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# Assessment of the exposure to cytotoxic *Bacillus cereus* group genotypes through high-temperature, short-time milk consumption

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## ABSTRACT

This study addresses the limited tools available for assessing food safety risks from cytotoxic *Bacillus cereus* group strains in contaminated food. We quantified the growth, in skim milk broth, of 17 cytotoxic *B. cereus* strains across 6 phylogenetic groups with various virulence gene profiles. The strains did not grow in HTST milk at 4 or 6°C. At 10°C, 15 strains exhibited growth; at 8°C, one strain grew; and all strains grew at temperatures ≥14°C. Using growth data from 16 strains, we developed linear secondary growth models and an exposure assessment model. This model, simulating a 5-stage HTST milk supply chain and up to 35 d of consumer storage with an initial contamination of 100 cfu/mL, estimated that 2.81 ± 0.66% and 4.13 ± 2.53% of milk containers would surpass $10^5$ cfu/mL of *B. cereus* by d 21 and 35, respectively. A sensitivity analysis identified the initial physiological state of cells as the most influential variable affecting predictions for specific isolates. What-if scenarios indicated that increases in mean and variability of consumer storage temperatures significantly affected the predicted *B. cereus* concentrations in milk. This model serves as an initial tool for risk-based food safety decision-making regarding low-level *B. cereus* contamination.
**Key words:** *Bacillus cereus* group, exposure assessment, microbial risk management, precision food safety

## INTRODUCTION

The *Bacillus cereus* group, also referred to as *B. cereus sensu lato* (*s.l.*), is a complex composed of closely related species that can be categorized into 8 phylogenetic groups (Carroll et al., 2020). This group of bacteria raises concerns within the food industry due to their ability to form endospores that withstand heat treatments, such as HTST pasteurization of milk (Buss da Silva et al., 2022; Tirloni et al., 2022). Certain strains within this group possess the potential to induce foodborne illness through emetic intoxication, a consequence of the production of heat-stable toxin cereulide within a food matrix. In contrast, other strains from this group can cause diarrheal intoxication. Upon ingestion by the human host, these bacteria, while growing in the small intestine, produce pore-forming enterotoxins, leading to cell death (Jessberger et al., 2020). Notably, the estimated annual number of foodborne illnesses caused by *B. cereus* group members in the United States is reported to be 63,623, with a hospitalization rate of 0.4% (CDC, 2018). Moreover, it is important to acknowledge that foodborne illnesses caused by *B. cereus* group members are often underreported due to their short-lived nature and mild symptoms (Stenfors Arnesen et al., 2008). Most foodborne illnesses caused by the *B. cereus* group have been associated with $10^5$ to $10^8$ cells/spores per gram of food. However, it has been proposed that any food that contains $>10^3$ *B. cereus* group cells or spores per gram cannot be considered safe for consumption (EFSA, 2005).

Members of *B. cereus* group are common biological hazards along the dairy production chain, and they often persist in dairy processing environment biofilms (Tirloni et al., 2022). These hazards are particularly relevant to perishable HTST milk, which requires refrigeration along the entire supply chain to ensure product quality and safety. Despite this requirement, HTST milk can be subject to temperature abuses due to an inefficient cold chain. For instance, although efficient temperature control of food products inside refrigerated trucks can usually be achieved, increases in temperature, sometimes to >10°C, during ground operations at the beginning and end

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