Original Research

Prevalence of *Bacillus* in Heat Treated Milk and Infant Food

Adel M. El-Kholy [1], Arafa M. S. Meshref [1], and Bahaa A.M. Morsy [2*]

1. Department of Food Hygiene, Faculty of Veterinary Medicine, Beni-Suef University, Beni-Suef 62511, Egypt.
2. Department of Food Hygiene, Fayoum University Hostels, Fayoum 63511, Egypt.

Correspondence*
Bahaa A.M. Morsy: Department of Food Hygiene, Fayoum University Hostels, Fayoum 63511, Egypt.
Email: dr.bahaamorsy@gmail.com.

Received: 17/9/2022
Accepted: 25/12/2022

ABSTRACT *Bacillus* species are widely distributed in nature and could be recognized as an important food contaminant in several types of raw food and dairy products. This spore forming pathogen can tolerate different types of heat treatment during dairy processing. Therefore, monitoring presence of this pathogen in dairy products seems to be of great importance. The bacteriological investigation in this study revealed that mean values of *Bacillus* species count in the examined one hundred and fifty samples fifty each of UHT milk, pasteurized milk and infant food (milk-based cereal) which were chosen at random from various supermarkets and dairy stores located throughout the city of Fayoum in Egypt were $5.9 \times 10^2 \pm 0.33 \times 10^3$, $1.2 \times 10^5 \pm 0.57 \times 10^5$ and $4.1 \times 10^3 \pm 0.79 \times 10^3$ (cfu / ml or g), respectively. The presence of *Bacillus* species is objectionable as it was implicated in various foodborne outbreaks, spoilage and responsible for defects such as sweet curdling and bitterness in dairy products

Keywords: *Bacillus cereus,* UHT milk, pasteurized milk, infant food, foodborne illness.

INTRODUCTION

Bacillus species are broadly disseminated in the environment because of their ability to withstand adversarial conditions; they are facultative anaerobic, Gram-positive, spore-forming bacterium **(Carlin *et al.*, 2010 and Ceuppens *et al.*, 2013)**. According to the food safety importance, the most important species is *Bacillus cereus*, known for its ability to form toxins and cause foodborne illnesses **(USEA, 2009)**.

It has been determined that the common soil dweller *Bacillus cereus (B. cereus)* is an opportunistic pathogen. Many uncooked foods, including grains, spices, milk and dairy products, vegetables, meat, cakes, and other desserts, frequently contain it (Larsen and Jorgensen, 1997). **(Larsen and Jorgensen, 1997)** on the other hand, it can be found in cooked meals and desserts **(Eglezos *et al.*, 2010 and samapundo *et al.*, 2011)**.

B. cereus is accountable for 2 kinds of foodborne illness: the emetic and diarrheal syndromes **(Kim *et al.*, 2010)**. A Severe attack of nausea and vomiting within 5 hrs. after having the meal containing performed toxin (cereulid) is the characteristic feature of the emetic syndrome. Due to its strong resistance to heat treatments, pH extremes, and proteolytic destruction, this toxin does not become inactive during the preparation and processing of food or throughout gastrointestinal passage **(Rajkovic *et al.*, 2008 and Stenfors-Arnesen *et al.*, 2008).** Moreover, one diarrheal (infection) due to enterotoxins production in intestines which consist of three toxins nonhemolytic enterotoxin (Nhe), hemolysin BL (Hbl), and cytotoxin K (CytK), which are heat labile, acid sensitive and proteolysis **(Lund *et al.*, 2000)**, diarrheal syndrome develops 8-16 hrs. after consumption of the infected food and characterized by abdominal pain and diarrhea **(Gilbert, 1979 and Agata *et al.*, 1995)**. However, *B. cereus* is not taken into account when diarrhea cases are diagnosed, even if the fecal samples gave negative results for the presence of *Salmonella, Shigella* and *Entamoeba* in the Middle East **(Organji *et al.*, 2015)**.

The most common pathogenic *Bacillus* species discovered in raw milk and throughout the entire dairy processing process is believed to be *B. cereus* **(Scheldeman *et al.*, 2006)**. Numerous isolated strains of *B. cereus* from milk are shown pathogenic properties that may responsible for a health hazard **(Nieminen *et al.*, 2007)**.

Bacillus cereus can tolerate boiling, UHT temperatures and pasteurization and in this way, find its way into final dairy products **(Ahmed *et al.*, 2011)**. It could produce protease, lipase and lecithinase which lead to spoilage of milk and dairy products **(Valerie De Jonghe *et al.*, 2010)**.

Bacillus species considered one of the most important spoilage microorganisms in dairy environment and its growth may result in various dairy defects. In addition, it is a great safety concern for dairy industry as it is contributed with

incidences of food poisoning by producing enterotoxin. Because of its outstanding ability to adhere to stainless steel surfaces of dairy plant and form biofilm, *B. cereus* can lead to serious hygiene problems and economic loss due to spoilage of dairy products and equipment impairment **(Kumari and sarker, 2016)**.

The aim of this work was planned to estimate microbiologically the prevalence of *Bacillus* species in some milk-based products by using HiCrome™ Bacillus Agar which are based on the formulation of Mannitol egg Yolk Polymyxin agar (MYP Agar).

MATERIALS AND METHODS

Collection and preparation of samples One hundred and fifty samples, comprising fifty each of UHT milk, pasteurized milk and infant food (milk-based cereal) were chosen at random for three months from various supermarkets and dairy stores located throughout the city of Fayoum in Egypt. The samples were brought as quickly as possible in an insulated ice box to the lab and looked at the same day. 11 ml/g a well-mixed sample was diluted by 1/10 by adding 0.1% to 99 ml of sterile peptone water, from which 10-fold serial dilutions were prepared (APHA, 1992). **Enumeration and identification of *Bacillus* species (FDA, 2012)** 0.1ml from each dilution was evenly spread over a dry surface of HiCrome™ *Bacillus* Agar plates by using a sterile bent glass rod. Cultured plates as well as control ones were incubated at 30ºC for 24-48 hrs. Suspected light blue, large, flat colonies with blue centers were counted, and then the plates were re-incubated for another 24 hrs. before being counted again for further growth. Suspected colonies were picked up and streaked onto nutrient agar slants, which were incubated at 30 ºC for 24 hrs. for further identification. Suspected colonies were biochemically confirmed by Nitrate broth, Modified VP medium, Phenol red glucose broth, Tyrosine agar, Lysozyme broth and Hemolytic activity.

RESULTS

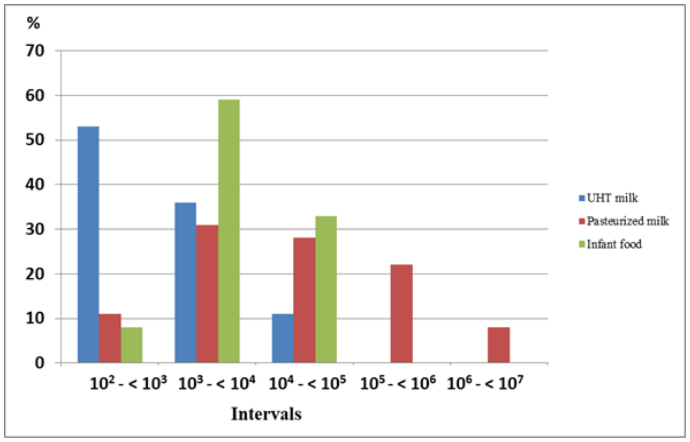
Table (1): Statistical analytical results of *Bacillus* spp. count (cfu /ml or g) in the examined samples:

Product	No. of samples	Positive samples		Min.	Max.	Mean ±SEM
		NO.	%			
UHT milk	50	19	38	1.5×10^2	1.6×10^4	$5.9 \times 10^2 \pm 0.33 \times 10^3$
Pasteurized milk	50	36	72	2.3×10^2	2.4×10^6	$1.2 \times 10^5 \pm 0.57 \times 10^5$
Infant food	50	39	78	1.6×10^2	1.9×10^4	$4.1 \times 10^3 \pm 0.79 \times 10^3$

Table (2): Frequency distribution of *Bacillus* spp. count (cfu /ml or g) in positive samples

Intervals	UHT milk		Pasteurized milk		Infant food	
	NO.	%	NO.	%	NO.	%
102 - < 103	10	53	4	11	3	8
103 - < 104	7	36	11	31	23	59
104 - < 105	2	11	10	28	13	33
105 - <106	-	-	8	22	-	-
106 - <107	-	-	3	8	-	-

Fig. (1): Frequency distribution of *Bacillus* spp. count (cfu/ml or g) in the positive samples



DISCUSSION

Prevalence of *Bacillus* spp.:

Bacillus cereus is the causative agent of two distinct forms of gastro-enteritic disease connected to food-poisoning. It produces one emesis-causing toxin and three enterotoxins that elicit diarrhea. Due to changing lifestyles and eating habits, *B. cereus* is responsible for an increasing number of foodborne diseases in the industrial world **(Schulz *et al.*, 2004)**.

Contamination by *B. cereus* in food may occur after heat treatment or food production, during food processing, preparation, transport, storage and distribution **(Bennett *et al.*, 2013)**.

Inspection of table (1) shows that *Bacillus spp.* existed in 19 (38%) of investigated UHT milk samples in count ranged from 1.5×10^2 to 1.6×10^4 with a mean of $5.9 \times 10^2 \pm 0.33 \times 10^3$ cfu/ml , lower counts were reported by **El-Toukhy(2017)** and **Khalid (2017)** who examined 25 UHT milk samples and results

were 40% with a mean of $3.6 \times 10^2 \pm 0.05 \times 10^2$ cfu /ml and 32% with a mean value of $1.6 \times 10^2 \pm 0.4 \times 10^2$ cfu /ml respectively, while higher count was indicated by **Shawky *et al*. (2017)** with an incidence of 40% and a mean of $9.4 \times l0^2 \pm 0.39 \times 10^3$ cfu/ml.

According to results of pasteurized milk in table (1) *Bacillus spp.* found in 36 (72%) of examined samples in count ranged from 2.3×10^2 to 2.4×10^6 with a mean of $1.2 \times 10^5 \pm 0.57 \times 10^5$ cfu /ml, lower results were stated by **El-Toukhy (2017)** and **Khalid (2017)** who examined 25 samples, results were 60% with a mean value of $3.7 \times 10^2 \pm 0.6 \times 10^2$ and 60% with a mean of $1.7 \times 10^2 \pm 0.4 \times 10^2$ cfu /ml, respectively.

As shown in table (1) *Bacillus spp.* existed in 39 (78%) of tested infant food samples in count ranged from 1.6×10^2 to 1.9×10^4 with a mean of $4.1 \times 10^3 \pm 0.79 \times 10^3$ cfu /g, lower results were stated by **Sadek *et al.*, (2018)**, **Pluta *et al.*, (2019)** and **El-Desoky (2021)** who found that samples contaminated at a percentage of 14.1%, 30% and 36.67%, respectively, on the other hand higher result was recorded by **El-Karamani (2017)** who found that 100% of samples were contaminated.

The spores of *Bacillus cereus* are resistant to heat, desiccation, disinfectants, ionizing radiation and UV light. They represent the major hygienic problem in processes where heat or other treatments kill the competing, non-sporeforming microflora. Raw milk is the usual source of spore-forming bacteria in finished dairy products. Their numbers before heat treatment determined its numbers after processing; however, they can also contaminate milk after processing **(Griffiths & Phillips, 1990)**.

CONCLUSION

Screening the results reflects that some dairy products were manufactured, handled, and distributed in an unhygienic manner. Some of the samples under examination had *Bacillus spp.* contamination.

The presence of *Bacillus spp.* is unacceptable, as they make the dairy products of inferior quality and unfit for human consumption. Likewise, *B.* cereus or its heat resistant enzymes are believed to be the major microbial causes of the spoilage of milk and milk products and reason for significant economic losses in the dairy industry. *B. cereus* is exposing the consumer to public health risks.

Therefore, some regulations should be taken into account in order to enhance the quality of these products so that consumers are protected from exposure to the risk of food contamination or intoxication including the following : to enhance product quality and guarantee the highest level of consumer safety, producers, processors, and handlers should be required to participate in educational programs like Good Hygienic Practice (GHP) and attention to detail which associated with practice of this products required and licenses should be given to establishment after all equipment, facilities and hygienic conditions are fulfilled, in addition suitable control measures through routine specialized inspection of facilities and dairies to guarantee error rectification and prevent repetition of problems.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

Agata, N., Ohta, M., Mori, M., & Isobe, M. (1995). A novel dodecadepsipeptide, cereulide, is an emetic toxin of *Bacillus cereus,* FEMS Microbiology Letters, 129: 112-117. [DOI: 10.1111/j.1574-6968.1995.tb07550.x]

Ahmed, M.M., Metwally, N., Dabiza, M.A., El-Kholy, W. I., & Sadek, Z.I. (2011). The Effect of Boiling on Milk Microbial Contents and Quality, Journal of American Science, 7(2):110–114.

APHA, (1992). Standard methods for the examination of dairy products. 3rd Ed., American public health association, Washington.

Bennett, S. D., 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(3), 425-433. [DOI: 10.1093/cid/cit244]

Carlin, F., Brillard, J., Broussolle, V., Clavel, T., Duport, C., & Jobin, M. (2010). Adaptation of *Bacillus cereus* , an ubiquitous worldwide distributed foodborne pathogen, to a changing environment, Food Research International 43: 1885-94**,** [DOI: 10.1016/j.foodres.2009.10.024]

Ceuppens, S., Boon, N., & Uyttendaele, M. (2013). Diversity of *Bacillus cereus* group strains is reflected in their broad range of pathogenicity and diverse ecological lifestyles. FEMS Microbiology Ecology. 84: 433-450. [DOI:10.1111/1574-6941.12110]

Eglezos, S., Huang, B., Dykes, G.A., & Fegan, N. (2010). The prevalence and concentration of *Bacillus cereus* in retail food products in Brisbane, Australia. Foodborne Pathogens and Disease , 7: 867-70.[DOI:10.1089/fpd.2009.0469]

El-Desoky, A. E. I. (2021). Safety and Qualty Aspects of Dried Milk Products, Ph.D. Thesis, Fac.Vet.Med., Cairo University, Egypt.

El-Karamani, Y. M. F. (2017). Studies on *Bacillus Cereus* group in Some Milk-Based Foods. Ph.D. Thesis, Fac.Vet.Med., Assiut University, Egypt.

El-Toukhy, H. E. E. (2017). Microbiological quality of heat-treated milk. M.V.S.c. Thesis, Fac.Vet.Med., Benha University, Egypt.

FDA (Food and Drug Administration), (January2012): *B. cereus*. Chapter 14. In: Bacteriological Analytical Manual. https://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm070875.htm.

Gilbert, R.J. (1979). *Bacillus cereus* gastroenteritis, In: Riemann, H., Bryan, F. (Eds.), Food-borne Infections and Intoxications, 2nd edn. Academic press, New York, pp. 495–518.

Griffiths, M. W., & Philips, J. D. (1990). Incidence, source and some properties of psychrotrophic *Bacillus* spp. found in raw and pasteurized milk. J Soc. Dairy Tech. 43, 62–66**.** [DOI:10.1111/j.1471-0307.1990.tb02446.x]

Khalid, S. N.M. (2017). Studies on aerobic spore formers in milk and some milk products. M.V.S.c. Thesis, Fac.Vet.Med., Benha University, Egypt.

Kim, J.B., Kim, J.M., Kim, S.Y., Kim, J.H., Park, Y.B., Choi, N.J., & Oh, D.H. (2010). Comparison of enterotoxin production and phenotypic characteristics between emetic and enterotoxic *Bacillus cereus*, Journal of Food Protection 73: 1219–1224. [DOI: 10.4315/0362-028X-73.7.1219]

Kumari, S., & Sarkar, P.K. (2016). Bacillus cereus hazard and control in industrial dairy processing environment. Food Control. (69), 20-29. [DOI:10.1016/j.foodcont.2016.04.012]

Larsen, H.D., & Jorgensen, K. (1997). The occurrence of Bacillus cereus in Danish pasteurized milk. International Journal of Food Microbiology (34), 179–186. [DOI:10.1016/S0168-1605(96)01182-8]

Lund, T., De Buyser, M., & Granum, B. (2000). A new cytotoxin from *Bacillus cereus* that may cause necrotic enteritis. Molecular microbiology 38 (2):254-261**.** [DOI:10.1046/j.1365-2958.2000.02147.x]

Nieminen, T., Rintaluoma, N., Andersson, M., Taimisto, A.M., Ali-Vehmas, T., Seppala, A., Priha, O., & Salkinoja-Salonen, M.S. (2007). Toxinogenic *Bacillus pumilus* and *Bacillus licheniformis* from mastitic milk, Veterinary Microbiology (124), 329–339. [DOI:10.1016/j.vetmic.2007.05.015]

Organji, S.R., Abulreesh, H.H., Elbanna, K., Haridy, G.E., Osman, G.E.H., & Khider, M. (2015). Occurrence and characterization of toxigenic *Bacillus cereus* in food and infant feces. Asian Pacific Journal of Tropical Biomedicine 5(7): 515–520.[DOI:10.1016/j.apjtb.2015.04.004]

Pluta, A.B., Garbowska, M., & Stefańska, I. (2019). Prevalence and Toxicity Characterization of *Bacillus cereus* in Food Products from Poland. Foods, 8(7): 269. [DOI:10.3390/foods8070269]

Rajkovic, A., Uyttendaele, M., Vermeulen, A., Andjelkovic, M., Fitz- James, I., Veld, P., Denon, Q., Verhe, R., & Debevere, J. (2008). Heat resistance of *Bacillus cereus* emetic toxin, cereulide, Letters in Applied Microbiology, 46: 536–541. [DOI:10.1111/j.1472-765X.2008.02350.x]

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**.** [DOI:10.1016/j.toxrep.2018.08.013]

Samapundo, S., Heyndrickx, M., Xhaferi, R., & Devlieghere, F. (2011). Incidence, diversity and toxin gene characteristics of Bacillus cereus group strains isolated from food products marketed in Belgium. Int. J. Food Microbiol. 150: 34-41. [DOI:10.1016/j.ijfoodmicro.2011.07.013]

Scheldeman, P., Herman, L., Foster, S., & Heyndrickx, M. (2006). *Bacillus sporothermodurans* and other highly heat-resistant spore formers in milk. Journal of Applied Microbiology 101: 542–555. [DOI:10.1111/j.1365-2672.2006.02964.x]

Schulz, M.E., Fricker, M., & Scherer, S. (2004). *Bacillus cereus*, the causative agent of an emetic type of food-borne illness. Mol. Nutr. Food Res. 48, 479 – 487. [DOI:10.1128/9781555816902.ch11]

Shawky, N.A., khalid, S.N.M., Ibrahim, E.M.A., & Mohammed, H.A. (2017). Thermophilic Bacilli in Commercially Heat-Treated Milks. Benha Journal of Applied Sciences (BJAS), Vol. (2) Issue (3) Dec. (2017), 61-64. [DOI:10.21608/bjas.2017.164546]

Stenfors-Arnesen, L.P., Fagerlund, A., & Granum, P.E. (2008). From soil to gut: *Bacillus cereus* and its food poisoning toxins. FEMS Microbiology Reviews, 32: 579–606**,** [DOI:10.1111/j.1574-6976.2008.00112.x]

(USEA) US Environmental protection Agency (2009). Bacillus subtilis final risk assessment. Biotechnology Program Under Toxic Substances Control Act (TSCA). Available at: http://www.epa.gov/biotech_rule/pubs/fra/fra009.htm Accessed May 28, 2010.

Valerie De Jonghe, An Coorevits, A., Block, J., Coillie, E.V., Koen Grijspeerdt, K., Herman, L., Vos, P., & Marc Heyndrickx, M (2010). Toxinogenic and spoilage potential of aerobic spore-formers isolated from raw milk. International Journal of Food Microbiology 136 (2010) 318–325, [DOI:10.1016/j.ijfoodmicro.2009.11.007]

Cite this Paper

Adel M. El-Kholy, Arafa M. S. Meshref, and Bahaa A.M. Morsy (2023). Prevalence of Bacillus in Heat Treated Milk and Infant Food. MJVM., Vol. (3), Issue (1) Special Issue of 2nd Sci Conference of Fac Vet Med Matrouh University, pages (1-4). DOI: 10.21608/MJVM.2022.163423.1011.

About the Journal

Matrouh Journal of Veterinary Medicine (MJVM)
The official journal of the faculty of veterinary medicine, Matrouh University, Egypt.
Publisher: *Matrouh University, Egypt.*
ISSN (Online):2735-458X
ISSN (Print): 2735-4903
Indexed in EKB Database