World Journal of Dairy & Food Sciences 18 (2): 16-23, 2023
ISSN 1817-308X

DOI: 10.5829/idosi.wjdfs.2023.16.23

Review on Prevalence of *Bacillus cereus* Enterotoxigenic Genes Isolated from *Food* of Animal Origin and its Public Health Significance

[1]*Enkeshe Loha Yada and* [2]*Yonas Dawule Menta*

[1]Wolaita zone Agricultural Office, Department of Animal Health, Sodo, Ethiopia
[2]Wolaita Zone, Boloso Sore Woreda, Agricultural Office, Areka, Ethiopia

Abstract: *Bacillus cereus* is a ubiquitous and highly resistant food poisoning bacterium that is an important food safety concern. It can contaminate a variety of foods throughout the world. In this systematic review and meta-analysis, a complete search was conducted on the prevalence of *B. cereus*, worldwide. Between 1990 and 2020, a total of 6035 articles were collected from the databases. By applying explicit criteria, the number of 98 studies was included in this study. The overall prevalence of *B. cereus* obtained in this study was 23.746% with a confidence interval of (95%) 22.979-24.513. However, the prevalence of *B. cereus* varied according to food type, identification method and continents. Our findings showed that the highest prevalence of *B. cereus* among eight groups of foods belonged to the cereals and beans, with 41.489% and 44.901%, respectively. Its prevalence was also high in vegetables (37.279%) and dairy products (36.385%). The detection methods used also affected reporting the prevalence of *B. cereus*. In general, the molecular tests such as PCR, reported twice the prevalence compared to other methods. The prevalence of *B. cereus* was also the highest in the Americas and lowest in Australia. However, with some exceptions, the prevalence of *B. cereus* in developing countries was significantly higher than in the developed countries. This study indicates that although the prevalence of *B. cereus* is generally high, depending on the type of food, the method of bacterial detection and also geographically, its prevalence can be quite different. The high prevalence of *B. cereus* in different food types can be attributed to *B. cereus* origin, the soil and non-compliance with hygienic protocols from farm to the fork, so it is still possible to prevent food contamination to this bacterium by observing hygienic principles. In addition, it is recommended to use molecular detection methods along with the Culture/Biochemical methods to obtain a more accurate prevalence of food contamination to *B. cereus*.

Key words: Antibiotic Susceptibility · *Bacillus cereus* · Bacterial Load · Prevalence · Raw Milk

INTRODUCTION

The genus *Bacillus* is the largest genus within the family *Bacillaceae*, presently consisting of at least 226 species most of which are saprophytes widely distributed in the environment and commonly isolated from soil, air, water, plants and animals [1]. *Bacillus cereus* is a Gram positive, facultative anaerobic bacterium characterized by large rod-shaped cells and an ability to form heat-resistant endospores. Since this bacterium is commonly widespread in the environment and is often found in soil, it is naturally present in a wide range of food products of both plant and animal origin. *B. cereus* grows best in a temperature range of 39°F (4°C) to 118°F (48°C). Optimal growth occurs within the narrower temperature range of 82°F (28°C) to 95°F (35°C) and a pH range of 4.9 to 9.3 [2]. Genera *Bacillus* are formed by Gram-positive rods able to produce endospores resistant to unfavourable external conditions [3], that can be distinguished from other spore-formers (*Sporolactobacillus*, *Clostridium*, *Desulfotomaculum*, *Sporosarcina* or *Thermoactinomycetes*) due to their aerobic nature (strict or facultative), rod-shaped cells and catalase synthesis [4].

Bacillus cereus sensu lato (or *B. cereus* group) consists of eight formally recognized species: *B. anthracis, B. pseudomycoides, B. mycoides, B. thuringiensis, B. weihenstephanensis, B. cytotoxicus, B. toyonensis* and *B. cereus sensu stricto* (the *B. cereus* species). Most of these species are very difficult to

Corresponding Author: Yonas Dawule, Wolaita Zone, Boloso Sore Woreda, Agricultural Office, Areka, Ethiopia.

distinguish, even with 16S rDNA sequencing. Many publications that refer to *B. cereus* actually refer to the *B. cereus* group. The *B. cereus* group is ubiquitous and abundant as spores in the soil. They are also found in large quantities in silage, feces and litter. *B. cereus* group are not responsible for zoonosis but due to their high presence in the environment, they can be carried by animals, including cows. They can contaminate raw milk by simple transfer during milking, when hygiene conditions are not fully observed and some strains of the species may cause mastitis in very rare cases. Pasteurization induces sporulation of *B. cereus* group species; the spores can subsequently survive the pasteurization process and, therefore, contaminate dairy products, causing quality and safety issues [1].

While there are numerous known species in the genus *Bacillus*, only two, *B. anthracis* and *B. cereus*, are associated with human diseases. *Bacillus anthracis*, though pathogenic, is rarely linked to foodhborne illness. The accurate number of food poisonings caused by *B. cereus* in different countries is not known because it is not a reportable illness and is not always diagnosed [5]. However, *Bacillus cereus* is the known source of two distinct types of foodborne illness. Both illnesses are associated with the ingestion of a distinct toxin produced by the bacteria. The first form is emetic, which is characterized by nausea, vomiting and abdominal cramps. The emetic form has a short onset time of about 1 to 6 hours after consumption of contaminated foods and is caused by the ingestion of a pre-formed toxin that contaminates food prior to eating. The temperature for emetic toxin (cereulide) production ranges from 77°F (25°C) to 86°F (30°F) [6]. The second form causes diarrhea and has a longer onset time of about 6 to 15 hours and can last approximately 24 hours. This form of the illness is caused by enterotoxins (toxins that specifically affect the intestinal mucosa) produced by *B. cereus* inside the host after ingestion [7]. *B. cereus* causes self-limiting (24-48 h) food-poisoning syndromes (a diarrheal type and an emetic type). Besides food related illnesses *B. cereus* may also cause non-gastrointestinal disease like endocarditis and endophthalmitis [8], respiratory tract and wound infections [9].

B. cereus produces one emetic toxin (ETE) and three different enterotoxins. Three pore-forming enterotoxins, responsible for the diarrhoeal type of food poisoning are Hemolysin BL (Hbl), Non-haemolytic enterotoxin (Nhe) and Cytotoxin K (CytK). Hbl and Nhe each consist of three different protein components, named L2, L1 and B and NheA, NheB and NheC, respectively, while CytK is a single-component toxin [10].

Most often, *Bacillus* sp. is detected and isolated by methods based on the resistance of spores to heating or ethanol. However, direct isolation of particular species requires a selective medium or other selective conditions that are available only for a few species. The cultivation methods for the determination of *B. cereus* as tested in this work are typical examples. These methods detect the failure to utilise mannitol, lecithinase activity or β-glucosidase activity, while other bacteria including some bacilli are inhibited by polymyxin B sulphate or trimethoprim [8]. Other methods for the detection and identification of *B. cereus* are e.g. serotyping, pyrolytic gas chromatography, pyrolytic mass spectrometry, ribotyping, phage typing, plasmid profiles, electrophoresis in pulse electric field and polymerase chain reaction (PCR) using genera-specific and species-specific primers [11].

Some regulatory authorities have set *B. cereus* group food safety limits of 10^3 cfu/g in dairy products for the general population, 10^2 cfu/g in infant formula. Alternatively, many countries have chosen not to impose *B. cereus* group standards for dairy products. Where standards exist, they tend to be restricted to products that are destined for consumption by infants and 'at risk' consumers. An amendment to European Regulation No 2073/2005 dated 2007 (Corrigendum No. 1441/2007) includes *B. cereus* group as a microbiological process hygiene criterion for powdered formulas and dried dietary foods for infants less than 6 months (n=5; c=1; m=50 cfu/g; M=500 cfu/g). In Europe, there are no specific regulations concerning *B. cereus* group in other milk and dairy products. However, dairy companies manufacturing powder for infant formula generally have specifications less than 100 cfu/g of *B. cereus* group strains [11].

Gastrointestinal illnesses caused by toxin producing bacteria, such as *B. cereus*, are almost certainly underestimated due to lack of diagnostic tools [12], due to this and related challenges notably data representing trends in food-borne infectious Gastro-intestinal (GI) disease is limited to a few developed countries [13]. Even though the problem of diseases caused by food-borne pathogens remains largely unknown, species of *Bacillus* and related genera have long been taxing to food producers because of their resistant endospores [14]. Therefore, the objective of this paper is to review the prevalence of Enterotoxigenic genes of Bacillus cereus isolated from food of animal origin, its food posing and none gastrointestinal illnesses, diagnosis, analysis, control and prevention [14].

MATERIAL AND METHODS

Description of the Organism

General Characteristics: *Bacillus cereus* is a Gram-positive, motile (flagellated), spore-forming (heat-resistant endospores), rod shaped bacterium that belongs to the *Bacillus* genus. Species within this genus include *B. anthracis, B. cereus*, *B. mycoides, B. thuringiensis, B. pseudomycoides and B. weihenstephanensis* [15, 16]. *B. cereus* group named also *B. cereus sensu lato* includes eight closely related species: *B. anthracis, B. cereus sensu stricto, B. cytotoxicus, B. mycoides, B. pseudomycoides, B. thuringiensis, B. toyonensis* and *B. weihenstephanensis* [17]. Since this bacterium is commonly widespread in the environment and is often found in soil, it is naturally present in a wide range of food products of both plant and animal origin. *B. cereus* grows best in a temperature range of 39°F (4°C) to 118°F (48°C). Optimal growth occurs within the narrower temperature range of 82°F (28°C) to 95°F (35°C) and a pH range of 4.9 to 9.3 [18].

Bacillus cereus have gone through huge taxonomic changes in the last 30 years, with number of genera and species now standing at 56 and over 545, respectively [19]. The bacterial spores do not swell the sporangium and sporulate readily only in the presence of oxygen [20]. The core of the spore is surrounded by the inner membrane, cortex and inner and outer coats, whereas the spores of B. cereus are devoid of metabolic activity. That's why they are refractory to extreme environmental conditions inclusive of heat, freezing, drying and radiation and may be regarded as the defensive agent for this bacterium [21].

Epidemiology: *Bacillus cereus* is known to cause bovine mastitis, although at lower incidence level than the major udder pathogens *Staphylococcus* spp., *Streptococcus* spp. and *Escherichia coli* [22]. The genus *Bacillus* is ubiquitous in nature because it does not have complex nutrient requirements it is frequently found in soils with low nutrients as well as on rice and straw [23]. Soil can contain between 10^3 and 10^5 spores of *B. cereus* per gram [24]. *Bacillus* species have also been found among normal flora of the teat skin [25]. Dairy industry is the spoilage potential of these species, especially in manufacturing of pasteurized milk [26], condensed sterilised milk [27], dried baby foods [28], milk powders and UHT milk [29].

Bacillus cereus is widespread in nature and readily found in soil, where it adopts a saprophytic life cycle; germinating, growing and sporulating in this environment [30]. Spores are more resistant to environmental stress than vegetative cells due to their metabolic dormancy and tough physical nature [31]. *B. cereus* food poisoning occurs year-round and is without any particular geographic distribution. Between 1973 and 1985, *B. cereus* caused 17.8 % of the total bacterial food poisonings in Finland, 11.5 % in the Netherlands, 0.8 % in Scotland, 0.7%in England andWales, 2.2%in Canada, 0.7%in Japan and 15.0 % (between 1960 and 1968) in Hungary [23]. In Norway, B. cereus was the most common microbe isolated from foodborne illnesses in 1990 [23]. In France, from 1998 to 2000, *B. cereus* represented 4 to 5 % of foodborne poisoning outbreaks of known origin [32]. As of 2008, 103 confirmed outbreak cases have been reported in the US [33]. In Northern America, *B. cereus* represented 1 to 2%of outbreaks of identified origin [34].

However, strains able to multiply below 7°C and strains able to multiply above 45°C, are not the most common. Emetic *B. cereus* is presumably unable to grow and produce their toxin cereulide below 10°C, or in the absence of oxygen [35]. It forms resistant spores and spreads easily; therefore there is a risk in its transmission through processed, pasteurized, sterilized and heat-treated food products [23]. The primary mode of transmission is via ingestion of *B. cereus* contaminated food, emetic type of food poisoning has been largely associated with the consumption of rice and pasta, while the diarrheal type is transmitted mostly by milk products, vegetables and meat [36, 37].

Isolation and Methods for Detection of *B. cereus*: Various selective solid media such as MYP (mannitolegg yolk-phenol red-polymyxin-agar) and PEMBA (polymyxinpyruvate egg yolk-mannitol-bromthymol blue-agar) were used for the isolation and detection of *B. cereus* from food. The selectivity of these media is based on the hydrolysis of egg yolk lecithin and the absence of the use of mannitol by *B. cereus* besides the presence of selective compound like polymyxin [38]. Several molecular typing methods that rely on DNA sequence differences have been used to reveal the genetic relationship of *B. cereus* group strains [39]. Enterobacterial repetitive intergenic consensus-PCR (ERIC-PCR) was used to screen for genetic relatedness. On the other hand, the application of pulsed-field gel electrophoresis (PFGE) has been proved to be useful for the discrimination and epidemiological characterization of *B. cereus* group strains [39].

Most often, *Bacillus* sp. is detected and isolated by methods based on the resistance of spores to heating or ethanol. However, direct isolation of particular species requires a selective medium or other selective conditions

Table 1: *B.cereus* food-borne outbreaks due to from foods of animal origin

Type of food	Region/Country	Affected persons	Years	Authors
Poultry	England & Wales	14	1992-2008	[43]
Chicken	USA & European Union	10	1988-2007	[43]
Red meat	England & Wales	9	1992-2008	[43]
Beef	USA, Australia, New Zealand	5	1988-2007	[44]
Pork	USA & European Union	2	1988-2007	[44]
Eggs	Australia	1	1988-2007	[44]
Seafood	USA & Asia	3	1988-2007	[44]
Dairy products	USA & European Union	3	1988-2007	[44]
Pasteurized milk	Netherlands	1(42)		

that are available only for a few species. The cultivation methods for the determination of *B. cereus* as tested in this work are typical examples. These methods detect the failure to utilise mannitol, lecithinase activity or β-glucosidase activity, while other bacteria including some bacilli are inhibited by polymyxin B sulphate or trimethoprim [40]. Various selective solid media such as MYP (mannitol-egg yolk-phenol red-polymyxin-agar) and PEMBA (polymyxin-pyruvate-egg yolk-mannitol-bromthymol blue-agar) were used for the isolation and detection of *B. cereus* from food. The selectivity of these media is based on the hydrolysis of egg yolk lecithin and the absence of the use of mannitol by *B. cereus* besides the presence of selective compound like polymyxin [38]. Other methods for the detection and identification of *B. cereus* are e.g. serotyping, pyrolytic gas chromatography, pyrolytic mass spectrometry, ribotyping, phage typing, plasmid profiles, electrophoresis in pulse electric field and polymerase chain reaction (PCR) using genera-specific and species- specific primers [41].

Outbreaks and Illnesses Caused Food Poisoning *Bacillus cereus* Group: Consumption of food contaminated with pathogens and microbial by-products such as toxins could result in serious diseases [42]. The reported incidence of foodborne outbreaks caused by *Bacillus* toxins is steadily increasing worldwide throughout the last decade. For instance, in the European Union about 600-700 confirmed cases of foodborne outbreaks linked to *B. cereus* toxins are annually reported [34]. In the European Union (EU), every year 500 to 700 confirmed human cases of foodborne diseases caused by *B. cereus* are reported [35]. In 2016, bacterial toxins ranked second among the causative agents in foodborne and waterborne outbreaks and 17.7% of the reported foodborne outbreaks were caused by bacterial toxins, including *B. cereus* emetic and diarrheal toxins [365]. Concerning the food matrices, involved in these outbreak situations, “mixed food” was reported in 23%, “other food” in 17% and “cereal products and legumes” in 14% of the outbreaks [35].

Regulation Related with B. Cereus Food Poisoning: Some regulatory authorities have set *B. cereus* group food safety limits of 10^3 cfu/g in dairy products for the general population, 102 cfu/g in infant formula. Alternatively, many countries have chosen not to impose *B. cereus* group standards for dairy products. Where standards exist, they tend to be restricted to products that are destined for consumption by infants and ‘at risk’ consumers [45]. An amendment to European Regulation No 2073/2005 dated 2007 (Corrigendum No. 1441/2007) includes B. cereus group as a microbiological process hygiene criterion for powdered formulas and dried dietary foods for infants less than 6 months (n=5; c=1; m=50 cfu/g; M=500 cfu/g). In Europe, there are no specific regulations concerning *B. cereus* group in other milk and dairy products. However, dairy companies manufacturing powder for infant formula generally have specifications of less than 100 cfu/g of *B. cereus* group strains. In order to meet this requirement, the corresponding specification for raw milk should be as low as 10 cfu/ml for *B. cereus* group. Methodology to determine this low number in a timely manner is lacking [45].

Treatment of *Bacillus cereus* Infections in Human: Any person thought have been exposed to *B. anthracis* can be administered a post exposure prophylactic course of oral antibiotics, usually ciprofloxacin or doxycyclin. Some strains of *B. cereus* showed intermediate sensitivity (mild resistance) to amoxicillin-clavulinic acid and resistance to beta lactam antibiotics such as pencillin is commonly observed in *B. cereus* strains food in the food chain [46, 47].

Prevention and Control of Bacillus Cereus Food Poisoning

Prevention: *B. cereus* spores are extremely heat resistant, so while cooking at proper temperatures would destroy most foodborne pathogens including the vegetative cells of *B. cereus*, it does not destroy the spores. Rapid cooling and proper reheating of cooked food are very essential if the food is not consumed immediately. Long-term storage must be at temperatures below 8°C (or preferably 4-6°C to prevent growth of B. cereus). Low pH foods (pH 4.3) can be considered safe from growth of the food-poisoning *Bacillus* spp. In most of the cases the mentioned food-borne outbreaks came from Chinese restaurants or takeaway shops which left the boiled rice to dry off at room temperature [43]. The predominance of cases in these types of restaurants is linked with the common practice of saving portions of boiled rice from bulk cooking. The boiled rice is then stored, usually at room temperature, overnight and these conditions supports the growth of bacteria.

Similarly in takeaway shops, the ready to eat foods are usually kept at room temperature which causes germination and multiplication of *B. cereus* [43]. The same problem may occur when foods such as pasta and pizza are stored for long periods of time at room temperature. According to the National Institutes of Health (NIH), the National Institute ofAllergy and Infectious Diseases (NIAID) and the National Food Processors Association (NFPA), there are some good suggestions to destroy *B. cereus* [47], & steaming under pressure, roasting, frying and grilling foods can destroy the vegetative cells and spores. & Foods infested with the diarrheal toxin can be inactivated by heating for 5 min at 133°F. & Foods infested with the emetic toxin need to be heated to 259°F for more than 90 min. Reheating foods until they are steaming is not enough to kill the emetic toxin. At present, the main problem with *B. cereus* seems to be in the dairy industry, where the keeping quality of milk is determined by the number of B. cereus cells/spores in the product. *B. cereus* may cause aggregation of the creamy layer of pasteurized milk because of lecithinase activity of bacterium, known as bitty cream. *B. cereus* is also responsible for sweet curdling (without pH reduction) in both homogenized and non-homogenized low-pasteurized milk. It seems impossible to completely avoid the presence of *B. cereus* in milk as raw milk already gets infected with bacterium at the farm. Soiling of the udders of cows is the principal source of contamination of milk with *B. cereus*. Soil has been shown to contain 105-106 spores per gram. It is very important, therefore, that the udder and the teats are cleaned to reduce the contamination of raw milk. Transport and further storage in the dairy may result in further contamination of the raw milk from *B. cereus* spores already present (adherent) in the tanks or pipelines. The problems the dairy industry is facing with *B. cereus* are difficult to solve with present knowledge, although we may limit it by monitoring the problem closely. First of all the number of *B. cereus* cells or spores may be limited in the rawmilk by proper cleaning of the udder and the teats before milking. The vegetative bacteria are killed in the pasteurization process, but the spores survive. Pasteurization might activate at least some of the spores (heat activation), which might start germinating. One of the main reasons why this bacterium causes problems in the food and dairy industry is the great ability of the spores to adhere to surfaces, in particular hydrophobic surfaces. The strong adhesion of *B. cereus* spores is mainly due to the high relatively hydrophobicity, low spore surface charge and the sporemorphology. The *B. cereus* spores have covering of long appendages and these promote adhesion [43].

Control Measures: Since *B. cereus* spores are widespread in the environment, application of control measures to prevent the contamination of the foods with the pathogen spores and toxins is important for the consumers' safety. Application of control measures, such as GMP and HACCP, in food processing lines can prevent food contamination with *B. cereus.* The dairy industry should apply control measures to avoid post-pasteurization contamination of milk with this pathogen [48]. Cooked foods should be consumed soon after preparation and in case of a later consumption, they should be kept hot (>63°C) or rapidly cooled. Refrigeration below 4°C is necessary to prevent growth of all types of *B. cereus,* including psychrotrophic strains. The governmental authors should, also, set limits for the population levels of *B. cereus* and its toxins in various foodstuffs [48].

CONCLUSION AND RECOMMENDATIONS

Bacillus cereus is the important specie in the genus *Bacillus* causing human and animal health threat. Its epidemiological studies would help in better understanding of the sources of infection and their risk assessment, routes of transmission, clinical forms and better management of the infection. Standard and hygienic operating methods in the farming, processing and marketing of foods are the way forward to reduce the incidence of *Bacillus cereus* isolated from food of animal origin animal with significant percentage. The contamination sources of foods of animal origin are more likely to be associated with insufficient hygienic practices and improper handling. The presence toxins produced from this organism in food products could be a potential risk for consumers. From the above conclusion the following recommendations are forwarded:

- Good Practices for Food Product Receiving, Handling, Processing and Storage.
- Effective cleaning and sanitation programs and safe handling procedures are important.
- Foods should be cooked thoroughly and cooled rapidly.
- Training for carrying out standard sanitary, hygiene and technical operations.
- Further studies on the occurrence of *Bacillus cereus* species in various food products should be carried out.

REFERENCES

1. International Dairy Federation (IDF), 2006. www.fil-idf.org.
2. Food and Drug Administration (FDA), 2012b. *Bacillus cereus*. Downloaded from http://www.fda.gov/ Food/Science Research/Laboratory Methods/ Bacteriological Analytical Manual BAM/ ucm 070875.
3. Liu, Y., Y. Cui, W. Peng, X. Huang, L. Ma, M. Zheng, S. Ding and K. Zhu, 2020. Prevalence of pathogens harboring mobile antimicrobial resistance genes and virulence factors in retail beef and mutton. FEMS Microbiol. Lett.
4. Shawish, R. and R. Tarabees, 2017. Prevalence and antimicrobial resistance of *Bacillus cereus* isolated from beef products in Egypt. Open Vet. J., 7(4): 337-341. https://doi.org/10.4314/ ovj.v7i4.9
5. Kotiranta, A., K. Lounatmaa and M. Haapasalo, 2000. Epidemiology and pathogenesis of B. cereus infections. Microbes Infect., 2: 189-198.
6. Food and Drug Administration (FDA), 2012a. *Bacillus cereus*. Downloaded from http://www.fda.gov/ Food/Science Research/Laboratory Methods/ Bacteriological Analytical Manual BAM/ ucm 070875.
7. Frenzel, E., M. Kranzler, T.D. Stark, T. Hofmann and M. Ehling-Schulz, 2015. The endospore-forming pathogen *Bacillus cereus* exploits a small colony variant-based diversification strategy in response to aminoglycoside exposure. MBio., 6(6): e01172-15. https://doi.org/ 10.1128/mBio.01172-15.
8. Mayada A.M. Abou Zeid and Shereen A. Yassin, 2017. Detection of Some Virulence Factors of *Bacillus cereus* in Heat-Treated Milk, 53: 72-78. DOI: 10.5455/ajvs.261157.
9. Mehrdad Tajkarimi, 2007. *Bacillus cereus*, PHR 250 4/25/, 6p.
10. Fagerlund, A., T. Lindback and P.E. Granum, 2010. *Bacillus cereus* cytotoxins Hbl, Nhe and CytK are secreted via the Sec translocation pathway. BMC Microbiol., 10: 304.
11. Te Giffel, M., R. Beumer, A. Christianson and M. Griffiths, 2000. *Bacillus cereus* in milk and milk products. Advances in detection, typing and epidemiology. Bulletin IDF, 357: 47-54.
12. Tewari, A. and S. Abdullah, 2014. Review on *Bacillus cereus* food poisoning: international and Indian perspective DOI 10.1007/s13197-014-1344-4.
13. Newell, D.G., M. Koopmans, L. Verhoef, E. Duizer, A. Aidara-Kane, H. Sprong, M. Opsteegh, M. Langelaar, J. Threfall, F. Scheutz, J. Giessen and H.. Kruse, 2010. Food-borne diseases- The challenges of 20 years ago still persist while new ones continue to emerge. Int. J. Food Microbiol., 139: S3-S15.
14. Ryu, J.H. and L.R. Beuchat, 2005. Biofilm formation and sporulation by *B. cereus* on a stainless steel surfaceand subsequent resistance of vegetative cells and spores tochlorine, chlorine dioxide and a peroxyacetic acid-based sanitizer. J. Food Prot., 68: 2614-2622.
15. Rajkovic, A., M. Uyttendaele, T. Courtens, M. Heyndrickx and J. Debevere, 2006. Prevalence and characterisation of *Bacillus cereus* in vacuum packed potato puree. International Journal of Food Science & Technology, 41(8): 878-884.
16. Mpatswenumugabo, P.J., C.L. Bebora, C.G. Gitao, A.V. Mobegi, B. Iraguha, O. Kamana and B. Shumbusho, 2017. Prevalence of Subclinical Mastitis and Distribution of Pathogens in Dairy Farms of Rubavu and Nyabihu Districts, Rwanda ID 8456713, 8 pages https:// doi.org/10.1155/2017/8456713.
17. Pfrunder, S., J. Grossmann, P. Hunziker, R. Brunisholz, M.T. Gekenidis and D. Drissner, 2016. *Bacillus cereus* group-type strain-specific diagnostic peptides. J. Proteome Res. 15, 3098-3107. doi: 10.1021/acs.jproteome.6b00216
18. Food and Drug Administration (FDA), 2012b. *Bacillus cereus*. Downloaded from http://www.fda.gov/ Food/Science Research/Laboratory Methods/ Bacteriological Analytical Manual BAM/ ucm 070875.
19. Logan N.A. and P. de Vos, 2009. Genus I. *Bacillus*. In: De Vos P., Garrity G.M., Jones D., Krieg N.R., Ludwig W., Rainley F.A., Schleifer K.H., Whitman W.B. (eds): Bergey's Manual of Systematic Bacteriology. Springer Science + Business Media, New York, pp: 21-108.

20. Blackburn, C.W. and P.J. McClure, 2005. Foodborne pathogens-hazards, risk analysis and control.Woodhead Publishing Limited, Cambridge, pp: 423-425.
21. Bottone, E.J., 2010. *Bacillus cereus*, a volatile human pathogen. Clin. Microbiol. Rev., 23: 382-398.
22. Radostits, O.M., C.C. Gay, D.C. Blood and K.W. Hinchcliff, 2000. Veterinary Medicine. W.B Saunders, Edinburgh, pp: 658.
23. Kotiranta, A., K. Lounatmaa and M. Haapasalo, 2000. Epidemiology and pathogenesis of B. cereus infections. Microbes Infect., 2: 189-198.
24. Guinebretière, M., S. Auger, N. Galleron, M. Contzen, B. De Sarrau, M. De Buyser, G. Lamberet, A. Fagerlund, P. Granum, D. Lereclus, P. De Vos, C. Nguyen-The and A. Sorokin, 2013. *Bacillus* cytotoxicus sp. nov. is a novel thermotolerant species of the Bacillus cereus group occasionally associated with food poisoning Int. J. Syst. Evol. Microbiol., 63(1): 31-40.
25. Woodward, W.D., A.C. Ward, L.K. Fox and L.B. Corbeil, 1988. Teat skin normal flora and colonization with mastitis pathogen inhibitors. Vet. Microbiol., 17: 357-365.
26. Zhou, G., H. Liu, J. He, Y.Yuan and Z. Yuan, 2008. The occurrence of *Bacillus cereus*, *B. Thuringiensis* and *B. mycoides* in Chinese pasteurized full fat milk. Int. J. Food Microbiol., 121: 195-200.
27. Clark, W.S., 2001. Concentrated and dry milks and wheys. In: Marth E.H., Steel J.L. (eds): Applied Dairy Microbiology. Marcel Dekker, Inc., New York, pp: 77-92.
28. Becker, H., G. Schaller, W. von Wiese and G. Terplan, 1994. *Bacillus cereus* in infant foods and dried milk products. International Journal of Food Microbiology, 23: 1-15.
29. Chen, L., T. Coolbear and R.M. Daniel, 2004. Characteristics of proteinases and lipases produced by seven *Bacillus* sp. isolated from milk powder production lines. International Dairy Journal, 14: 495-504.
30. Svensson, B., K. Ekelund, H. Ogura and A. Christiansson, 2004. Characterisation of *Bacillus cereus* isolated from milk silo tanks at eight different dairy plants. Int. Dairy J., 14: 17-27.
31. Marxen, S., T.D. Stark, E. Frenzel, A. Rutschle, G. Lucking and G. Purstinger, 2015. Chemodiversity of cereulide, the emetic toxin of *Bacillus cereus*. Anal Bioanal Chem., 407: 2439-53.
32. Hanashiro, A., M. Morita, G.R. Matte, M.H. Matte and E.A.F.S. Torres, 2005. Microbiological quality of selected street foods forms a restricted area of Sao Paulo city, Brazil. Food Control., 16: 439-444.
33. Vangoitsenhoven, R., D. Rondas, I. Crevecoeur, W. D'Hertog, P. Baatsen and M. Masini, 2014. Foodborne cereulide causes beta-cell dysfunction and apoptosis. PLoS One, 9: e104866.
34. Koizumi, Y., T. Okuno, H. Minamiguchi, K. Hodohara, H. Mikamo and A. Andoh, 2020. Survival of a case of *Bacillus cereus* meningitis with brain abscess presenting as immune reconstitution syndrome after febrile neutropenia. A case report and literature review. BMC Infect. Dis., 20: 15.
35. European Food Safety Authority (EFSA), 2006. Opinion of the Scientific Panel on Biological Hazards on *Bacillus cereus* and other *Bacillus* spp. in foodstuffs. (Question N° EFSA-Q-2004-010).EFSA J. (2006) 175, 1-48 EFSA.
36. Mayada A.M. Abou Zeid and Shereen A. Yassin, 2017. Detection of Some Virulence Factors of *Bacillus* cereus in Heat-Treated Milk Vol. 53: 72-78. DOI: 10.5455/ajvs.261157.
37. Logan N.A. and P. de Vos, 2009. Genus I. *Bacillus*. In: De Vos P., Garrity G.M., Jones D., Krieg N.R., Ludwig W., Rainley F.A., Schleifer K.H., Whitman W.B. (eds): Bergey's Manual of Systematic Bacteriology. Springer Science + Business Media, New York, pp: 21-108.
38. Heilkenbrinker, U., R. Dietrich, A. Didier, K. Zhu, T. Lindback, P.E. Granum and E. Martlbauer, 2013. Complex formation between NheB and NheC is necessary to induce cytotoxic activity by the three-component *Bacillus cereus* Nhe enterotoxin. PLoS ONE 8, e63104.
39. Eglezos, S., B. Huang, G.A. Dykes and N. Fegan, 2010. The prevalence and concentration of *Bacillus cereus* in retail food products in Brisbane, Australia. Foodborne Pathogens and Disease, 7: 867-870.
40. Logan N.A. and P. de Vos, 2009. Genus I. Bacillus. In: De Vos P., Garrity G.M., Jones D., Krieg N.R., Ludwig W., Rainley F.A., Schleifer K.H., Whitman W.B. (eds): Bergey's Manual of Systematic Bacteriology. Springer Science + Business Media, New York, 21-108.
41. Te Giffel, M., R. Beumer, A. Christianson and M. Griffiths, 2000. *Bacillus cereus* in milk and milk products. Advances in detection, typing and epidemiology. Bulletin IDF, 357: 47-54.

42. Osman, M.K., D.A. Kappell, A. Orabi, S.K. Al-Maary, S.A. Mubarak, M.T. Dawoud, A.H. Hemeg, M.I.I. Moussa, M.A.Hessain, M.Y.H. Yousef and R.K. Hristov, 2018. Poultry and beef meat as potential seedbeds for antimicrobial resistant enterotoxigenic *Bacillus* species: a materializing epidemiological and potential severe health hazard doi:10.1038/s41598-018-29932-3.
43. HPA (Health Protection Agency, UK), 2010. Foodborne Outbreak Surveillance and Risk Assessment http://www.hpa.org.uk [accessed 22 February.
44. Greig, J.D. and A. Ravel, 2009. Analysis of foodborne outbreak data reported internationally for source attribution. Inter. J. Food Microbiol., 130: 77-87.
45. Ryu, J.H. and L.R. Beuchat, 2005. Biofilm formation and sporulation by *B. cereus* on a stainless steel surfaceand subsequent resistance of vegetative cells and spores tochlorine, chlorine dioxide and a peroxyacetic acid-based sanitizer. J. Food Prot., 68: 2614-2622.
46. International Dairy Federation (IDF), 2006. www.fil-idf.org.
47. Pan, Q., Y. Liu, R. Wang, T. Chen, Z. Yang, Y. Deng, Z. Zhao, X. Hu, X. Chen and W. Wei, 2017. Treatment of *Bacillus cereus* endophthalmitis with endoscopy-assisted vitrectomy. Medicine, 96: e8701
48. European Food Safety Authority (EFSA), 2005. Opinion of the Scientific Panel on Biological Hazards on *Bacillus cereus* and other *Bacillus* spp. in foodstuffs. (Question N° EFSA-Q-2004-010).EFSA J. (2005) 175, 1-48 EFSA.