

Bacillus cereus food poisoning: international and Indian perspective

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Abstract Food borne illnesses result from eating food or drinking beverages that are contaminated with chemical matter, heavy metals, parasites, fungi, viruses and Bacteria. *Bacillus cereus* is one of the food-borne disease causing Bacteria. Species of *Bacillus* and related genera have long been troublesome to food producers on account of their resistant endospores. Their 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, so that its significance in such episodes are clearer. However, it is still unclear why such cases are so rarely reported worldwide.

Keywords *Bacillus cereus* · Toxin · Meat · Milk · Food poisoning

Introduction

The problem of diseases caused by food-borne pathogens remains largely unknown. Notably data representing trends in food-borne infectious Gastro-intestinal (GI) disease is limited to a few developed countries (Newell et al. 2010).

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Even though, food-borne bacteria have to date been the most well investigated and monitored causes of GI infectious disease, our understanding of the microbial agents of GI illness remains limited. Comprehensive diagnostic studies of intestinal infections (Tompkins et al. 1999) indicate that between 50 and 60 % of all causative agents are unidentified. In addition, GI illnesses caused by toxin producing bacteria, such as *B. cereus*, are almost certainly underestimated due to lack of diagnostic tools.

Species of *Bacillus* and related genera have long been taxing to food producers because of their resistant endospores (Andersson et al. 1995; Ryu and Beuchat 2005). These organisms 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 (Logan and Halket 2011).

B. cereus is a large, Gram-positive, motile, aerobic-to-facultative, spore-forming rod. The bacterial spores do not swell the sporangium and sporulate readily only in the presence of oxygen (Blackburn and McClure 2005).

The study of *B. cereus* in relation to food has gained significance in the light of its ability to form heat resistant endospores and capacity to grow and produce toxins in a wide variety of foods. Transmission electron microscopy of the vegetative cells reveals a cytoplasmic membrane surrounding the cellular content. In addition, some strains contain an outermost crystalline surface protein (S layer) (Kotiranta et al. 1998, 2000). 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 (Bottone 2010).

The bacteria is widely distributed environmentally and bears a close phenotypic and genetic (16S rRNA) relationships to several other *Bacillus* species, especially *B. anthracis*

(Ash et al. 1991). There are several *Bacillus* species closely related physiologically and share a significant genetic similarity to *B. cereus* (Blackburn and McClure 2005), as such these related species are included in a heterogeneous “*B. cereus* group”. 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) and *B. pseudomycoides* (Nakamura 1998). The natural habitat for most species is soil, and direct contamination of agricultural products from soil is of importance with respect to foodborne infection or intoxication and food spoilage (Kramer and Gilbert 1989). Analysis of rRNA sequences 16S and 23S, suggest that these species have diverged from a common evolutionary lineage (Ash et al. 1991; Ash and Collins 1992). The most important *Bacillus* species with respect to food is *B. cereus*.

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 (Drobniewski 1993; Logan and Rodriguez-Diaz 2006). 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).

Historical perspective

The genus *Bacillus* was of significant importance in the early history of microbiology, Ferdinand Cohn (1876) was finally able to discredit the theory of spontaneous generation after observing *Bacillus subtilis* and its spores (Logan 2011) and Robert Koch’s (1876) study of the *B. anthracis* marked the genesis of clinical bacteriology.

B. cereus as one of the most ubiquitous bacteria on the earth (Jackson et al. 1995), and the natural environment of *B. cereus* mainly consists of decaying organic material, fresh water, soil, marine water, vegetables and the intestinal tract of invertebrates (Todd 1996). Rasko et al. (2005) and Bottone (2010) found that due to this ubiquitous presence, soil and food is contaminated with *B. cereus* and the colonization of the human intestine is also possible.

Although, there were reports in the European literature in the beginning of the 20th century about food-borne illness caused by *B. cereus* or *B. cereus*-like organism, there was no definite proof that *B. cereus* could cause food-poisoning. Hauge (1955) was the first to establish *B. cereus* as a food-poisoning organism causing a diarrhoeal type of illness on the consumption of vanilla Sauce. His findings were further confirmed by different other European workers in the early 1950s. In the United States and Canada, *B. cereus* food-poisoning was first documented in 1968 (Szabo et al. 1984). Until

1970’s, outbreaks caused by *B. cereus* were only characterized by watery diarrhea, occurring 8–16 h after ingestion of the contaminated food. However in 1971, a new form of *B. cereus* food-poisoning, characterized by nausea and vomiting was identified in United Kingdom (UK) due to the consumption of rice from Chinese restaurants and take-away outlets. As many as 192 such incidences involving more than 1,000 cases were reported in the UK between a period of 1971 and 1984 (Kramer and Gilbert 1989).

B. cereus was recorded to be the third most common cause of the food-poisoning outbreaks in Hungary (117 outbreaks) between 1960 and 1968, followed by Finland (50 outbreaks), Netherlands (11 outbreaks) and Canada (9 outbreaks) (Gilbert 1979; Shinagawa 1990). Besides these, there are many reports of food-borne outbreaks of *B. cereus* from a large variety of foods in many countries including the USA (Bean and Griffin 1990), UK, Scandinavia, Japan (Johnson et al. 1984) and Norway (Kotiranta et al. 2000). Although Norway is almost free of *Salmonella* and *Campylobacter* food poisoning, *B. cereus* is most commonly reported in food-poisoning syndromes (Blackburn and McClure 2005). As per Turnbull (1981) reviewed the literature and concluded that there were at least 230 outbreaks of the diarrhoeal type of *B. cereus* food-poisoning reported world-wide between 1950 and 1976.

More than 30 separate incidents of food poisoning outbreaks associated with cooked rice (usually fried) from Chinese restaurants or ‘take away’ shops were reported in Great Britain since 1971 (PHLS 1972, 1973). In London more than 40 incidences of food-poisoning, associated with the consumption of cooked rice, usually from Chinese restaurants and “take-away” shops, were reported where all of these cases were attributed to *B. cereus* (PHLS 1972, 1973; Gilbert and Taylor 1975a).

In Japan, a total of 5,141 outbreaks of food-poisoning occurred between 1982 and 1986 involving 1, 85,301 cases; out of these, bacterial pathogens were responsible for 3,740 outbreaks. *B. cereus* caused 73 of these outbreaks, involving 1,323 cases (Shinagawa 1990). Between 1986 and 1995, 852 outbreaks of food-borne diseases involving 26,173 cases and 20 deaths were reported in Taiwan. Out of these, 555 cases (65 %) were caused by bacterial pathogens. The third most common bacterial pathogen in these poisonings was *B. cereus* (18 % cases) (Pan et al. 1997).

While summarizing the data generated on food-borne illnesses due to the consumption of Chinese-Indonesian food and meat products during 1991 to 1994 at the regional Food Inspection Services in the Netherlands, Simone et al. (1997) reported 2,621 incidences, involving 7,567 ill people. Of the incidents of known etiological agent, 19 % were attributed to *B. cereus*, which was the highest.

B. cereus is reported not only in the food of restaurants and take aways, but also from other places serving food. Hatakka (1998) reported that between the year 1991 and 94, *B. cereus*

was the most common pathogen (3 %) found in the samples taken from the hot meals served on aircraft. Daniels et al. (2002) reported *B. cereus* to be the causative agent in 7 % of school food-borne disease outbreaks in North America over the period of 1998–2000.

Pirhonen et al. (2005) investigated food-poisoning outbreaks occurred after eating a dish of pasta and minced meat, involving both emesis and diarrhea in two adult persons. Emetic toxin producing strains of *B. cereus* formed the majority (68 %) of strains identified in tested food. Haemolytic diarrhoeal toxin was produced by 26 % of the strains studied and 6 % of the strains produced neither emetic nor haemolytic diarrhoeal toxin.

As per the latest European Food Safety Authority (2007) report on food-borne outbreaks, *B. cereus* was the causative agent in 77 outbreaks and caused 17.1 % of the cases due to bacterial toxins.

Epidemiology

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 and Wales, 2.2 % in Canada, 0.7 % in Japan, and 15.0 % (between 1960 and 1968) in Hungary (Kotiranta et al. 2000). In Norway, *B. cereus* was the most common microbe isolated from foodborne illnesses in 1990 (Kotiranta et al. 2000). In France, from 1998 to 2000, *B. cereus* represented 4 to 5 % of foodborne poisoning outbreaks of known origin (Haeghbaert et al. 2001, 2002a, b). As of 2008, 103 confirmed outbreak cases have been reported in the US (Venkitanarayanan and Doyle 2008). In Northern America, *B. cereus* represented 1 to 2 % of outbreaks of identified origin (Granum and Baird-Parker 2000).

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 (Kotiranta et al. 2000). Soil can contain between 10^3 and 10^5 spores of *B. cereus* per gram (Guinebretiere and Nguyen-The 2003). *Bacillus* species has also been isolated from extreme environments like hot lakes with temperature more than 60 °C, deep sea (Jannasch and Taylor 1984; Gaill 1993), refrigerated foods and high pressure environments (Csonka 1989). Growth may occur from pH 4.5 to 9.3; water activity must be higher than 0.92 for growth and the temperature range for growth (4–50 °C) is very wide (Kramer and Gilbert 1989). 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 (EFSA 2005).

B. cereus is easily spread from its natural environment to many types of foods, especially of plant origin, because of the resistance of its endospores to various stresses and their long-term survival capacity. Its spores and vegetative forms are frequent inhabitants of many food types, especially cereals and its derivatives (Barkley and Delaney 1980) rice, vegetables (Portnoy et al. 1976); spices, herbs and additives (Baxter and Holzapfel 1982). These forms are also present in milk and dairy products (Ahmed et al. 1983), raw meat, eggs, and processed foods (Goepfert et al. 1972) as well as in ready to eat food stuffs (Kramer and Gilbert 1989). It forms resistant spores and spreads easily, therefore there is a risk in its transmission through processed, pasteurized, sterilized, and heat-treated food products (Kotiranta et al. 2000).

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 (Murray et al. 2007; Logan and Rodriguez-Diaz 2006).

A somewhat different distribution for emetic and diarrhoeal disease is observed between countries, which could partly be a reflection of the association of the two types of disease with different food vehicles. In Japan and the UK, the emetic disease dominates (Gilbert and Kramer 1986; Shinagawa et al. 1995), while in Northern Europe and North America, the diarrhoeal disease seems more prevalent (Kotiranta et al. 2000). At least a part of this difference in disease pattern is probably due to different eating habits, but it is difficult to document whether the distribution is truly different or a result of reporting differences (Kotiranta et al. 2000).

B. cereus and foodborne illness

Mainly two types of disease syndromes are caused by *B. cereus*. 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 (Logan and Rodriguez-Diaz 2006). This syndrome is mild and primarily manifested by abdominal cramps and diarrhea following an incubation period of 8 to 16 h and lasting for 6 to 12 h (Hauge 1955; Murray et al. 2007; Logan and Rodriguez-Diaz 2006). Diarrhea may be mild or profuse and watery. This type is referred to as the “long-incubation” or diarrheal form of the disease and it resembles food poisoning caused by *Clostridium perfringens* (Drobniewski 1993).

Another form is the emetic syndrome, which is more severe and acute than diarrheal syndrome and is referred to as “short-incubation” or emetic form of the disease. 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 h

of ingestion (average 2 to 5 h) (Mortimer and McCann 1974). The toxin is preformed and indigested with food. In emetic type of illness, the dose is about 10^5 – 10^8 cells per gram in order to produce sufficient toxin (Logan and Rodriguez-Diaz 2006). It resembles *Staphylococcus aureus* food poisoning in its symptoms and incubation period. The number of organisms necessary to cause this syndrome seems to be higher than that of the diarrheal syndrome (Gilbert 1979).

In either syndrome, the illness usually lasts less than 24 h. In a few patients symptoms may last longer (Murray et al. 2007; Logan and Rodriguez-Diaz 2006). Both syndromes arise as a result of the fact that *B. cereus* spores can survive normal cooking procedures. Under improper storage conditions after cooking, the spores germinate and the vegetative cells multiply (Logan 2011).

Toxins

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 (Stenfors Arnesen et al. 2008; Fagerlund et al. 2010).

The emetic syndrome, due to ETE, is an intoxication that is caused by a single highly heat-, proteolysis-, acid- and alkali-resistant toxin, that is pre-formed when ingested, leading to rapid onset of the syndrome. The emetic toxin (ETE) is dodecadepsipeptide, cereulide (Shinagawa et al. 1995; Agata et al. 1995) and having a ring-shaped structure of three repeats of four amino acids with a molecular weight of 1.2 kDa. The mechanism and site of action of this toxin is unknown, although the small molecule forms ion channels and holes in membranes.

The long-incubation form of illness is mediated by the heat-labile diarrhoeagenic enterotoxin Nhe and/or hemolytic enterotoxin Hbl, which cause intestinal fluid secretion, probably by several mechanisms, including pore formation and activation of adenylatecyclase enzymes (Jalalpour 2012). The hemolytic enterotoxin, Hbl, is encoded by the hblCDA operon. The three protein components, L1, L2 and B, constitute the hemolysin. B is for binding whereas L1 and L2 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. Genes encoding Hbl are carried by about 50–66 % of strains tested (Granum 2002; Ngamwongsatit et al. 2008; Ankolekar et al. 2009), and it was formerly believed to be the primary virulence factor in *B. cereus* diarrhoea, but outbreaks associated with strains lacking this toxin have occurred (Granum et al. 1996). It is believed to cause osmotic lysis by forming a

transmembrane pore, following independent binding of its three components B, L1 and L2 to the host cell (Stenfors Arnesen et al. 2008).

Non-haemolytic enterotoxin (Nhe) is another three component proteinaceous, pore-forming toxin that is structurally similar to Hbl. 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). Production of both Hbl and Nhe is believed to be restricted to members of the *B. cereus* group (From et al. 2005). It consists of a cytolytic protein NheA and two binding components NheB and NheC. The three genes encoding the Nhe components constitute an operon. It appears that all *B. cereus* strains carry genes encoding Nhe (Ngamwongsatit et al. 2008; Stenfors Arnesen et al. 2008; Ankolekar et al. 2009).

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 haemolytic, and nearly 90 % of *B. cereus* strains may carry the gene for it (Ngamwongsatit et al. 2008). The toxin is able to form pores which are weakly anion selective and exhibit an open channel probability close to one. It is a potent cytotoxin against human intestinal Caco-2 epithelia. CytK, like other L-barrel pore-forming toxins, spontaneously forms oligomers which are resistant to sodium dodecyl sulphate (SDS), but not to boiling (Stenfors Arnesen et al. 2008; Fagerlund et al. 2010). 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) and is the more aggressively cytotoxic (Fagerlund et al. 2004).

Additionally, a protein, first isolated from the *B. cereus* FM1 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 (Asano et al. 1997; Boonchai et al. 2008). However, very few studies have been performed on this protein, and its specific role during *B. cereus* virulence has not been reported. The entFM gene is located on the chromosome and appears to be common to *B. thuringiensis* and *B. cereus* strains. Prevalence studies revealed that entFM is detected in most outbreak-associated strains (Hsieh et al. 1999; Ngamwongsatit et al. 2008).

Regulation of production of *B. cereus* toxins and its implications for food safety were reviewed by Ceuppens et al. (2011); they concluded that the complexity of toxin expression is still not well understood and that the influences of food components, temperature and other environmental factors need investigation within the relevant foods and intestinal environments.

The capacity of the concerned strain to produce toxin(s) influences the infective or intoxicating dose in either types of

illness. Cases with both diarrhoeal and emetic symptoms may be caused by single strains producing both diarrhoeal and emetic toxins, or by the presence of both diarrhoeal and emetic strains in the food (Pirhonen et al. 2005).

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). Over the past 20–30 years in many countries, the total number of food-borne illnesses showed an increasing trend (Kafenstein et al. 1997). In a study conducted in south eastern China during 1986–1987, the overall incidence of diarrhoeal illnesses was 730 episodes per 1,000 population (Kangchuan et al. 1991).

B. cereus being ubiquitous in the environment makes it difficult to link clinical cases to its environmental sources. It represents one of the major pathogens in mass catering, causing problems both by deteriorating the products and by endangering people's life upon consuming them (Granum et al. 1993; Te Giffel et al. 1996). Because 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-vassilidou 1992). When *B. cereus* has been exposed to sublethal acid treatment conditions by Kim et al. (2013), it adapted or resistant strains have developed. Acid resistance of the strain can cause a food safety problem because it may result in enhanced protection for cells which are subsequently exposed to lethal heat or hydrogen peroxide stress.

The factors that make *B. cereus* a potential threat to food processing is its ability to form thermotolerant endospore, ability to grow and survive at refrigeration temperature and toxin production (Griffiths 1990; Van Netten and Kramer 1992; Granum and Lund 1997; McKillip 2000). 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). It can also compromise the microbiological quality of eggs and their products which are likely to be contaminated with psychrotrophic *B. cereus* group bacteria (Techer et al. 2014).

Meat and meat products

In Hungary, during the period 1960–68, it was presumed spore-forming aerobes possibly *B. cereus* was the third most important cause of food-poisoning while staphylococci and salmonellae being the primary causes of outbreaks. Foods found to be associated with *B. cereus* food-poisoning were mainly meat dishes (Ormay and Novotny 1969).

B. cereus is found to be present in several spices and additives, so that contamination of meat with *B. cereus* increases with each additional stage in the processing of the raw meat (Volkova 1971). Meat additives play an important role in increasing the *B. cereus* load in final products as it is reported that vegetables, cheese and spices have the incidence of 14, 10.3 and 10 % respectively, but the highest counts were up to 500/g are reported in hamburgers and minced meat which are the finished products (Cantoni and Bresciani 1987). Smykal and Rokoszewska, in the year 1976 indicated that over a 7 year period (1964–1971) prevalence of *B. cereus* was 13.3 % in meat and meat products and 27.2 % of soups and meat-based sauces.

This bacterium survives not only at room temperature, it is also found in heat treated food. *B. cereus* was recovered from 28 % of the meat products samples including heat-treated products. Of these food samples, heat treated meat products alone showed 48 % positivity for the *B. cereus* (Schlegelova et al. 2003). This is because bacteria are resistant to heat stress. Bolstad (1990) reported a case of food-poisoning involving two people in Norway, who consumed ready-grilled chicken, held at ambient temperature in the local food shop, the product revealed the presence of *B. cereus* numbering approximately 10,000/g.

B. cereus also occurs in frozen food and food products. Mira and Abuzied (2006) collected five types of ready-to-eat chicken products and frozen half cooked chicken products from fast food shops and supermarkets, and showed 100 % prevalence rate of *B. cereus*. They found highest incidence of isolation of *B. cereus* from ready-to-eat chicken product followed by frozen half cooked chicken product samples. Similarly Smith et al. (2004) analyzed 60 samples of chicken meat products for the presence of *B. cereus* and found that 27 of these were harboring the organism. Guven et al. (2006) and Kursun et al. (2011) found 22.4 and 36 % incidence of *B. cereus* in retail samples of meat and meat products and in rabbit meat samples respectively.

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). 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 psychrotrophic strains, are recognized to limit the keeping quality of pasteurized milk (Svensson et al. 2004; Hanson et al. 2005; Barbano and Santos 2006; Aires et al. 2009).

Contamination of pasteurized milk has been mainly traced to raw milk (Lin et al. 1998; Huck et al. 2007; Banyko and Vyletelova 2009) and/or equipment surfaces. The role of processing equipment as a reservoir for *B. cereus* milk

recontamination is well documented (Te Giffel et al. 1997; Svensson et al. 1999, 2000, 2004; Schlegelova et al. 2010) notably post-pasteurization contamination (Eneroth et al. 2001; Sharma and Anand 2002; Salustiano et al. 2009).

As per Nagarajan et al. 1990, psychrotrophic *B. cereus* isolated from milk when inoculated into fresh sterile milk is found to survive pasteurization, mainly as endospores. The organism grows in milk during storage at 4 °C and elaborates a high protease activity. Though apparent milk spoilage due to this organism is not detected during storage at 4 °C, deterioration in milk quality is observed.

Smykal and Rokoszewska (1976) analyzed milk and various milk products and cakes over a period of 7 years (1964–1971), and recovered *B. cereus* at high incidences. Soegaard and Peterson (1977) tested whole milk, low-fat milk, skimmed milk and cream samples obtained from five dairies for *B. cereus* for 1 year. On the day of packaging, *B. cereus* was found in an average 8.1 % of samples (9 % of low-temp. pasteurized and 7.3 % of high-temp. pasteurized samples). The level of contamination at individual dairies ranged from 2.1 to 14.5 % of samples.

Bacterial load of milk and milk products varies with the type of husbandry and management and also with the kind of the products. It also varies from farm to farm and between regions. The bacterial load may increase during the transport storage and disposal of the milk and milk products. The same holds true for *B. cereus* as well. Raevuori and Koironen (1978) detected *B. cereus* in 15 of 3,500 mastitis milk samples tested at the Finnish State Veterinary Institute, Helsinki, during a 3-month period, but no organism was detected in any of 3,106 samples tested by 11 municipal milk-testing laboratories. At the same time analysis of commercial milk products revealed *B. cereus* in 3 out of 80 farm milk samples, 4 of 48 pasteurized milk samples, 2 of 39 pasteurized cream samples and 2 of 13 dried milk samples.

El-Naway et al. (1982) found each of 70 samples of market milk, scalded cream and Domiati cheese contained *B. cereus*, with average cell counts of 2.55×10^2 , 6.70×10^2 and 2.40×10^3 /g, respectively.

Ahmed et al. (1983) analyzed 400 samples of milk and milk products over a 5-month period from different retail outlets in Madison, Wisconsin and isolated *B. cereus* from 9, 35, 14 and 48 % of raw milk, pasteurized milk, Cheddar cheese and ice cream samples respectively.

B. cereus was also detected in various kinds of baby food products, produced at a factory in Hradec Kralove, Czechoslovakia (Jarchovska 1987).

Raimundo and Robbs (1988) observed that out of 30 samples of milk and milk products, procured from supermarkets in Rio de Janeiro City, Brazil *B. cereus* was present in 66.6 % of pasteurized milk samples, 80.0 % of full-fat dried milk and 13.3 % of dairy cream samples.

Kamat et al. (1989) analyzed pasteurized milk and milk products and protein-rich food powders containing milk or cocoa with average *B. cereus* count from 2×10^2 to 5×10^5 /g.

Homleid (1993) examined a number of samples of skim, low-fat and whole milk, whipping cream and 20 % fat cream from Norwegian dairies at the KIM (Milk Products Control Institute) immediately after production and after 10 days storage and reported that *B. cereus* was present in about 8–10 % of samples of each product type, except 20 %-fat cream.

Te Giffel et al. (1997) reported that *B. cereus* was present in both raw and pasteurized milk with a prevalence of 2–37 %. Similarly Carp-Carare et al. (2000) reported its presence in milk powder and found *B. cereus* in 76.1 % of the samples. Floristean et al. (2004) found 17.59 % of milk and dairy products samples positive for *B. cereus* with the highest prevalence of 37.5 % in powdered milk samples.

Reyes et al. (2007) observed 45.9 % incidence of *B. cereus* in dried milk products (milk with rice, milk substitute, milk powder, milk-cereal-rice, pudding milk, flan, and mousse) which were used by the Chilean School Feeding Program. Chitov et al. (2008) found all pasteurized milk samples positive for *B. cereus* and the bacterial count varied between 50 and 1.7×10^3 cfu/g.

Other foods

B. cereus has also been isolated from a wide variety of foods other than meat and milk, such as desert mixes (Warburton et al. 1987), infant foods (Becker et al. 1994), spices (Konuma et al. 1988; Choo et al. 2007; Kim et al. 2013), ready to serve foods (Harmon and Kautter 1991), seafood (Wijnands et al. 2006; Rahmati and Labbe 2008), coca/chocolate (Te Giffel et al. 1996), pulses, cereals and cereal derivatives (Te Giffel et al. 1997), fresh vegetables (Valero et al. 2002) and rice (Sarrias et al. 2002).

Rusul and Yaacob (1995) analysed the enterotoxigenic *B. cereus* strains in dried foods and wet wheat noodles in Malaysia and found that 50 % of the 164 enterotoxigenic strains were able to grow at +5 °C. In Netherlands, the incidence of *B. cereus* and *B. subtilis* in various food products (milk, yeast, flour, pasta products, Chinese meals, cocoa, chocolate, bakery products, meat products, herbs and species) was found to be 48 %, with the contamination level from 10^2 to 10^6 bacteria/g or per ml (Te Giffel et al. 1996).

Desai and Varadaraj (2009) found 46 % traditional foods samples positive for *B. cereus* by PCR with 16S rDNA and phosphatidylinositol phospholipase C primers in PCR. They also stated that toxigenic traits appear to be well spread within *B. cereus* cluster and have become stable traits among food isolates of *B. cereus* prevalent in the food chain.

In Iran, Rahimi et al. (2013) reported 42 % samples of infant foods positive for the presences of *B. cereus* and its enterotoxigenic genes. *B. cereus* count ranged from 30 to 93

spores per gram sample. This study is the first prevalence report of *B. cereus* and its enterotoxigenic genes in infant foods in Iran.

Scenario of *B. cereus* food poisoning in India

In India, majority of outbreaks of foodborne disease go unreported, unrecognized or un-investigated and may only be noticed after major health or economic damage. In such a condition controlling the outbreaks, detection and removal of implicated foods, identification of the factors that contribute to the contamination, growth, survival and dissemination of the suspected agent, prevention of future outbreaks and strengthening of food safety policies and programs are not possible. The reported bacterial foodborne disease outbreaks in India during 1980–2009 indicated that 24 outbreaks have occurred involving 1,130 persons. As per Park (2011), 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). In such a scenario the sure shot identification of the causative agent becomes very difficult and as such there are a few reports of food poisonings due to *B. cereus* in this country.

In 1978 Kulshreshtha, 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.

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. Such a case of food poisoning due to *B. cereus* was reported by Lakhani (1979) 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.

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). Symptoms appeared 2 h after ingestion. The foods implicated were gulabjamun and samosa, a kind of a ready to eat food in India. Analysis of remaining suspect food indicated that the outbreak could have been due to staphylococcal enterotoxins and/or *B. cereus* toxins. One other incident of *B. cereus* food poisoning was recorded by Singh et al. (1995) 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.

Bulk milk and milk products can also serve as a source of *B. cereus* food poisoning, where by a proper hygiene is not maintained during the preparation, transport and storage of the raw materials and end 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) 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), 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. Victims developed nausea and vomiting symptoms 3 h after consumption of the food.

Bachhil and Negi (1984), isolated *B. cereus* from 35 % of fresh buffalo meat and 65 % of cooked and semi-cooked buffalo meat samples, while Konuma et al. (1988) found *B. cereus* with $<10^2$ /g contamination levels in 18.3 % meat products and 6.6 % raw meat samples. In an another study Bachhil and Jaiswal (1988) reported incidence of *B. cereus* in 35 % of fresh buffalo meat, 100 % of kababs and 30 % of curry sample, with a mean counts of 9.65×10^4 , 1.07×10^3 and 6.4×10^2 *B. cereus*/g, respectively. Contamination of *B. cereus* is also reported Yadava 2004, from variety of foods from India viz. fish (40 %), chicken and meat products (80 %) (Kamat et al. 1989) fried rice (24 %) and chow Mein (23 %).

Anamika and Kalimuddin (2004) found that a total of 81 samples out of 120 samples of khoa, paneer and mushroom obtained from local and standard shops in Ranchi, Jharkhand, India, were contaminated with *B. cereus*.

Bedi et al. (2004) found an overall incidence of 56.3 % of *B. cereus* in chicken, mutton, butter chicken, chicken soup and mutton soup.

Bedi et al. (2005) reported an overall incidence of 53.8 % of *B. cereus* in raw milk, burfi and skimmed milk powder samples and in 20 % of the positive samples, the level of *B. cereus* contamination was more than 10^5 cfu/g.

Analysis of 200 raw and cooked mutton samples by the Willayat et al. (2007) revealed the presence of *B. cereus* in 47 samples and the prevalence of 30 % and 15 % respectively.

Fish, shrimp and clam samples procured from local markets of Cochin were screened by Das et al. (2009) for the presence of *B. cereus* and found 25 samples positive for *B. cereus* and 42 isolates of this bacterium were recovered from those positive fish samples.

Meat samples (mutton tikka and chutney samples) collected from Kashmir valley, India showed 45 % prevalence of *B. cereus* in mutton tikka and 32.5 % in the chutney samples (Hafiz et al. 2012) and 16 % in the raw milk (Altaf et al. 2012).

A study was carried by Sudershan et al. (2012) to identify microbiological hazards and to assess their exposure associated with consumption of poultry based street food served in different localities of Hyderabad and found that most prevalent pathogenic bacteria isolated were *S. aureus* and *B. cereus*. They observed that rice and noodles were kept at room temperature for about 5–6 h which was a critical control point.

In another study, Tewari et al. (2013) reported 30.9 % overall incidence of *B. cereus* in various meat and meat products, while the recorded incidences of *B. cereus* from raw meat and meat products samples were 27.8 and 35 %, respectively.

Prevention 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. 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 (Lee et al. 1995). 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* (Sandra et al. 2012). 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 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):

- 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 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. 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 raw milk 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 spore morphology. The *B. cereus* spores have covering of long appendages and these promote adhesion (Husmark and Ronner 1992).

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.

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