

Review Article

Growing Role of *Bacillus Cereus* as an Emerging Potential Food Pathogen of Humans: A Review

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Received: August 28, 2024

Accepted: September 19, 2024

Published: September 26, 2024

Abstract

The genus *Bacillus* includes gram-positive and gram variable rod-shaped bacteria that sporulate under aerobic conditions. *B. cereus* (*Bacillus cereus*) is a Gram-positive spore-forming bacterium commonly found in the environment. *B. cereus* causes two types of food poisoning, the emetic and diarrheal syndromes, and a variety of local and systemic infections. *B. cereus* is widespread in nature and frequently contaminates a wide variety of food products. The incidence of both the diarrheal and emetic syndromes caused by *B. cereus* probably has been underestimated because the illnesses are usually self-limiting with mild symptoms. Despite the recognition of *B. cereus* as a foodborne pathogen over 50 years ago, its virulence mechanisms are still not fully elucidated. Cereulide has been identified as the causative agent in the emetic syndrome, and HBL is associated with diarrheal food poisoning. Nhe, a homolog of HBL, probably possesses biological activities similar to those of HBL and could be a factor in the diarrheal syndrome; however, this hypothesis has not been tested. In addition to causing food poisoning, HBL can play a role in non-gastrointestinal infections caused by *B. cereus*. The *in vivo* roles of many of the putative and potential virulence factors produced by *B. cereus*, such as hemolysins, phospholipases, and proteases, have not been defined. These variables are probably involved in infections and diseases caused by *B. cereus* based on their biological activity. The genus and several species of *B. cereus* are included in this review, but the strains and toxins that cause foodborne illness are the main focus. Many putative virulence factors are produced by *B. cereus*, however most of these factors' involvement in particular infections are yet unknown. Thus far, only two substances have been specifically identified as emetic and diarrhoeal toxins, respectively: cereulide and the tripartite haemolysin BL. Another homolog of haemolysin BL that has been linked to the diarrhoeal illness is nonhemolytic enterotoxin. Apart from food poisoning, *B. cereus* has been linked to a number of infectious diseases in the past and present, such as periodontal diseases, ocular infections (such as endophthalmitis, panophthalmitis, and keratitis), skin infections, post-operative and post-traumatic wound infections (with or without bone involvement), necrotising fasciitis, salpingitis, meningitis, and endocarditis.

Keywords: *Bacterium*; *Bacillus*; *B. cereus*; *Hemolysin*; *Pathogen*

Abbreviation: ATR: Acid Tolerance Response; GIT: Gastro-Intestinal Tract; MYP: Mannitol-Egg Yolk-Phenol Red-Polymyxin-Agar; PEMBA: Polymyxin Pyruvate-Egg Yolk-Mannitol-Bromothymol Blue-Agar

Introduction

Bacillus cereus is a Gram-positive spore-forming bacterium commonly found in the environments. This bacterium is also a major contaminant of raw or processed foods of plant or animal origin [58]. *B. cereus* exists as a soil saprophyte that can adapt and proliferate in the lower sections of the Gastro-Intestinal Tract (GIT) [90]. It is also an opportunistic pathogen responsible for local and systemic infections [88].

Bacillus cereus causes two types of food poisoning (the emetic and diarrheal syndromes) and a variety of local and systemic infections such as endophthalmitis, endocarditis, meningitis, periodontitis, osteomyelitis, wound infections, and septicemia [24]. The pathogenesis of *B. cereus* is still largely undefined. The organism produces a large number of potential virulence factors, including multiple hemolysins, phospholipases, and proteases [11]. However, the roles of these factors in specific infections have not been established. The emetic toxin has been identified as cereulide [4] and the tripartite Hemolysin BL (HBL) has been established as a diarrheal enterotoxin [12]. A homolog of HBL, nonhemolytic enterotoxin (Nhe), also has been associated with the diarrheal syndrome [56]. While certain *B. cereus* strains have been used as probiotics [48], others may cause food poisoning in humans [25]. The pathogenicity of *B. cereus* is attributed to the species' production of extracellular factors such as phospholipase cereulide (emetic toxin), enterotoxin Hbl, non-haemolytic toxin (Nhe), haemolysin IV, which has a strong disruptive effect on cellular membranes, and associated with the induction of necrotic enterocolitis cytotoxin (CytK) [41].

Its name derived from the cell shape (bacillus, rod) and colony appearance (cereus, wax). The facultative anaerobic *B. cereus* has been isolated from almost all categories of foodstuff, as it is able to grow in very diverse habitats like soil and sediments [88]. *B. cereus* spores can reach concentrations of up to 103-105 cells per gram soil [99]. Moreover, *B. cereus* has been isolated from the insect gut of several arthropod species in high frequency and a commensal lifestyle has been proposed for this bacterium [79].

A factor that plays a role in acid resistance is the mechanism of cross-protection between the different stresses microorganisms are exposed. For example, exposure of the microorganism to the various stresses they encounter in the course of food production, e.g., heat processing, dehydration, or acidification, can elicit a higher tolerance to stresses encountered passing through the stomach [66]. Indeed, a sub lethal acidic environment can trigger an adaptive response that protects the bacterium during subsequent incubations at lethal acidic pH. This mechanism is known as Acid Tolerance Response (ATR) and plays an important role in the adaptation of intestinal pathogens to the pH of the stomach [21]. *B. cereus* vegetative cells are also able to induce ATR [79]. The ATR of *B. Cereus* may involve (i) FOF1 ATPase and/or glutamate decarboxylase (implicated in pHi homeostasis), (ii) modifications of metabolism and (iii) synthesis of proteins which act as protect and/or repair factors [79]. The ability to generate protecting biofilms and to form endospores, which are metabolic inactive and resistant to harsh conditions such as heat (>100°C), many chemicals, radiation as well as desiccation, allows *B. cereus* to survive [1].

The toxicological profile of *B. cereus* strains ranges from non-pathogenic strains used as probiotics in animal feed [53]. *B. cereus* is mainly known to evoke two types of gastrointestinal food borne poisonings. The emetic type indicated by nausea

and forceful vomiting shortly after ingestion is caused by the small dodecadepsipeptide cereulide [3], which is produced by a subgroup of *B. cereus* [28]. The diarrheal syndrome is caused by several heat labile enterotoxins produced during growth of *B. Cereus* in the intestine. The Hemolysin BL (HbL) and the pore forming non-hemolytic toxin (Nhe) belong to the class of three-component enterotoxins, whereas cytotoxic K (CytK) represents a β -barrel channel forming one-component enterotoxin [57]. In general, 6 to 12 hours after ingestion of about 10⁵ to 10⁷ cells, abdominal cramps and diarrhea occur, but the course of the disease is normally relatively mild and symptoms disappear within 24 hours [88]. The extent and duration of the disease depend on the infection dose and the number of produced enterotoxins, which seem to differ strongly among different *B. cereus* strains [26]. Besides the known toxins, *B.Cereus* also produces several enzymes like sphingomyelinase, phosphatidylinositol and phosphatidylcholine-specific phospholipases and several proteases that are so far not directly associated with gastrointestinal diseases, but may play an important role in non-gastrointestinal infections such as wound and eye infections, bacteremia, pneumonia, meningitis, periodontitis, and endocarditis [74]. Most notably, the high hydrophobic character of the spores seems to increase their adherence to the surface of food processing machines and equipment, pipelines as well as tanks leading to contamination of food products by direct contact with these different sources [30]. Consequently, once spores have entered the food, pasteurization or normal sanitation processes will not contribute to their elimination [27].

B. cereus is one of six members of the *Bacillus cereus* group within the genus *Bacillus*. The other members of this genetically closely related group are *Bacillus anthracis*, *B. thuringiensis*, *B. weihenstephanensis*, *B. mycoides* and *B. pseudomycoides* [22]. Although a clear separation of these species by phenol-typing or classical DNA hybridization studies failed, these bacilli differ significantly in their ecological features such as the synthesis of virulence factors, specialized morphology and cold adaption [8]. These special features are mainly encoded by genes located on mega plasmids like e.g. pXO1 and pXO2 of *B. anthracis*. The causative agent of the fatal mammalian disease anthrax, *B. anthracis*, arrested attention in 2001 for its use as bioterrorism agent and biological weapon developed by several countries [46]. The insect pathogen *B. thuringiensis* produces toxic crystals (δ -endotoxins), which are encoded on a plasmid and lyse midgut epithelial cells [14]. *B. thuringiensis* is routinely used as agent to control agricultural insect pests [18].

The prevalence of *B. cereus* induced food-borne illnesses is difficult to determine, because the symptoms associated with *B. cereus* infections or intoxication are mild, so it is conceivable that many *B. cereus* infections are not reported and that the prevalence of these infections is largely under estimated [39]. *B. cereus* illness recognized that there may be significant under reporting due to the generally mild, short duration and self-limiting symptoms, in addition to its being infrequently tested for in routine laboratory analyses of stool samples [41] and due to lack of effective surveillance, *B. cereus* associated food poisoning may be largely under reported, and probably confused with *Staphylococcus aureus* and *Clostridium perfringens* food poisoning due to similar symptoms [88].

Objectives

To review on *Bacillus cereus* nature, morphology source of contamination, Pathogenic virulence, mode of transmission and its public health importance.

Organisms and Growth Condition

The Organism: Characteristics and Identifications

B. cereus was originally described as a mesophilic organism, growing between 10 and 50°C and with an optimum temperature of 35 and 40°C (Claus & Berkeley, 1986). The Latin term *ce-reus* indicates wax-like, whereas the word *bacillus* denotes little rod. The name refers to *B. cereus*'s easily identifiable shape under a microscope or on blood agar plates. *B. cereus* is a big rod-shaped (1.0–1.2 mm by 3.0–5.0 mm) Gram-positive bacterium that grows to enormous colonies (3–8 mm diameter) on ordinary agar medium. Its appearance is fairly fawny, greyish, and reminiscent of "ground glass," with frequently uneven borders.

On blood agar, the colonies are surrounded by zones of beta hemolysis [52], the size of which is often large, but can vary depending on culturing conditions. On widely used agar media, the majority of strains will develop endospores in a few days. *B. cereus* spores are ellipsoidal, positioned centrally or par centrally, and do not spread the cell [36]. Employing phase contrast microscopy or spore staining techniques, the placement and morphology of the spores are much used criteria to distinguish the species of the genus *Bacillus* [34].

Other commonly used features for identification are motility, haemolysis, carbohydrate fermentation (*B. cereus* does not ferment mannitol) and the very active lecithinase (phospholipase) production [51]. Various plating media are used for the isolation, detection and enumeration of *B. cereus* from foods, including MYP (mannitol-egg yolk-phenol red-polymyxin-agar) and PEMBA (polymyxin pyruvate-egg yolk-mannitol-bromothymol blue-agar) [47]. These media make use of the bacterium's lecithinase synthesis (the egg-yolk reaction that results in precipitate zones) and absence of mannitol fermentation in addition to specific chemicals like polymyxin.

A thorough description of these media is found in Kramer & Gilbert [52]. More recently, chromogenic media have been developed for several food pathogens, including *B. cereus* (for instance *Cereus-Ident-Agar* from heipha Dr Muller GmbH, and chromogenic *B. cereus Agar* from Oxoid Ltd). These new media have been evaluated together with standard plating media by Fricker *et al.* [32].

Colony Morphology

Colony Morphology When grown under aerobic conditions on 5% sheep blood agar at 37°C, *B. cereus* colonies are dull gray and opaque with a rough matted surface (Figure 1).



Figure 1: Gray, opaque, granular, spreading colonies with irregular perimeters growing on 5% sheep blood agar. Note the smaller smooth colonies admixed among spreading growth Wong *et al.*, [12].

Colony perimeters are irregular and represent the configuration of swarming from the site of initial inoculation, perhaps due to *B. cereus* swarming motility [35]. Zones of beta-hemolysis surround and conform to the colony morphology [95].

Reservoirs and Lifestyles

B. cereus is described as being of ubiquitous presence in nature and can be found in many types of soils, sediments, dust and plants (Schoeni & Wong, 2005). Spores may be passively spread and thus found also outside natural habitats. It is thought that *B. cereus sensulato* is present in soil as spores, which when in touch with organic matter, an insect or animal host, or both, germinate and thrive. A study revealing that *B. cereus* could germinate, develop, and sporulate in soil, indicating a saprophytic life cycle, was motivated by interest in the ecology of this bacteria [98].

Additionally, a filamentous mode of growth in a multicellular phenotype was seen and proposed as a route of translocation via soil [98]. In the intestines of insects, a multicellular filamentous style of growth has also been seen. When spore-forming bacteria that were later identified as *B. cereus* were recovered from the guts of various soil-dwelling arthropod species, it was proposed that the bacteria lived in the intestines of insects. These bacteria appear to live in symbiosis with their invertebrate host [61].

The existence of different morphological modes used by *B. cereus*, such as the filamentous mode, may be adaptations to different life cycles like the 'normal' cycle of life as a symbiotic or the more infrequent pathogenic life cycle with rapid growth. *Bacillus cereus* has been reported to be present in stools of healthy humans at varying levels [49]. *B. cereus* would have a transitory existence in the mammalian gut due to its widespread low-level prevalence in surroundings, feed, and foods [52].

The suggested probiotic effect of *B. cereus* may stem from its potential adaption to the conditions of the animal gut. Since all strains of *B. cereus* are capable of producing at least one toxin linked to diarrhoeal illness, such use cannot be categorically deemed safe for humans [48]. However, the European Food Safety Authority (EFSA) has allowed the use of probiotics including particular strains that produce insignificant quantities of toxicity at 37 OC. Since *B. cereus* is widespread in so many different settings, it seems to reason that it would also be present in water. Nevertheless, there are few data on *B. cereus*'s occurrence in water sources, and there are no established techniques for detecting the bacteria in water. Norwegian surface waters were investigated for presence of *B. cereus* spores, and cytotoxic strains were isolated from several rivers. This suggests the possibility that the water supply may be a means by which *B. cereus* enters the food processing chain microbial communities as a natural niche for part of the *B. cereus* life cycle is further [72].

Emetic and Diarrheal Food Poisoning Caused by *B. cereus*

Two distinct foodborne disease types, emetic and diarrheal, are associated with *B. cereus*. Both are generally mild and self-limiting, although more serious and even lethal cases have occurred [23]. *Bacillus cereus* was established as an organism of foodborne disease in the 1950s, with the first described outbreaks of the diarrheal type of disease in hospitals in Norway in 1947–1949 [43]. Earlier descriptions of disease which could probably be attributed to *B. cereus* lack the nomenclature and epidemiological framework that would allow this attribution,

Table 1: Selected major outbreaks associated with *B. cereus*.

Year	No. of cases (fatalities)	Food	Syndrome type	Country	Comments	References
2008	1 (1)	Spaghetti with tomato sauce	Emetic	Belgium	Food stored at room temperature for 5 days after preparation. <i>B. cereus</i> and cereulide isolated from pasta	Naranjo <i>et al.</i> [68]
2007	2 (1)	Asparagus sauce	Emetic	Australia	Prior to serving, the sauce was stored for 2 hours in a hot kitchen (up to 37°C), permitting <i>B. cereus</i> growth	NSW Food Authority 2013
2003	4 (1)	Pasta salad	Emetic	Belgium	Food stored for 3 days in fridge at 14°C, permitting <i>B. cereus</i> growth. <i>B. cereus</i> isolated from food	Dierick <i>et al.</i> [23]
2000	173	Cake	Diarrhoeal	Italy	<i>B. cereus</i> isolated from food and rolling board. Rolling board likely source of contamination	Ghelardi <i>et al.</i> [35]
1998	44 (3)	Vegetable puree	Diarrhoeal	France	Cytotoxin K produced by <i>B. cereus</i> involved	Jenson and Moir [49]
1991	139	Barbequed pork	Diarrhoeal	US	<i>B. cereus</i> spores from dried foods, slaughtered animals or worker hands likely source of contamination. Unrefrigerated storage of cooked pork for >18 hours permitted <i>B. cereus</i> growth	Luby <i>et al.</i> [55]
1989	55	Cornish game hens	Diarrhoeal	US	Inadequate thawing and cooking, cross-contamination from basting brush used before and after cooking, inadequate refrigeration	Slaten <i>et al.</i> [87]

Source: FDA [31].

however there is little doubt that *B. cereus* has been implicated in foodborne disease historically [52]. The emetic syndrome was first identified after several outbreaks caused by eating cooked rice in the United Kingdom in the early 1970s [64]. This disease is an intoxication caused by the *B. cereus* emetic toxin, named cereulide, produced in foods before ingestion. The course of the disease is characteristic, with nausea and emesis occurring only a few hours after the meal. [52]. The most important differential diagnosis is intoxication with *Staphylococcus aureus* enterotoxins, which causes similar symptoms; however, in this disease emesis is commonly accompanied by diarrhea [85]. Several severe and even lethal cases of emetic foodborne *B. cereus* disease have been reported [33]. The diarrheal syndrome is thought to be a toxic infection caused by vegetative cells, ingested as viable cells or spores, producing protein enterotoxins in the small intestine [20]. It is easily confused with the foodborne disease caused by another spore forming bacterium, *Clostridium perfringens* (Granum, 1990), and typically presents with abdominal pain, watery diarrhea and occasionally nausea and emesis. The incubation time is over 6h, normally in the range of 8–16h, and on average 12h, but in rare cases longer incubation times have been observed [52].

Non gastrointestinal illness of *B. Cereus*

B. cereus has been associated with illness other than food poisoning, although these infections are not common. The bacterium has been found contaminating post-surgical or traumatic wounds and burns and causes a variety of opportunistic infections, especially in immunocompromised patients, including bacteremia, septicemia, endocarditis, meningitis, pneumonia, pleuritis, osteomyelitis, and endophthalmitis [24]. These infections can be highly fulminant and sometimes fatal. *B. cereus* can also play a role in sepsis caused by use of contaminated needles by intravenous drug abusers or by following penetrating wounds [11].

Involvement of *B. cereus* in pneumonia is rare and is usually associated with other risk factors such as leukemia. However, two unusual cases were reported that involved two previously healthy middle-aged individuals who experienced fulminant

bacteremia and pneumonia caused by *B. cereus*, with symptoms similar to those of *B. anthracis* pulmonary infection [63]. Both individuals experienced chills for three to five days, fever, cough, and bloody expectoration before hospitalization. Their conditions deteriorated in the hospital, and both patients died. *B. cereus* is a common cause of eye infections, often causing irreversible tissue damage in a short time (within 24 h). It is one of the most common causes of post traumatic endophthalmitis, where the organism is introduced into the eye by foreign bodies as a consequence of traumatic injury [45]. It also causes metastatic endophthalmitis from hematogenous spread of the organism to the eye from other sites. Metastatic endophthalmitis is often highly fulminant and sometimes fatal. The rapid progression and fulminance of *B. cereus* endophthalmitis is attributed to multiple toxins elaborated by the organism, including the diarrheal enterotoxin HBL [13].

Pathogenic Strain and Pathogenicity of *Bacillus cereus*

The pathogenicity of *B. cereus* is known to be intimately associated with production of tissue-destructive/reactive exoenzymes; among these secreted toxins are three distinct phospholipases, an emesis-inducing toxin (which is responsible for the vomiting syndrome), and three pore-forming enterotoxins: the cytotoxin K, the Hemolysin BL (HBL), and a Non-Hemolytic Enterotoxin (NHE) [17].

The toxicological profile of *B. cereus* strains ranges from non-pathogenic strains used as probiotics in animal feed [53] to highly pathogenic isolates, which are responsible for major outbreaks such as 173 cases of intoxication after a banquet [84] or severe individual cases leading to hospitalization and even death. *B. cereus* is mainly known to evoke two types of gastrointestinal food borne poisonings. The emetic type indicated by nausea and forceful vomiting shortly after ingestion is caused by the small dodecade psipeptide cereulide [3], which is produced by a subgroup of *B. cereus* [28]. The diarrheal syndrome is caused by several heat labile enterotoxins produced during growth of *B. cereus* in the intestine. The hemolysin BL (HbL) and the pore forming non-hemolytic toxin (Nhe) belong to the class of three-component enterotoxins, whereas cytotoxin K (CytK)

Table 2: Summary for differentiation of the *Bacillus cereus* from another common Bacillus cereus group.

Tests	<i>B. anthracis</i>	<i>B. cereus</i>	<i>B. mycooides</i>	<i>B.thuringiensis</i>
Motility	-	+	-	+
Hemolysis	-	+	Weak	+
Penicillin susceptibility (10 unit disc)	S	R	R	R
Gelatin stab culture	Inverted fir tree type of growth	Rapid liquefaction	Rapid liquefaction	Rapid liquefaction
Lecithinase activity (egg-yolk agar)	+ weak	+	+	+
Nutrient agar with 0.7% Na-bicarbonate under 10% CO ₂	Mucoid colonies	Unchanged	Unchanged	Unchanged
Susceptibility to cherry gamma phage	+ (lysis)	-	(+) lysis may occur	-
Pathogenicity for mice or guinea-pigs (subcut. or i.v.)	+ (death in 24-48 hrs)	+ large dose (non-invasive)	-	-

Source: Qunnin et al., [78].

represents a β -barrel channel forming one-component enterotoxin [57].

In general, 6 to 12 hours after ingestion of about 10^5 to 10^7 cells, abdominal cramps and diarrhoea occur, but the course of the disease is normally relatively mild and symptoms disappear within 24 hours (Stenfors Arnesen *et al.*, 2008). The extent and duration of the disease depend on the infection dose and the amount of produced enterotoxins, which seem to differ strongly among different *B. cereus* strains. Besides the known toxins, *B. cereus* also produces several enzymes like sphingomyelinase, phosphatidylinositol and phosphatidylcholine-specific phospholipases and several proteases that are so far not directly associated with gastrointestinal diseases, but may play an important role in non-gastrointestinal infections such as wound and eye infections, bacteremia, pneumonia, meningitis, periodontitis, and endocarditis [74].

In recent years, the number of food borne outbreaks caused by *B. cereus* has increased in industrial countries [88]. The ability to generate protecting biofilms and to form endospores, which are metabolic inactive and resistant to harsh conditions such as heat (>100°C), many chemicals, radiation as well as desiccation, allows *B. cereus* to survive e.g. treatments of the food industry like conservation, chemical disinfection and preservation [1]. Most notably, the high hydrophobic character of the spores seems to increase their adherence to the surface of food processing machines and equipment, pipelines as well as tanks leading to contamination of food products by direct contact with these different sources [30]. Consequently, once spores have entered the food, pasteurization or normal sanitation processes will not contribute to their elimination [27].

Isolation and Identification of *B. Cereus*

B. cereus can be isolated and identified from foods and from clinical samples taken from cases of food poisoning. Bennett and Belay (2001) and Kramer and Gilbert [52] have provided detailed descriptions of these methods. Mannitol-egg yolk-polymyxin agar and the same agar supplemented with pyruvate and bromothymol blue are two often used differential media.

These formulations take advantage of the fact that unlike many Bacillus species, *B. cereus* does not ferment mannitol but does produce phosphatidylcholine-preferring phospholipase C (lecithinase). Polymyxin B is used as the primary selective agent for the *B. cereus* group. Classical identification schemes for members of the Bacillus genus have been described extensively [94].

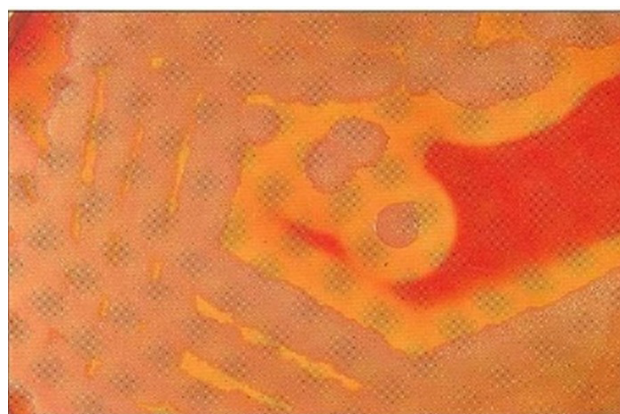
Generally, *B. cereus* colonies on solid media are 5 to 6 mm in diameter and have a ground glass or matte appearance, with edges that range from circular and entire to irregular and fimbriate. Colonies often appear greenish on blood agar. The

spores are ellipsoidal or cylindrical and do not swell the sporangia. Confirmation of *B. cereus* involves a battery of biochemical tests. These tests can be performed using conventional methods (Bennett and Belay, 2001) or using miniaturized commercial systems that combine biochemical profiles with information in large databases. Serotyping has been a useful tool in epidemiological studies. Forty-two serotypes have been identified based on serological classification of spore, somatic, and flagellar antigens. Twenty-three of these 42 serotypes are associated with *B. cereus*-related disease [24]. The flagellar (H) serotypes most commonly associated with diarrheal food poisoning are 1, 2, 6, 8, 10, 12, and 19. The H serotypes 1, 5, 8, 12, and 19 are commonly associated with the emetic food poisoning syndrome. Some *B. cereus* strains may cause both forms of food poisoning [40]. Phage typing as a means of *B. cereus* identification was explored because of the specificity of Bacillus-associated bacteriophages for their host strain [97].

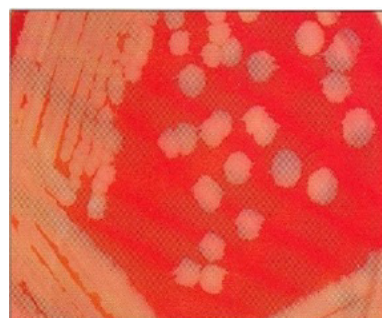
Biochemical Test

Bacillus Cereus colony similar with Bacillus anthrax and other bacillus groups

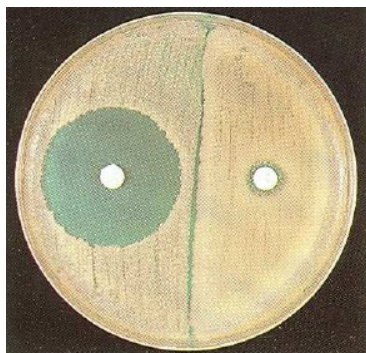
differentiation of *B.cereus* and *B.anthrax* on sheep blood agar



B.Cereus Complete Hemolysis on Blood Agar



B. anthracis on sheep blood agar illustrating nonhemolytic, flat, 'ground-glass', dry (dull) colonies with irregular edges on let P.J.Qunnin *et al.*, [78].



B. anthracis (left) and *B. cereus* (right) on demonstrating the susceptibility of *B. anthracis* to penicillin (10 unit disc) compared to the resistance of *B. Cereus*

Susceptibility to penicillin



Strong lecithinase activity by *B. cereus* (top) on egg yolk agar after 24 hrs incubation

B. anthracis (left) gives a weak opaque zone after 48 hrs

B. licheniformis (right) is unreactive in this medium

lecithinase activity on egg yolk agar (*B.cereus*, *B. anthracis* and *B.licheniformis*)

Source: P.J.Qunnin *et al.*, [78]

Epidemiology

B. Cereus is a ubiquitous environmental rod; specifically, its natural reservoir is represented by decomposing organic matter and vegetables, fresh and marine waters, and the invertebrate intestinal tract, which can contaminate soil and food and cause temporary colonisation of the human gut) [17]. When bacilli come into contact with organic matter or within an insect or animal host, they may bacilli lose their flagella, attach to the arthropod enteric epithelium, and sporulate [17]. *B. cereus* also has a saprophytic life cycle where spores germinate in the soil, with the production of vegetative bacilli, that could then sporulate, keeping the cycle; following host defecation or death, cells and spores are released into the soil, where vegetative organisms may sporulate again, then surviving until the uptake by another host [17]. Survival of *B. cereus* in the environment is then strictly related to the spore; the latter is in fact resistant to extreme conditions including heat, radiation, drying, freezing, and may be considered to be the infective agent for this organism. In the food industry, such spores are particularly troublesome since they can be refractory to pasteurization and gamma radiation; moreover, their hydrophobic nature permits them to adhere to surfaces, and this feature enables the bacterium to spread to all kinds of food. Given the ubiquitous distribution of *B. cereus* in food products, therefore, the organism is ingested in small amounts and becomes part of the transitory human enteric flora; it is unclear, however, if the recovery of *B. cere-*

us from man stools is a function of germinating spores or the growth of vegetative cells [17].

Outbreaks of *Bacillus Cereus* Foodborne Disease

The incidence of foodborne *B. cereus* disease is significantly underreported due to a number of causes. It is a result of the disease's often brief and mild course, which deters patients from seeking medical care and makes it difficult to distinguish between illnesses brought on by food poisoning and *S. aureus* intoxication (Kotiranta *et al.*, 2000). The percentage of food poisoning outbreaks associated with *B. cereus* varies from country to country and is dependent on the reporting system. In The Netherlands, from 1991 to 1994 *B. cereus* was identified as the most common cause (19%) of food poisoning outbreaks [86]. In Taiwan, from 1986 to 1995 *B. cereus* outbreaks ranked third, behind those caused by *Vibrio para haemolyticus* and *Staphylococcus aureus* (Horng *et al.*, 1997). Mead *et al.* [62] estimated that more than 27,000 foodborne illnesses annually in the United States are caused by *B. cereus*, which from 1993 to 1997 ranked seventh among the etiological agents causing reported bacterial foodborne outbreaks (14 of 655; 0.5%) and sixth as the causative agent for reported cases (691 of 43,821; 0.8%) [71]. IN Ethiopia *Bacillus cereus* in raw milk samples was 38.8% and 15.4% reported by Ashabir *et al.*, and Kassa *et al.*, respectively [2,9].

Aside from food poisoning, several infectious processes have been attributed to *B. cereus* in the past and recent years, including periodontal diseases, ocular infections (endophthalmitis, panophthalmitis and keratitis, that develop after the microorganism introduction into the eye due to the occurrence of a traumatic event), skin as well as post-operative and posttraumatic wound infections (with or without bone involvement), osteomyelitis, necrotizing fasciitis, salpingitis, meningitis, endocarditis, bacteremia [82].

Mode of Transmission

B. cereus food poisoning can be caused by either ingesting large numbers of bacterial cells and/or spores in contaminated food (diarrheal type) or by ingesting food contaminated with pre-formed toxin (emetic type). Food contamination, incorrect food handling and storage, and inadequate chilling of cooked food items are the main causes of this disease's spread [83]. Most remarkably, the spores' extreme hydrophobicity appears to boost their adherence to the surfaces of tanks, pipelines, and machinery used in food processing, contaminating food products when they come into direct touch with these various sources [30]. Consequently, once spores have entered the food, pasteurization or normal sanitation processes will not contribute to their elimination [26]. The diarrhoeal syndrome is thought to be a toxic infection caused by vegetative cells, ingested as viable cells or spores, producing protein enterotoxins in the small intestine [20].

Symptoms of Disease

Due to the typically mild, transient, and self-limiting symptoms of *B. cereus* infection, as well as the fact that it is rarely screened for in standard laboratory analysis of stool samples, there may be a large underreporting of cases [41]. *B. cereus* is linked to two different foodborne illness types: diarrhoeal and emetic. Both are generally mild and self-limiting, although more serious and even lethal cases have occurred [23]. This disease is an intoxication caused by the *B. cereus* emetic toxin, named cereulide, produced in foods before ingestion. The course of the

disease is characteristic, with nausea and emesis occurring only a few hours after the meal [52].

Other than food poisoning the *B. Cereus* has been found contaminating post-surgical or traumatic wounds and burns causes a variety of opportunistic infections, especially in immunocompromised patients, including bacteremia, septicemia, endocarditis, meningitis, pneumonia, pleuritis, osteomyelitis, and endophthalmitis [24]. *B. cereus* is a common cause of eye infections, often causing irreversible tissue damage in a short time (within 24 h). It is one of the most common causes of post traumatic endophthalmitis, where the organism is introduced into the eye by foreign bodies as a consequence of traumatic injury (Boldt *et al.*, 1989).

Diagnosis and Culturing

B. cereus and its spores have very diverse contamination sources like soil, bedding, feed, dust, air, feces, dirty teats and milking equipment [88]. After the sample collected by peptone water from clinical diseased or food sample, then spread on to Bacillus cereus selective medium plate (Oxoid Ltd, London, UK) in duplicates. After that, the inoculation plates were monitored for colony growth for 18 to 24 hours while being incubated aerobically at 30 °C. The incubation period was increased by another 24 hours and the growth of the colonies was re-examined if none appeared. *B. Cereus* colonies were presumed to be based on colour, morphology, and egg yolk precipitation on selective medium: they were described as crenate colonies with a diameter of around 5 mm, a colour of turquoise blue, and an opaque zone surrounding them [69].

Confirmatory and Differential Tests

Two or three colonies were selected from positive plates and moved to nutrient agar slants. These were aerobically incubated for 24 hours at 30 °C. The *B. cereus* group was recognized by Gram staining as a big, rod-shaped, Gram-positive organism with short to long chains. *B. cereus* was differentiated from the group members as based on strong β -haemolysis on sheep blood agar (Oxoid Ltd, London, UK), diffuse growth in semisolid SIM medium (HiMedia Ltd, India) and characteristic pale green endospores without bulged sporangium and with no parasporal crystal bodies in red stained cytoplasm using rapid staining methods (warm 0.5% basic Fuchsin (BD Difco BBL Stains), Malachite green (Fluka) and Sudan Black B (Sigma-Aldrich) [2].

Antibiotics and Therapy

The antibiotic susceptibility profile of the isolated (and accurately identified) strain typically determines the therapeutic options for *B. cereus* illnesses; however, species-specific criteria for assessing and interpreting the in vitro response to medication have not yet been published. However, while waiting for the results of an antibiotic sensitivity test, empirical antimicrobial therapy may be essential in the event of a suspected *B. cereus* infection [17]. In general, most *B. cereus* isolates are resistant to penicillin and cephalosporin as a consequence of β -lactamase production; in particular, it is likely that resistance to penicillin, ampicillin and cephalosporin's should be considered to be constant, nowadays, like that to trimethoprim [19].

A chromosomal Metallo- β -Lactamase (MBL) is widespread in *B. cereus* (the enzyme is called 'BclI') [19]. Of interest, *B. cereus* MBL has been the first enzyme of this category that was discovered, in 1966 [80], while MBLs were later described in *Stenotrophomonas maltophilia*, *Aeromonas* spp, *Bacteroides fragilis*,

Acinetobacter spp, *Pseudomonas aeruginosa*, and some flavobacteria, and have been known to inhibit all β lactams except for the monobactam (aztreonam, to which Gram positive organisms are however intrinsically resistant) [82]. Furthermore, *B. cereus* usually produces Bush group 2a penicillinases I and III that typically hydrolyze penicillin and are inhibited by clavulanic acid [82].

Prevention and control

Canning is the only method that will remove *B. cereus* spores from food products because it is a common occurrence. Before being stored, spores can be found in practically every type of food, though usually in amounts too small to result in food-borne illness. A very significant initial food contamination risk exists, although temperature abuse-induced *B. cereus* multiplication is typically the cause of human health risks. Cleaning is a crucial step in preventing *Bacillus cereus* from growing on machinery and other equipment used to transport food inside the processing facility.

Whenever possible the use of hypochlorite (pH < 8) is recommendable at least in pipelines. This will eliminate or dramatically reduce the number of spores. The use of weak acids at 30-40 °C for 20-30 min can be an alternative to chemicals that can harm the pasteurizer or other equipment's (Anderson *et al.*, 1995). Spores of *Bacillus cereus* have a pronounced ability to adhere to the surface of stainless-steel material commonly used to build processing equipment for the food industry, which may become a reservoir of spores. Therefore, the attachment of *Bacillus cereus* to online processing equipment may present a major problem for the food industry [73]. It is a primary factor in both its existence and its challenging control. Controlling microorganisms that are present in food products and processing equipment is crucial to delivering wholesome and safe products to consumers. This could mean doing more frequent and thorough cleanings [75].

Conclusion

B.cereus are well-known opportunistic human pathogens, which can cause two different types of foodborne illnesses, emesis and diarrhea. Although the three main diarrhea-associated toxins, Nhe, HBL, and CytK, as well as the emetic toxin cereulide, are known over a decade and considerable progress has been made on the understanding of toxin gene regulation, the exact mechanisms of toxin syntheses and toxin actions are far from understanding. However, there is still a significant knowledge vacuum and no known causative genotypes for other disorders, such as food poisoning diarrhoea and (opportunistic) infections caused by pathogenic *B. cereus* strains. In conclusion, *B. cereus* is a complex microbe that exhibits a wide range of pathogenicity and ecological lifestyle variations. The virulence and food poisoning potential of these mysterious bacteria must thus be investigated using a multidisciplinary approach that integrates studies in epidemiology, molecular biology, taxonomy, metabolism, microbiology, ecology, and host-interaction.

Apart from food poisoning, *B. cereus* has been linked to a number of infectious diseases in the past and present, such as periodontal diseases, ocular infections (such as endophthalmitis, panophthalmitis, and keratitis, which arise from the introduction of microorganisms into the eye as a result of a traumatic event), skin infections, wound infections following surgery and trauma, osteomyelitis, necrotising fasciitis, salpingitis, meningitis, and endocarditis.

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