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Foodborne *Bacillus cereus*: Some New Observations

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Abstract

Bacillus cereus is a foodborne pathogen. It is a Gram-positive *Bacillus* featuring a non-bulging central spore. It has the ability to express many powerful toxins like Cereulide, Non-hemolytic enterotoxin, Hemolysin BL and Cytotoxin K. Foods responsible for *Bacillus cereus* food poisoning are rice, chowmein and chicken preparations. Different foods elaborate different types of toxins after harbouring the *Bacillus*. Cases are more common in some regions of the world and comparatively less common in some other regions. These have been reviewed here in some detail.

***Keywords**: Food; Bacillus cereus; Cereulide; Emetic Syndrome; Diarrheal Syndrome; Enterotoxins; Detection; Food Safety*

Abbreviations

CFU: Colony Forming Unit; Nhe: Non-Hemolytic Enterotoxin; UHTST: Ultra High Temperature-Short Time

Introduction

Bacillus cereus is a Gram-positive rod with central spore. This bacterium is implicated in many infections like post-operative endophthalmitis, foodborne infections like nausea and vomiting, and diarrhoeagenic toxin causing diarrhoea.

The toxin cereulide is called 'waxy' toxin. The term "cereulide" literally means "cyclic peptide of *Bacillus cereus*". The bacterium has earned the epithet *Bacillus cereus*, and this, however, refers to the wax-like appearance of colonies, not to the toxin [1].

Materials and Methods

Search strategy: Thorough and meticulous scientific literature search using MeSH (Medical subject headings) terms was used for citing and quoting literature.

The agent

Bacillus cereus is a Gram positive *Bacillus* with central on-bulging spore. The bacterium was first isolated from air and described by Frankland and Frankland in the year 1887. Earlier thought to be a strict aerobe, *B. cereus* has now been found to be facultatively anaerobic. *B. cereus* can be detected in a wide variety of foods, like starchy foods and rice, and other foods such as raw and pureed and minimally processed vegetables, bread, milk, and dairy products, meat products, as well as ready-to-eat foods [2].

Epidemiology

B. cereus is responsible for approximately 1.4 to 12% of all global foodborne illness outbreaks [2]. The emetic form of illness is common in Great Britain and Japan, while diarrhoeagenic form is commoner in Northern Europe and the USA. However, recently both forms of illnesses are reported commonly from North America and Great Britain. The bacterium produces spores which can withstand normal cooking temperatures [3]. Generally, all *B. cereus* isolates can be categorized into seven different toxin profiles: A (*nhe*+, *hbl*+, *cytK*+), B (*nhe*+, *cytK*+, *ces*+), C (*nhe*+, *hbl*+), D (*nhe*+, *cytK*+), E (*nhe*+, *ces*+), F (*nhe*+), and G (*cytK*+). HBL stands for Hemolysin BL while NHE stands for non-hemolytic enterotoxin. CES gene encodes for cereulide. The emetic strains are mainly associated with starchy foodstuffs like rice, pasta and pastries, while the enteropathogenic strains are abundant in all kinds of foods like milk products, vegetables, meat products, puddings, sauces, soups, spices, poultry, and sprouts [4].

Below is a table 1 containing details of the recent outbreaks of foodborne *Bacillus cereus* in India and across the world.

Place	Year	Source	Remarks
Denmark, France, Spain and Luxembourg	December 2025 to February 2026	Infant feeding formula containing cereulide	----
Diphu, Assam, India	2021	Fried rice	Molecular detection of entero-toxin and emetic toxin genes was done
Jodhpur, Rajasthan, India	2025	Powdered human milk fortifier (HMF)	Other bacilli were also found

Table 1: *Recent outbreaks of foodborne Bacillus cereus.*

Pathogenesis

The cereulide toxin present in rice and noodles irritates the stomach mucosa to cause vomiting [5]. Cereulide is a sticky, wax-like toxin which sticks well to foods like rice and chowmein. Cereulide was originally described by Agata., *et al*. in 1994. It is a potassium binding depsipeptide that is structurally similar to the ionophore valinomycin. Due to its characteristic structure, which is characterized by alternating peptide and ester bonds, it is highly lipophilic and extremely heat resistant and stable from pH 2 to 11. It will not lose activity even after two hours at 121°C [6]. Because of its small size, it is not removed by normal hygienic procedures in food processing, like bactofugation or filtration. It will not also be inactivated by heat treatments. Because of its resistance to cleavage by pepsin and trypsin, it is not inactivated during stomach passage in the host.

The non-hemolytic enterotoxin and cytotoxin K, present in chicken preparations, causes invasive infections in small gut and hence bloody diarrhoea [7]. Hemolysin BL is also responsible for invasive infection. It is a pore forming toxin made of 3 components [8].

The bacterium is present in ambient air and enters food, like rice, when it is kept open for too long after preparation [9]. Being spore producing, it survives boiling very well. In rice, pasta and noodles, the cereulide toxin of *B. cereus* is expressed [10]. In chicken preparations, the non-hemolytic enterotoxin and cytotoxin are expressed more. Thus, the nature of the food also determines the type of toxin produced.

Toxin biology

The toxin cereulide is heat stable, acid stable and lipophilic [1]. The last property makes it easy to cross cell membranes and induce nausea and vomiting within hours. Cereulide is produced by *B. cereus* via a non-ribosomal polypeptide synthetase (NRPS) enzyme, which is encoded by the cereulide synthetase (*ces*) gene cluster. The toxin Hemolysin BL is actually composed of 3 separate components: the B subunit, and two L subunits that help with the construction of pore and action of the toxin. Nhe or non-hemolytic enterotoxin is also a tripartite toxin. CytK or Cytotoxin K, a member of the Pore forming toxin (PFT) family, is a single protein having dermonecrotic, cytotoxic, and hemolytic activities. The bacterium is psychrophilic and produces proteases that affect the quality and taste of milk, especially UHTST-pasteurized milk.

Food safety

In European Union, the upper safe limit of *B. cereus* in food is 50 CFU per gram. The same in France is 1 x 10^5 CFU per gram of food. Indian guidelines also mandate that for labelling the infection as food poisoning, the upper safe limit should be 1 x 10^5 CFU per gram of food or vomitus.

Lab diagnosis

Specimens needed for laboratory diagnosis are food or vomitus or stool which can be cultured on MacConkey agar and Egg yolk agar. Presence of *B. cereus* at concentrations exceeding 10^5 CFU per ml or gram of food is diagnostic of food poisoning [11]. The bacterium is lecithinase positive on egg yolk agar, motile, and cannot break down mannitol [12]. It is motile and haemolytic. Gram stain from advancing end of *B. cereus* colonies shows Gram positive bacilli arranged in chains with no spore [13]. Gram stain from lagging end of the colony reveals Gram positive bacilli with non-bulging spore, individually and not in chains, since this is the part where nutrients are depleted [13]. This is because the advancing end has newly forming active vegetative cells while the lagging end has old cells.

The ISO 7932:2004 method uses a horizontal test for the enumeration of presumptive *B. cereus* colonies. This method comprises a serial dilution of the samples and a subsequent dilution spreading on *B. cereus* selective Mossel medium agar (MYP or Mannitol-Egg-yolk-polymyxin-Agar) [14]. Incubation of the colonies at 30°C during 18 - 24 hours is needed. On MYP medium, colonies of *B. cereus* produce a pink-purple colour, surrounded by a characteristic halo formed of pink precipitate, which permits their identification. Finally, a hemolysis test should be performed on bacterial colonies to confirm *B. cereus* strains. This method confirms only presumptive *B. cereus* cells, because not all strains of *B. cereus* will be hemolytic. On egg yolk agar, halo of lecithinase is produced by *B. cereus*, since it breaks down lecithin of egg, releasing free fatty acids, which combine with calcium of egg to produce the zone of opacity or haziness around the colonies.

The image below shows lecithinase of *B. cereus* on egg yolk agar.

Figure 1: *Lecithinase of B. cereus on egg yolk agar (Image: Author).*

Molecular techniques

Toxin genes of *B. cereus* can be detected by PCR from powdered milk [15]. Real time PCR can also be used [16]. Specific primers have been designed for the housekeeping gene *gyrB* to establish a quantitative polymerase chain reaction (qPCR) method, which tries to achieve the purpose of detecting all *B. cereus* strains in raw milk. Researchers across the world have developed a PCR technique as a reliable and rapid identification tool for the *Bacillus cereus* group species, based on a unique conserved sequence of the *motB* gene (which encodes flagellar motor protein) from *B. cereus, Bacillus thuringiensis* and Bacillus anthracis [17]. Studies have detected hemolytic (*hblA*, *hblC*, and *hblD*) and non-hemolytic (*nheA*, *nheB*, and *nheC*) enterotoxin genes in many *B. cereus* strains isolated from indoor air, which indicate that airborne isolates may be able to cause diarrhea rather than vomiting [18].

Treatment, prevention and risk management

Treatment is mainly supportive. Some techniques are good for prevention, like rapid cooling, appropriate cold and hot holding practices, minimizing room-temperature storage, hygienic food handling, as well as risk-based control strategies for high-risk foods and vulnerable subjects. Mass-scale cooking of rice should be done carefully and food should always be covered after cooling.

Discussion

The *Bacillus cereus* group, also known as *B. cereus* sensu lato, is a subdivision of the *Bacillus* genus that consists of eight formally recognised species: *B. cereus* sensu stricto, *B. anthracis*, *B. thuringiensis*, *B. weihenstephanensis*, *B. mycoides*, *B. pseudomycoides*, *B. cytotoxicus* and *B. toyonensis*. Many groups and clades of *Bacillus cereus* are there. *B. cereus* strains are thin, straight or slightly curved bacilli having size 1 × 3-4 μm with square ends, which usually form chains [19]. The bacterium expresses many virulence factors like lecithinase and potent toxins. Lecithinase is helpful for identification, too. Molecular methods are also present. Food safety, adherence to critical control points during food handling and covering cooked food may prevent or avert foodborne *Bacillus cereus* infections. Conventional techniques and emerging molecular methods help in identification of this pathogen in a better manner. Only isolation does not imply infection, and colony counts have to be remembered.oravelsue of pink precipitation Overall, it is an emerging pathogen that needs to be monitored. More research needs to be focussed on this pathogen to unravel new mechanisms of action and newer diagnostic modalities.

Conclusion

Bacillus cereus continues to baffle clinicians and microbiologists alike. More research is awaited regarding its pathogenesis and laboratory diagnosis.

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