from infant dry milk.

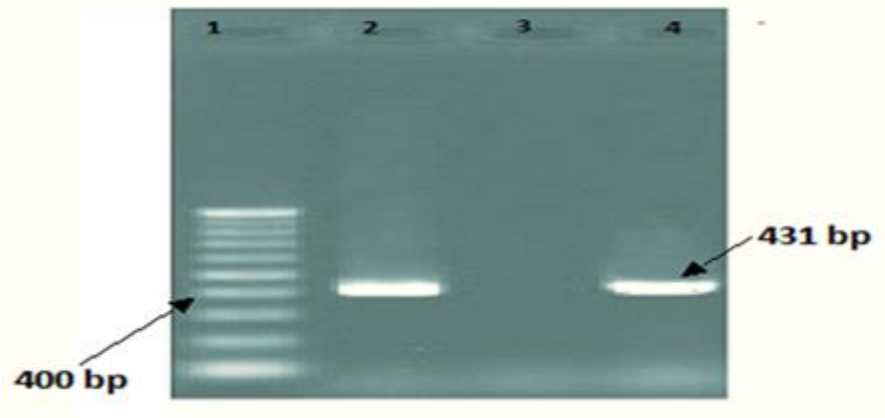

Figure 2. PCR amplification products of β-lactamase genes (bla_{TEM}). (Lane 1:100 bp DNA ladder; Lane 2:Positive control; Lane 3: Negative control; lane 4:TEM positive isolate).

Discussion

Foodborne diseases are the growing health issue in infants, worldwide. Therefore, various studies were conducted in the past for checking the *B. cereus* genes in baby foods. There were many studies conducted in the previous years to isolate and evaluate the prevalence of beta-lactamase gene of *B. cereus* in the food products, but very few studies have been studied for detection of beta-lactamase gene in infant dry milk (2, 6).

The most recent study was conducted by Rahimifard et al. to investigate *B. cereus* in 60 samples of infant formula. In this study, out of 60 samples, 11 contained >10 CFU/g and confirmed the presence of *B. cereus* in infant formula (21). In China, Gao et al. investigated the prevalence, antimicrobial susceptibility, genetic diversity and virulence genes of *B. cereus* in total 265 pasteurized milk samples (22, 23). In this study, high prevalence of *nheA* (99%), *nheB* (99%), *entFM* (96%), and *nheC* (94%) toxin-related genes of *B. cereus* were reported from pasteurized milk. Another study was conducted in Egypt by Sadek et al, reported that *hblA* (11.1%), *nheC* (71.1%) and *cytK* (95.5%) genes of *B. cereus* were detected in the milk-based infant food (2).

In Iran, Ranjbar et al. investigated 300 samples of baby milk-based foods. The study detected the prevalence of *nheA* (88.8 %), *nheC* (55.5 %) and *entFM* (55.5 %) enterotoxigenic genes were found in the *B. cereus* strains of milk-based infant food (24). In the same years, one more study was conducted in Ghana by Owusu-Kwarteng, et al, to investigate the 114 milk sample collected from local dairy farms. The study reported the presence of *cytK* (75%), *entFM* (67%), and *ceS* (9%) genes of *B. cereus* from dairy products (25). In Malaysia, Lesley et al. investigated the 32 samples of formula milk and ultra-high temperature (UHT) milk products. The study detected the presence of the *gyrB* gene of *B. cereus* in the formula milk and UHT milk products (26).

In Iran, Soltan Dallal et al. detected *B. cereus* strain in 125 samples of powdered infant milk. In this study, 53.63% *NHE* and 79% *EM*virulence genes were isolated from the powdered infant milk (27). In China, Cui et al. investigated that *B. cereus* gene in the 306 milk samples collected from dairy farms. The study detected the toxin genes *nhe* (100%), *hbl* (78.3%) and *ces* (1.1%) from the local dairy farms (28). In Hong Kong, Zhang et al. investigated the viable enterotoxin gene producing *B. cereus* in the ready-to-eat foods and infant milk powder. The study detected three *B. cereus* gene i.e. *cytK* (96%), *nheA* (97%), and *hblD* (97%) from the infant formula milk powder and ready-to-eat foods (29).

In Brazil, Reis et al. investigated the virulent gene of *B. cereus* in 260 samples of dairy products. The study also detected the *hblA* gene (41.3%), *hblC* gen (54%), and *hblD* gene (54%) from the 63 isolates of *B. cereus* from the milk and dairy products (30). In Italy, Di Pinto et al. investigated the enterotoxigenic *B. cereus* in the infant milk powder. All isolated strains had the genes of the *cytK*, *NHE*, and *HBL* enterotoxins (31). In Iran, Rahimi et al. investigated the enterotoxigenic genes of *B. cereus* in infant food. In this study, a total of 200 different infant foods collected and investigate the enterotoxigenic gene of *B. cereus* (1). The study reported *entFM* (61.90%) and *hblA* (13.09%) in the infant food and showed that kid's foods are the main source of virulent genes of *B. cereus*.

In Finland and Germany, Shaheen et al. investigated the production of *B. cereus* emetic toxin from infant food formulas (32). The study confirmed that heat-stable toxin present in the infant food formulas. In Germany, Becker et al. investigated *B. cereus* from the 265 infant foods & dried milk products samples. It is detected that 54% sample contaminated with *B. cereus* and positive samples (44%) had 0.3-10 *B. cereus*/g (3). In Scotland, Rowan et al. detected the growth of *B. cereus* and synthesis of diarrheal enterotoxin in the Infant Milk Formula (33).

Alanbar et al (2019) analysed different powdered infant formula products and their study revealed that there are presence of mono-antibiotic and multi-antibiotic resistant *Bacillus cereus* along with other *Bacillus* strains. Hence, they supported that *Bacilllus* strains are capable of producing Extended-spectrum beta-lactamase (ESBLs), since *Bacillus* strains from their study showed resistance against even third and fourth generations of antibiotic like; cephalosporins (ceftazidime and cefepime, respectively) (6).

Conclusion

In conclusion, MBL genes producing *B. cereus* detected in the infant dry milk. That means, *B. cereus* was not properly eradicated from the dry milk and might be contaminated during cross-contamination process, using contaminated raw milk, mishandling or poor packing. Considering that infants are the primary consumers of the dry milk, it is important to detect the antibiotic resistance genes like; bla_{TEM} gene (100%), $bla_{\text{CTX-M}}$ (84.2%) and bla_{SHV} genes (4%) of *B. cereus*, which are responsible for causing more severe foodborne diseases, by encoding such enzymes that can hydrolyse nearly all β-lactam antibiotics (34). Therefore, it is suggested that constant surveillance of infant dry milk is important, to reduce the risk of *B. cereus*; causing outbreaks. Besides this, preventive measurement studies also need to implement effective prevention against such bacterial strains. Such bacterial strains acquire MBL genes either by transformation and conjugation to become an opportunistic pathogens, because of their exposure to the selective pressure of antibiotics. Hence, such studies can help in preventing foodborne outbreaks in the future.

Acknowledgment

The authors would like to thank all those who participated in this study and the central patobiology laboratory.

Funding information

Not Declared.

Ethics approval and consent to participate

Not applicable.

Conflict of interest

There is no conflict of interest

References

1. Rahimi E, Abdos F, Momtaz H, et al. *Bacillus cereus* in infant foods: prevalence study and distribution of enterotoxigenic virulence factors in Isfahan Province, Iran. *Sci world J* 2013; **2013**:1-5.
2. Sadek ZI, Abdel-Rahman MA, Azab MS, et al. Microbiological evaluation of infant foods quality and molecular detection of *Bacillus cereus* toxins relating genes. *Toxicol reports* 2018; **5**: 871-77.
3. Becker H, Schaller G, von Wiese W, et al. *Bacillus cereus* in infant foods and dried milk products. *Int J food microbial* 1994; **23**(1):1-5.
4. Senesi S, Ghelardi E. Production, secretion and biological activity of *Bacillus cereus* enterotoxins. *Toxins* 2010; **2**(7):1690-703.
5. Bottone EJ. *Bacillus cereus*, a volatile human pathogen. *Clin Microbiol Rev* 2010; **23**(2):382-98.
6. Alanber MN, Alharbi NS, Khaled JM, et al. Evaluation of multidrug-resistant *Bacillus* strains causing public health risks in powdered infant milk formulas. *J Infect public Health* 2019; 1-7.
7. Ojdana D, Sacha P, Wieczorek P, et al. The occurrence of *blaCTX-M*, *blaSHV*, and bla_{TEM} genes in extended-Spectrum β-lactamase-positive strains of *Klebsiella pneumoniae*, *Escherichia coli*, and *Proteus mirabilis* in Poland. *Int J Antibiot* 2014; **2014**; 935842.
8. Tallent SM, Kotewicz KM, Strain EA, et al.

Efficient isolation and identification of *Bacillus cereus* group. *J AOAC Int* 2012; **95**(2): 446-51.
9. Banykó J, Vyletělová M. Determining the source of *Bacillus cereus* and *B. licheniformis* isolated from raw milk, pasteurized milk and yoghurt. *Lett Appl Microbiol* 2009; **48**:318-23.
10. Tallent S, Rhodehamel E., Harmon, S. et al. *Bacillus cereus*, FDA bacteriological analytical manual online, https://www. fda.gov/food/laboratory-methods-food /bam-chapter-14-*bacillus-cereus* 2019.
11. Tewari A, Singh SP. Cultural and biochemical characterization of *Bacillus cereus* isolates collected from Pantnagar, *J Vet Public Health* 2015; **13**(1):5-8.
12. Reiner K. Catalase Test Protocol, American Society For Microbiology, [Created on: 11 November 2010], Revised on 2016; 1-9.
13. Mahon CR, Lehman DC, Manuselis G. Textbook of diagnostic microbiology, 4th ed. W. B Saunders Co., Philadelphia, PA, 2011.
14. Mohamed AS, Alnakip MEA, Abd-El Aal SF. Occurrence of *Bacillus cereus* in raw milk and some dairy products in Egypt. *Jpn Vet Res* 2016; **64**(2):S95-102.
15. Khatun M, Bera P, Mitra D, et al. Estimation of Heavy metal tolerance and antibiotic susceptibility of *Bacillus cereus* isolated from municipal solid waste. *Int J Pharm Bio Sci* 2012; **3**(4):819–29.
16. Griffiths MW, Scraft H. *Bacillus cereus* Food Poisoning. In Foodborne Diseases, Third Ed., Revised by Christine E R Dodd., 2017; 395-405.
17. Hwang JY, Park JH. Characteristics of enterotoxin distribution, hemolysis, lecithinase, and starch hydrolysis of *Bacillus cereus* isolated from infant formulas and ready-to-eat foods. *J. Dairy Sci* 2015; **98**: 1652–1660.
18. CLSI guideline on methods for antimicrobial susceptibility testing for human Mycoplasmas (M43), 2011.
19. Oliwa-Stasiak K, Molnar CI, Arshak K, et al. Development of a PCR assay for identification of the *Bacillus cereus* group species. *J Appl Microbiol* 2010; **108**(1):266-73.
20. Senda K, Arakawa Y, Nakashima K, et al. PCR detection of metallo-beta-lactamase gene (*blaIMP*) in gram-negative rods resistant to broad-spectrum beta-lactams. *J Clin Microbiol* 1996 ; **34**(12):2909-13.
21. Rahimifard N, Fatholahzadeh B, Noory Z, et al. *Bacillus cereus* contamination in infant formula: a study in food and drug control laboratory. *Tehran Univ Med J TUMS Pub* 2007; **65**(8):64-8.
22. Gao T, Ding Y, Wu Q, et al. Prevalence, virulence genes, antimicrobial susceptibility, and genetic diversity of *Bacillus cereus* isolated from pasteurized milk in China. *Front Microbiol* 2018; **9**:533.
23. Mansury D, Motamedifar M, Sarvari J, et al. Antibiotic susceptibility pattern and identification of extended spectrum β-lactamases (ESBLs) in clinical isolates of Klebsiella pneumoniae from Shiraz, Iran. *Iran J Microbiol* 2016; **8**(1):55.
24. Ranjbar R, Shahreza MH. Prevalence, antibiotic-resistance properties and enterotoxin gene profile of *Bacillus cereus* strains isolated from milk-based baby foods. *Trop J Pharm Res* 2017; **16**(8):1931-7.
25. Owusu-Kwarteng J, Wuni A, Akabanda F, et al. Prevalence, virulence factor genes and antibiotic resistance of *Bacillus cereus* sensu lato isolated from dairy farms and traditional dairy products. *BMC microbiol* 2017; 17(1):65.
26. Lesley MB, Ernie SR, Kasing A, et al. Detection of *Bacillus cereus* in formula milk and ultra-high temperature (UHT) treated milk products. *Int Food Res J* 2017; **24**(3).
27. Soltan Dallal MM, Nezamabadi S, Mardaneh J, et al. Detection of toxigenic *Bacillus cereus* strains in powdered infant formula (PIF) milk by PCR assay. *Tehran Med J TUMS Pub* 2017; **75**(3):179-86.
28. Cui Y, Liu X, Dietrich R, et al. Characterization of *Bacillus cereus* isolates from local dairy farms in China. *FEMS*

Microbiol Lett 2016; **363**(12):fnw096.
29. Zhang Z, Feng L, Xu H, et al. Detection of viable enterotoxin-producing *Bacillus cereus* and analysis of toxigenicity from ready-to-eat foods and infant formula milk powder by multiplex PCR. *J Dairy Sci* 2016; **99**(2):1047-55.
30. Reis AL, Montanhini M, Bittencourt JV, et al. Gene detection and toxin production evaluation of hemolysin BL of *Bacillus cereus* isolated from milk and dairy products marketed in Brazil. *Braz J Microbiol* 2013; **44**(4):1195-8.
31. Di Pinto A, Bonerba E, Bozzo G, et al. Occurence of potentially enterotoxigenic *Bacillus cereus* in infant milk powder. *Europ Food Res Technol* 2013; **237**(2):275-9.
32. Shaheen R, Andersson MA, Apetroaie C, et al. Potential of selected infant food formulas for production of *Bacillus cereus* emetic toxin, cereulide. *Int J Food Microbiol* 2006; **107**(3):287-94.
33. Rowan NJ, Anderson JG. Maltodextrin stimulates growth of *Bacillus cereus* and synthesis of diarrheal enterotoxin in infant milk formulae. *Appl Environ Microbiol* 1997; **63**(3):1182-4.
34. Torkar KG, Bedenic B. Antimicrobial susceptibility and characterization of metallo-β-lactamases, extended-spectrum β-lactamases, and carbapenemases of *Bacillus cereus* isolates. *Microb Pathog* 2018; 1-19.