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Bacillus cereus food poisoning in Indian perspective: A review

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Abstract
Bacillus cereus is one of food-borne disease causing bacteria. Bacillus spores may be present on various types of raw and cooked foods and their ability to survive high cooking temperatures requires that cooked foods be served hot or cooled rapidly to prevent the growth of this bacteria. *Bacillus cereus* is well known as a cause of food poisoning and much more is now known about the toxins produced by various strains of this species. *Bacillus cereus* is widespread in nature and frequently isolated from soil and growing plants, but it is also well adapted for growth in the intestinal tract of insects and mammals. From these habitats it is easily spread to foods, where it may cause an emetic or a diarrhoeal type of food-associated illness that is becoming increasingly important in the industrialized world. The emetic disease is a food intoxication caused by cereulide, a small ring-formed dodecadepsipeptide. The genetic determinants of cereulide are plasmid-borne. The diarrhoeal syndrome of *B. cereus* is an infection caused by vegetative cells, ingested as viable cells or spores, thought to produce protein enterotoxins in the small intestine. These are cytotoxins, have been associated with diarrhoeal disease are haemolysin BL, nonhaemolytic enterotoxin and cytotoxin K. This review will focus on the toxins associated with foodborne diseases frequently caused by *B. cereus*.

Keywords: Bacillus, poisoning, causing, dodecadepsipeptide, *Bacillus cereus*

Introduction
Ready to eat foods, such as cooked meats, cold vegetable dishes and noodles in sauce and fried rice, are very approachable as they are intended for direct consumption but these foods and food products have been shown to be frequently contaminated with pathogenic bacteria such as *Bacillus cereus*, *Listeria monocytogenes* and *Staphylococcus aureus* (Yang *et al.*, 2016) [47]. *Bacillus cereus* is a large, Gram-positive, motile, aerobic to facultative anaerobic, spore-forming rod that causes foodborne diseases and is ubiquitous in nature (Marrollo, 2016) [33]. The bacterial spores do not swell the sporangium sporulate readily only in the presence of oxygen (Blackburn and McClure, 2005) [9]. Electron microscopy of the vegetative cells reveals a cytoplasmic membrane surrounding the cellular content. In addition, some strains contain an outermost crystalline surface protein (Kotiranta *et al.*, 2000) [26]. The core of the spore is surrounded by the inner membrane, cortex, 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 (Bottone, 2010) [11]. *Bacillus cereus* has been isolated from a variety of foods such as cooked rice and mixed salad (Park *et al.*, 2005; Gao *et al.*, 2018, Yu *et al.*, 2019) [37, 18, 48]. The natural habitat for most species is soil (Kramer and Gilbert 1989) [27]. The most important Bacillus species with respect to food is *B. cereus* which is responsible for self-limiting (24–48 hours) 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 (Drobniewski, 1993; Logan and Rodrigez-Diaz, 2006) [14, 31]. 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 (Kotiranta *et al*., 2000) [26]. The most important pathogenic species belong to the *B. cereus* group, consisting of *B. cereus*, *B. mycoides*, *B. thuringiensis*, *B. Anthracis* and the recently described *B. Weihenstephanensis* (Lechner *et al.*, 1998) [30] and *B. pseudomycoides* (Nakamura, 1998)[35].

Historical perspective
Robert Koch's (1876) study of the *B. Anthracis* marked the genesis of clinical bacteriology. (Hauge, 1955) [22] was the first to establish *B. cereus* as a food poisoning organism causing a diarrhoeal type of illness on the consumption of vanilla Sauce.

In the United States and Canada, *B. cereus* food-poisoning was first documented in 1968 (Szabo *et al.*, 1984) [44].

Epidemiology

Etiological agent

The genus bacillus includes 51 recognized species and is divided into three groups based on the morphology of the spore and sporangium. The species of group 1 have ellipsoidal or cylindrical spores that are centrally or terminally located and do not distend the sporangium. Group 1 is further subdivided into subgroups 1A and 1B based on cell size and presence of poly-beta-hydroxybutyrate globules in the protoplasm. The *B. cereus* group, consisting of *B. cereus*, *Bacillus mycoides*, *B. thuringiensis* and *B. Anthracis*, belongs to subgroup 1A, members of which have bacillary body widths of 1 mm and protoplasmic globules. Recently, two additional species, *Bacillus pseudomycoides* and psychrotolerant *Bacillus Weihenstephanensis* were described. Hemolysis, motility, penicillin resistance, tyrosine degradation and phosphatase production have been used traditionally to distinguish *B. cereus* and *B. thuringiensis* from *B. Anthracis*. *B. cereus* and *B. thuringiensis* are positive and *B. Anthracis* is generally negative for all five characteristics.

Transmission

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 (Logan and Rodrigez-Diaz, 2006) [31]. In Japan and the UK, the emetic disease dominates (Shinagawa *et al.*, 1995) [41], while in Northern Europe and North America, the diarrheal disease seems more prevalent (Kotiranta *et al.*, 2000) [26] and this difference in disease pattern is probably due to different eating habits. Only spores able to germinate will contribute to the onset of disease (Clavel *et al.*, 2004; Wijnands *et al.*, 2006) [13, 46].

Bacillus cereus and food borne illness - Mainly two types of disease syndromes are caused by *B. cereus*. These are as follows:

1. Diarrheal syndrome is due to the production of heat labile enterotoxins during growth of vegetative cells in the small intestine of the host and the infective dose is 10^4 – 10^9 cells per gram of food. This syndrome is mild and primarily manifested by abdominal cramps and diarrhea after an incubation period of 8 to 16 hours and lasting for 6 to 12 hours. It is referred to as "long-incubation" or diarrheal form of the disease (Logan and Rodrigez-Diaz, 2006) [31].
2. Emetic syndrome, which is more severe and acute than diarrheal syndrome and is referred to as "short-incubation" or emetic form of the disease. The emetic illness is triggered by the toxin known as cereulide, which is usually preformed in food before ingestion, resulting in a foodborne intoxication (Ehling-Schulz *et al.*, 2004) [15]. Emetic syndrome is characterized by nausea and vomiting and abdominal cramps. The toxin responsible for this syndrome is a small cyclic heat-stable peptide which causes vomiting after 1 to 6 hour of ingestion (average 2 to 5 hours) (Mortimer and McCann, 1974) [34]. In emetic type of illness, the dose is about 10^5 –10^8 cells per gram in order to produce sufficient toxin (Logan and Rodrigez-Diaz, 2006) [31].

Table 1: Characteristics of the two types of disease caused by *Bacillus cereus* (Agata *et al.*, 1995; Granum, 1997) [1, 20].

	Diarrhoeal syndrome	Emetic syndrome
Infective dose	10^5–10^7 (total)	10^5–10^8 (cells g^{-1})
Toxin produced	In the small intestine of the host	Preformed in foods
Type of toxin	Protein	Cyclic peptide
Incubation period	8–16 h (occasionally >24 h)	0.5–5 h
Duration of illness	12–24 h (occasionally several days)	6–24 h
Symptoms	Abdominal pain, watery diarrhoea and occasionally nausea	Nausea, vomiting and malaise (sometimes followed by diarrhoea, due to additional enterotoxin production?)
Foods most frequently implicated	Meat products, soups, vegetables, puddings/sauces and milk/milk products	Fried and cooked rice, pasta, pastry and noodles

Toxins produced by *B. cereus*

B. cereus produces one emetic toxin (ETE) and three different enterotoxins.

Diarrheal type of food poisoning: Three pore-forming enterotoxins, responsible for the diarrheal type of food poisoning are as follows:

Hemolysin BL (HBL): Hemolysin BL has three protein components, L_1, L_2 and B, constitute the Hemolysin. B is for binding whereas L_1 and L_2 are lytic components. It is a proteinaceous toxin that also has dermonecrotic and vascular permeability activities and causes fluid accumulation in ligated rabbit ILEAL loops (Beecher and MacMillan, 1990; Beecher *et al.*, 1995) [7, 8]. Genes encoding HBL are carried by about 50–66% of strains tested (Granum, 2002; Ngamwongsatit *et al.*, 2008; Ankolekar *et al.*, 2009) [19, 36, 6] and it was formerly believed to be the primary virulence factor in *B. cereus* diarrhea (Granum *et al.*, 1996) [21]. It is believed to cause osmotic lysis by forming a transmembrane pore, following independent binding of its three components B, L_1 and L_2 to the host cell (Stenfors Arnesen *et al.*, 2008)[43].

Non-Haemolytic enterotoxin (NHE): The long-incubation form of illness is mediated by the heat-labile diarrhoeagenic enterotoxin and hemolytic enterotoxin which cause intestinal fluid secretion, probably by several mechanisms, including pore formation and activation of adenylatecyclase enzymes (Jalalpour, 2012) [24]. Non-hemolytic enterotoxin (NHE) is structurally similar to HBL. NHE consists of the proteins NHE A, NHE B and NHE C. It was discovered following a Norwegian outbreak that was caused by the Hbl-negative strain and it is now believed to be the most dominant diarrhoeal toxin (Stenfors Arnesen *et al.*, 2008) [43]. Production of both HBL and NHE is believed to be restricted to members of the *B. cereus* group.

Cytotoxin K (CYTK): Cytotoxin K (CYTK) is a single-

component, b-barrel pore forming toxin that belongs to the same family of toxins as Clostridium perfringens beta-toxin. It is dermonecrotic, cytotoxic and hemolytic, and nearly 90% of *B. cereus* strains may carry the gene for it (Ngamwongsatit *et al.*, 2008) [36]. It consists of a catalytic protein NHE A and two binding components NHE B and NHE C. The three genes encoding the Nhe components constitute an operon. It appears that all *B. cereus* strains carry genes encoding NHE (Ankolekar *et al.*, 2009) [6]. Additionally, a protein, first isolated from the *B. cereus* FM_1 strain, was named enterotoxin FM (entFM) because at high doses it was suspected to cause fluid accumulation in rabbit and mouse ligated intestinal loop tests (Boonchai *et al.*, 2008) [10]. It is a potent Cytotoxin against human intestinal Caco-2 epithelia. CytK, like other L-barrel pore-forming toxins, spontaneously forms oligomers are resistant to sodium dodecyl sulphate (SDS), but not to boiling (Stenfors Arnesen *et al.*, 2008; Fagerlund *et al.*, 2010) [43, 17]. This toxin occurs in two forms, CytK-1 and CytK-2, which have 89% amino acid sequence homology. The former was associated with the French necrotic enteritis outbreak (Lund *et al.*, 2000) [32] and is the more aggressively cytotoxic (Fagerlund *et al.*, 2004) [16].

Table 2: Diarrheal type of toxins produced by *B. cereus* (Granum *et al.*, 1997) [20]

	Enterotoxin HBL	Enterotoxin NHE	Enterotoxin T
Number of components	3	3	1
Size of active component(s)			
L_2	46 kDa	45 kDa	41 kDa
L_1	38 kDa	39 kDa	
B	37 kDa	105 kDa	
Haemolytic	Yes	No	No

2. Emetic type of food poisoning

The emetic syndrome is caused by the toxin known as cereulide. The emetic toxin (ETE) is dodecadepsipeptide, cereulide (Agata *et al.*, 1994) [2] and having a ring-shaped structure of three repeats of four amino acids with a molecular weight of 1.2 KDA Cereulide, which is structurally very similar to the antibiotic vancomycin that belongs to the dodecadepside group. Cereulide has been shows to be a potent ionosphere, with high affinity for potassium (Andersson *et al.*, 2007) [5]. The mechanism and site of action of this toxin is unknown, although the small molecule forms ion channels and holes in membranes.

***B. cereus* in food and food products**

Though there are various food-borne pathogens known to cause food-borne illnesses, *B. cereus* has been generally found in most of the cases to be responsible for food-borne outbreaks (Velusamy *et al.*, 2010) [45]. It easily contaminates various food samples and as its elimination is not guaranteed by pasteurization and sanitation procedure, it causes spoilage and food-poisoning by its proteolytic, Lipolytic and saccharolytic activities (Kalogridou-vassiliodou, 1992) [25]. Milk and rice perhaps are the two most commonly contaminated food items. It constitutes 90% of the paddy soil bacteria and also contaminates milk and milk products via contact with soil (Kramer and Gilbert, 1989) [27].

Milk and milk products

B. cereus is considered to be a common contaminant of raw milk and has been reported since 1916 (Ahmed *et al.*, 1983) [3]. Most of the *B. cereus* contamination results from the raw milk in which the organism is partly present as spores and able to survive pasteurization. In the dairy industry, *B. cereus* group spp., especially psychrothrophic strains, are recognized to limit the keeping quality of pasteurized milk (Aires *et al.*, 2009) [4].

Scenario of *B. cereus* food poisoning in India

Incidents of food poisoning are more common in India during various cultural and religious events when food is prepared in bulk as it becomes difficult to maintain hygiene during preparation and storage of food. There are a number of reports of food poisoning among students, who take midday meals in their schools in various states of India and in many of these, *B. cereus* were reported to be present in the food. An incident in which 35 of 50 students, who had attended a party suffered from food poisoning was by (Ram *et al.*, 1987) [39]. The foods implicated were gulabjamun and samosa, a kind of a ready to eat food in India. Symptoms appeared 2 hour after ingestion. In India during 1980–2009 indicated that 24 outbreaks have occurred involving 1,130 persons. As per (Park, 2011) [38], one third of total pediatric admissions in hospitals in India are due to diarrheal diseases and 17% of all deaths in indoor pediatric patients are diarrhea related (Park, 2011) [38]. In India, majority of outbreaks of foodborne disease go unreported and uninvestigated and may only be noticed after major health or economic damage. Kulshreshtha (1978) [28] reported the first outbreak of *B. cereus* food-poisoning among the children due to the consumption of milk powder in India, where *B. cereus* was isolated from stools and vomitus of the victims and from implicated food. Food poising due to *B. cereus* was reported by Lakhani (1979) [29] in a village near Poona at a religious function, in which around 500 people of different age groups developed nausea and vomiting after consuming contaminated rice having viable count ranging from 2.0 to 7.0×10^7 cfu/g. Analysis of remaining suspect food indicated that the outbreak could have been due to staphylococcal enterotoxins and *B. cereus* toxins. One other incident of *B. cereus* food poisoning was recorded by (Singh *et al.*, 1995) [42] where six person of a family were involved in food poisoning who had consumed bakery bun contaminated with *B. cereus*. There are several reports from almost all the parts of India about the presence of *B. cereus* in various food and food products. In India till date milk is supplied by the local milkman and the quality of the milk is usually low and hygiene of the milk is most of the times compromised. (Chopra *et al.*, 1980) [12] found contamination of *B. cereus* in all 10 milk, 8 of 10 burfi and 7 of 10 milk cake samples obtained from Ludhiana city market. Similarly an episode of gastrointestinal illness was recounted by (Hussain *et al.*, 2007) [23], due to consumption of *B. cereus* contaminated food in a fast food restaurant in India. The food included hot cholapuri, made of flour and Bengal gram.

Diagnosis

B. cereus is a large Gram-positive motile facultative anaerobic, endospore forming bacillus of 1.0-1.2 x 3.0-5.0 µm in size. The spores are ellipsoidal in a central or paracentral position.

Bacillus cereus count

High number of organisms is required for producing the disease, colony count is taken to establish the clinical significance of *B. cereus* in foods.

Procedure

1. Weigh 10 g sample and homogenize with 90 ml diluent (NSS/PBS) and make 10-fold serial dilution.
2. Inoculate by spread technique on selective agar (MEYP/PEMBA) and enumerate the organisms as per method described in chapter 2.
3. Pick up about 5 characteristic colonies from the selected dilution and process for identification of *B. cereus* as described above.

Isolation and identification

For prevalence studies isolation is attempted which could be done either by enrichment followed by plating on selective agar media.

Enrichment

Enrichment can be done in non-selective media such as nutrient or lactose broths or selective medium such as trypticase-soy-polymyxin broth.

Selective plating

The mannitol egg yolk polymyxin agar (MEYP) and polymyxin pyruvate egg yolk mannitol Bromothymol blue agar (PEMBA) are the selective media for *B. cereus* isolation. The colonies are gray-white flat or slightly raised with an opaque zone of turbidity.

Procedure

1. Inoculate food sample in nutrient broth or trypticase-soy-polymyxin broth are incubate at 37°C for 18h. A heat shock of 70°C for 15 min before incubation is recommended, if enriched in nutrient broth, which enhances germination of spores and reduces contaminating bacteria.
2. Streak an inoculum from enrichment broth/homogenate onto selective agar [mannitol egg yolk polymyxin (MEYP) or Polymyxin pyruvate egg-yolk mannitol–Bromothymol blue agar (PEMBA)] and incubate at 37°C for 24 hour.
3. Subject the characteristic colonies to Gram's staining. *B. cereus* are Gram-positive bacilli with endospores. Old cultures may appear as Gram-negative; hence fresh culture (up to 24 hour old) is recommended for staining.
4. Pick up presumptive colonies on nutrient agar slants for further characterization.

Colony characteristics of *Bacillus cereus*

5. *Bacillus* species grow readily on nutrient agar or peptone media. The optimum temperature for growth varies from 20 °C to 40 °C, mostly 37 °C. *B. cereus* is mesophilic and is capable of adapting to a wide range of environmental conditions.
6. Nutrient Agar - It forms large (2-5 mm) grey-white, granular colonies with a less wavy edge and less membranous consistency. On 5% sheep blood agar at 37°C, *Bacillus cereus* colonies are large, feathery, dull, gray, granular, spreading colonies and opaque with a rough matted surface and irregular perimeters.
7. Blood agar - It is beta-hemolytic. Colony perimeters are irregular and represent swarming motility. In some instances, smooth colonies develop either alone or in the midst of rough colonies. When grown apart from the initial inoculum, smooth colonies are surrounded by a uniform zone of beta-hemolysis framing the centrally situated colony.
8. Mannitol egg yolk polymyxin agar (MYP) – Considered as the standard media for plating of *B. cereus*, but it has little selectivity so background flora is not inhibited and can mask the presence of *B. cereus*. *B. cereus* colonies are usually lecithinase-positive and mannitol-negative on MYP agar.
9. Bacara agar – It is a chromogenic selective and differential agar that promotes the growth and identification of *B. cereus*, but inhibits the growth of background flora. *Bacillus cereus* colonies turn pink-orange with an opaque halo. The chromogenic agar has been suggested for the enumeration of *cereus* group as a substitute for MYP. Typical colonies will grow as pink-orange uniform colonies surrounded by a zone of precipitation.

Table 3: Identifying characteristics for *B. cereus*

S. No.	Characteristics	Reaction
1.	Motility	+
2.	Reduction of nitrate	+
3.	Hydrolysis of starch	+/-
4.	Citrate	+/-
5.	Catalase	+
6.	Voges Proskauer	+
7.	Haemolysis (rabbit RBC)	+
8.	Acid from carbohydrates	
	Glucose	+
	Mannitol	-
	Arabinose	-
	Xylose	-

Differential diagnosis

Differential diagnosis of *B. cereus* infections include, viral infections (e.g., rotavirus), Bacterial infections (*Campylobacter*, *Shigella*, *Salmonella*, *E. coli*, *Y. enterocolitica*, *V. cholera*, *C. difficult*), Parasitic infections (e.g., *Giardia lamblia*, *Cryptosporidium*, *Entameba*, *Microsporidium*, *Cyclospora*), Toxins (*Staphylococcus aureus*), GI bleeding, Appendicitis, Diverticulitis, Adrenal crisis, Mesenteric ischemia, Thyroid storm, Antibiotic adverse effect.

Counteraction and control of Bacillus food poisoning

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.

1. Rapid cooling and proper reheating of cooked food are very essential if the food is not consumed immediately.
2. Long-term storage must be at temperatures below 8°C (or preferably 4–6°C to prevent growth of *B. cereus*).
3. Low pH foods (pH 4.3) can be considered safe from growth of the food-poisoning Bacillus spp.
4. According to the National Institutes of Health (NIH), the National Institute of Allergy and Infectious Diseases (NIAID) and the National Food Processors Association (NFPA), there are some good suggestions to destroy *B. cereus* for example (Schneider *et al.*, 2004) [40] and 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. and 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.

5. 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.
6. Soiling of the udders of cows is the principal source of contamination of milk with *B. cereus*. Soil has been shown to contain 10^5–10^6 spores per gram. It is very important, therefore, that the udder and the teats are cleaned to reduce the contamination of raw milk.
7. To control *B. cereus*, it is very important to trace the presence of spores from farmer to package. The storage temperature is the most important factor in keeping *B. cereus* numbers to a minimum. Besides this, Food poisoning generally occurs as a result of poor hygiene and/or food handling practice. Hence, it is important to educate food handlers about their responsibilities for food safety and train them on personal hygiene policies and basic practices for safe food handlings.

Conclusion

B. cereus is a normal soil inhabitant and is most frequently isolated from foods. *B. cereus* food poisoning is not a reportable disease and is therefore highly underestimated in official statistics but psychotropic strains of *Bacillus cereus* is reported most frequently from the dairy industry thus becoming an in increasing threat to dairy industry as it produces two different type of toxin that are cyclic peptide which is responsible for emetic (intoxication) syndrome and second is enterotoxins which are responsible diarrhoeal (infection) syndrome. The minimal infective dose for the diarrhoeal syndrome ranges between 10^5 and 10^7 *B. cereus* cells. Two different enterotoxins of *B. cereus* were characterized. Both are having three-component proteins, one haemolytic and one non-haemolytic. The haemolytic and non-haemolytic enterotoxin are transcribed in one operon. But non-haemolytic enterotoxin is regulated by *plcR*.

References

1. Agata N, Ohta M, Mori M, Isobe M. A novel dodecadepsipeptide, cereulide, is an emetic toxin of Bacillus cereus FEMS microbiology letters. 1995;129(1):17-9.
2. Agata N, Mori M, Ohta M, Suwan S, Ohtani I, Isobe M. A novel dodecadepsipeptide, cereulide, isolated from Bacillus cereus causes vacuole formation in HEp-2 cells, FEMS microbiology letters. 1994;121(1):31-4.
3. Ahmed AA, Moustafa MK, Marth EH. Incidence of Bacillus cereus in milk and some milk products, Journal of Food Protection. 1983;46(2):126-8.
4. Aires GS, Walter EH, Junqueira VC, Roig SM, Faria JA. Bacillus cereus in refrigerated milk submitted to different heat treatments, Journal of food protection. 2009;72(6):1301-5.
5. Andersson MA, Hakulinen P, Honkalampi-Hämäläinen U, Hoornstra D, Lhuguenot JC, Mäki-Paakkanen J *et al.* Toxicological profile of cereulide, the Bacillus cereus emetic toxin, in functional assays with human, animal and bacterial cells Toxicon. 2007;49(3):351-67.
6. Ankolekar C, Rahmati T, Labbé RG. Detection of toxigenic Bacillus cereus and Bacillus thuringiensis spores in US rice, International Journal of food microbiology. 2009;128(3):460-6.
7. Beecher DJ, MacMillan JD. A novel component Hemolysin from Bacillus cereus Infection and immunity. 1990;58(7):2220-2227.
8. Beecher DJ, Schoeni JL, Wong AC. Enterotoxic activity of hemolysin BL from Bacillus cereus Infection and immunity. 1995;63(11):4423-8.
9. Blackburn C, McClure PJ, editors. Foodborne pathogens: hazards, risk analysis and control. Elsevier, 2009 Jun 30.
10. Boonchai N, Asano SI, Bando H, Wiwat C. Study on cytotoxicity and nucleotide sequences of enterotoxin FM of Bacillus cereus isolated from various food sources. Journal of the Medical Association of Thailand Chotmaihet thangphaet. 2008;91(9):1425-32.
11. Bottone EJ. Bacillus cereus, a volatile human pathogen, Clinical microbiology reviews. 2010;23(2):382-98.
12. Chopra P, Ajit S, Kalra MS. Occurrence of Bacillus cereus in milk and milk products, Indian Journal of Dairy Science. 1980;33(2):248-52.
13. Clavel T, Carlin F, Lairon D, Nguyen-The C, Schmitt P. Survival of Bacillus cereus spores and vegetative cells in acid media simulating human stomach, Journal of applied microbiology. 2004;97(1):214-9.
14. Drobniewski FA. Bacillus cereus and related species, Clinical microbiology reviews. 1993;6(4):324-38.
15. Ehling-Schulz M, Knutsson R, Scherer S. Bacillus cereus, Genomes of foodborne and waterborne pathogens. 2010;20:147-64.
16. Fagerlund A, Ween O, Lund T, Hardy SP, Granum PE. Genetic and functional analysis of the cytK family of genes in bacillus cereus microbiology. 2004;150(8):2689-97.
17. Fagerlund A, Lindbäck T, Granum PE. Bacillus cereus cytotoxins HBL, NHE and CYTK are secreted via the Sec translocation pathway BMC Microbiology. 2010;10(1):1-8.
18. Gao T, Ding Y, Wu Q, Wang J, Zhang J, Yu S *et al.* Prevalence, virulence genes, antimicrobial susceptibility, and genetic diversity of Bacillus cereus isolated from pasteurized milk in China Frontiers in microbiology. 2018;26(9):533.
19. Granum PE. Bacillus cereus and food poisoning. Applications and systematics of Bacillus and relatives. 2002;9:37-46.
20. Granum PE, Lund T. Bacillus cereus and its food poisoning toxins FEMS Microbiology Letters. 1997;157:223–8.
21. Granum PE, Andersson A, Gayther C, Te Giffel M, Larsen H, Lund T *et al.* Evidence for a further enterotoxin complex produced by Bacillus cereus FEMS microbiology letters. 1996;141(2-3):145-9.
22. Hauge S. Food poisoning caused by aerobic spore-forming bacilli, Journal of Applied Bacteriology. 1955;18(3):591-5.
23. Hussain SA, Munshi ZH, Hussain I. Food poisoning due to Bacillus cereus: A case report, Journal of Veterinary Public Health. 2007;5(1):57-9.
24. Jalalpour S. Food borne diseases bacteria; frequency antibiotic resistance bacteria in Iranian foods, African

Journal of Microbiology Research. 2012;6(4):719-23.
25. Kalogridou-Vassiliadou D. Biochemical activities of Bacillus species isolated from flat sour evaporated milk, Journal of dairy science. 1992;75(10):2681-6.
26. Kotiranta A, Lounatmaa K, Haapasalo M. Epidemiology and pathogenesis of Bacillus cereus infections Microbes and infection. 2000;2(2):189-98
27. Kramer JM, Gilbert RJ. Bacillus cereus and other Bacillus species. Foodborne bacterial pathogens 1989;24(19):21-70.
28. Kulshreshthra SB. Annual report Div Publ Hlth IVRI, Izatnaga, Cited by Singh CM (1979) in problems of food borne infection and intoxications & their working preposition in South East Assi. WHO Inter Country Workshop on Advanced Microbiological Methods in food Hygiene Compendium, Izatnagar, 1978; 1:15–55.
29. Lakhani AG. Foodborne outbreaks and examination of incriminated specimens. WHO Inter-country workshop on advanced microbiological methods in food hygiene. Compendium, IVRI, Izatnagar, 1979; 2:267–279.
30. Lechner S, Mayr R, Francis KP, Pru BM, Kaplan T, Wiebner-Gunkel ELKE *et al.* Bacillus Weihenstephanensis sp. a new psychrotolerant species of the Bacillus cereus group, International Journal of Systematic and Evolutionary Microbiology. 1998;48(4):1373-1382.
31. Logan NA, Rodrigez-Diaz M. Bacillus spp. and related genera. Principles and practice of clinical bacteriology. 2006;20(2):139-58.
32. Lund T, De Buyser ML, Granum PE. A new Cytotoxin from Bacillus cereus that may cause necrotic enteritis Molecular microbiology. 2000;38(2):254-61.
33. Marrollo R. Chapter 1 - Microbiology of Bacillus cereus. In: Savini V, editor, The diverse faces of bacillus cereus, Academic Press, 2016, 1–13.
34. Mortimer PR, McCann G. Food-poisoning episodes associated with Bacillus cereus in fried rice The Lancet. 1974;303(7865):1043-5.
35. Nakamura LK. Bacillus pseudomycoides sp. Nov. International Journal of Systematic and Evolutionary Microbiology. 1998;48(3):1031-1035.
36. Ngamwongsatit P, Buasri W, Pianariyanon P, Pulsrikarn C, Ohba M, Assavanig A *et al.* Broad distribution of enterotoxin genes (HBL CDA, NHE ABC, cytK, and entFM) among Bacillus thuringiensis and Bacillus cereus as shown by novel primers, International Journal of food microbiology. 2008;121(3):352-6.
37. Park K. Park's textbook of preventive and social medicine, Preventive Medicine in Obstet, Pediatrics and Geriatrics, 2005.
38. Park K. Acute diarrhoea diseases. In: Park's textbook of preventive and social medicine, 21st edn. Banarsidas Bhanot, Jabalpur, 2011.
39. Ram S, Singh A, Kalra MS. Food poisoning outbreak caused by Staphylococcus aureus and B. cereus, J Res Punjab Agric Univ. 1987;24(3):469-71.
40. Schneider KR, Parish ME, Goodrich RM, Cookingham T. Preventing foodborne illness: Bacillus cereus and Bacillus Anthracis. EDIS. 2005, 1.
41. Shinagawa K, Konuma H, Sekita H, Sugii S. Emesis of rhesus monkeys induced by intragastric administration with the HEp-2 Vacuolation factor (cereulide) produced by Bacillus cereus FEMS microbiology letters. 1995;130(1):87-90.
42. Singh BR, Kulshreshtha SB, Kappor KN. A bakery product associated B. cereus food poisoning outbreak, Indian J Comp Microbiol Infect Dis. 1995;16:151–152.
43. Stenfors Arnesen LP, Fagerlund A, Granum PE. From soil to gut: Bacillus cereus and its food poisoning toxins, FEMS microbiology reviews. 2008;32(4):579-606.
44. Szabo RA, Todd EC, Rayman MK. Twenty-four hour isolation and confirmation of Bacillus cereus in foods, Journal of food protection. 1984;47(11):856-60.
45. Velusamy V, Arshak K, Korostynska O, Oliwa K, Adley C. An overview of foodborne pathogen detection: In the perspective of biosensors biotechnology advances. 2010;28(2):232-54.
46. Wijnands LM, Dufrenne JB, Rombouts FM, In't Veld PH, Van Leusden FM. Prevalence of potentially pathogenic Bacillus cereus in food commodities in The Netherlands, Journal of food protection. 2006;69(11):2587-94.
47. Yang X, Zhang J, Yu S, Wu Q, Guo W, Huang J *et al.* Prevalence of Staphylococcus aureus and methicillin-resistant Staphylococcus aureus in retail ready-to-eat foods in China Frontiers in microbiology. 2016;7(7):816.
48. Yu P, Yu S, Wang J, Guo H, Zhang Y, Liao X *et al.* Bacillus cereus isolated from vegetables in China: incidence, genetic diversity, virulence genes, and antimicrobial resistance Frontiers in microbiology. 2019;15(10):948.