Virulence potential of *Bacillus cereus* isolates isolated from Italian infant formulae

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Background

Spore-forming bacteria can survive the production processes of powdered formulae [1]. Among the *Bacillus* spp. frequently isolated from these products, *Bacillus cereus* is of particular concern, as it can compromise product safety through spoilage, toxin production and infection [2]. However, limited data are available on this pathogen in powdered formulae, since microbiological safety assessments mainly focus on *Cronobacter sakazakii* and *Salmonella* spp.

Methods

A total of 42 *B. cereus* strains isolated from powdered formulae were tested for proteolytic activity (1.2% agar plates containing 1.5% skim milk incubated at 37°C for 18 h), phosphatidylcholine-specific phospholipase C (PC-PLC) activity (Blood Agar Base supplemented with 0.15% L-α-phosphatidylcholine incubated at 25°C for 16 h) and hemolysin BL production (Columbia blood agar supplemented with 5% sheep blood incubated at 30°C for 18 h) [3].

To investigate toxin-related genes, strains were grown in liquid culture, DNA was extracted and purified and conventional PCR was performed targeting the following genes: cereulide (ces), sphingomyelinase (sph), enterotoxins (entFM, entS, bceT), non-hemolytic enterotoxin components (nheA, nheB, nheC), cytotoxin K (cytK) [4,5].

Antimicrobial susceptibility was evaluated by broth microdilution (Sensititre™ automated system with the GPALL1F plate) and minimum inhibitory concentrations were interpreted according to the 2025 EUCAST clinical breakpoints.

Gene	Primers Pairs	
ces	cesF1: ggtgacacacattatcatataaggtg	cesR2: gtaagcgaacctgtctgtaacaaca
sph	Ph1: cgtgccgatttaattggggc	Ph2: caatgttttaaacatggatgcg
bceT	ETF: ttacattaccaggacgtgctt	ETR: tgtttgtgattgtaattcagg
entS	TY123: ggtttagcagcagcttctgtagctggcg	TY125: gtttcgttagatacagcagaaccacc
EntFM	ENTA: atgaaaaaagtaatttgcagg	ENTB: ttagtatgcttttgtgtaacc
cytK	F2: aacagatatcggtcaaaatgc	R7: cgtgcatctgtttcatgagg
nheA	344S: tacgctaaggaggggca	843A: gtttttattgcttcatcggct
nheB	1500S: ctatcagcacttatggcag	2269A: actcctagcggtgttcc
nheC	2820S: cggtagtgattgctggg	3401A: cagcattcgtacttgccaa

Table 1: Specific primers pairs for Identification of target genes

Results

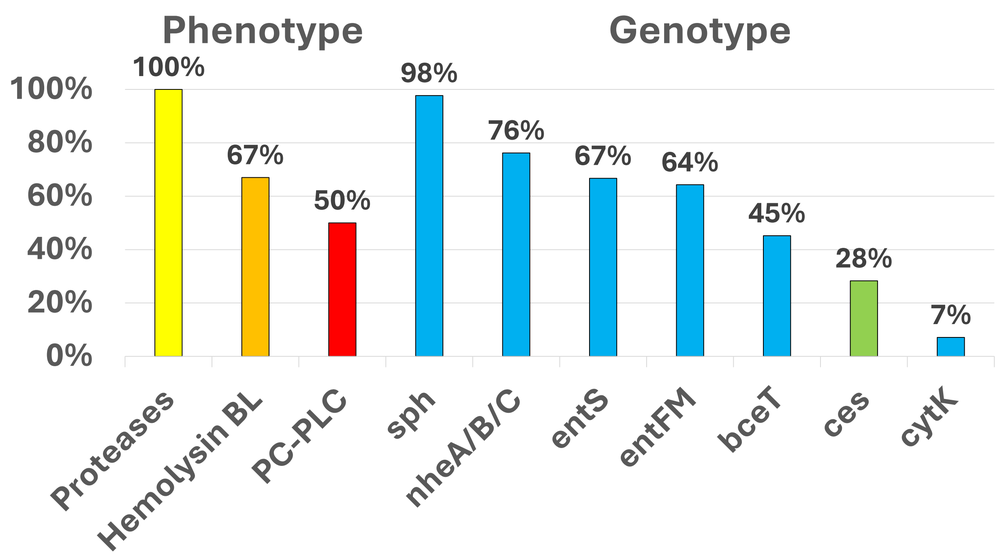

Table 2: Phenotypic and genotypic characterization of the 42 B. cereus isolates

Phenotypic and genotypic characteristics of the isolates are reported in Table 2.

Twelve strains expressed all three virulence factors, although only two carried all targeted toxin-related genes except ces or cytK. Excluding virulence-factor expression, seven isolates simultaneously harbored all toxin-related genes except one, while three isolates carried nheA, nheB, nheC, bceT, and cytK simultaneously.

A high prevalence of proteolytic activity has also been reported in *B. cereus* dairy isolates, while PC-PLC production was lower in our study [6]. The prevalence of bceT was lower than that reported in *B. cereus* isolates from Italian fresh and short-ripened cheeses [7], and cytK prevalence was markedly lower than the 100% reported in isolates from powdered infant formula [8]. The occurrence of nheA/B/C and entFM was comparable to other studies [7,9].

The detection of ces-positive strains raises concern because cereulide may be pre-formed in reconstituted formula, as demonstrated by a recent outbreak [10].

Regarding antimicrobial susceptibility, a high proportion of isolates exhibited resistance to β-lactam antibiotics, a phenotype generally considered intrinsic to *B. cereus*. Excluding intrinsic resistance, most of the strains were fully susceptible and no multidrug-resistant isolates were detected, as all resistant strains showed resistance to only one antibiotic class (Table 3). Our findings are consistent with previous Italian data on *B. cereus* strains from various food sources, including dairy products, but lower erythromycin resistance (6.8%) and higher clindamycin resistance (19.5%) have been reported [11]. In contrast, erythromycin resistance was higher than that reported for Chinese dairy isolates (16.7%) [9].

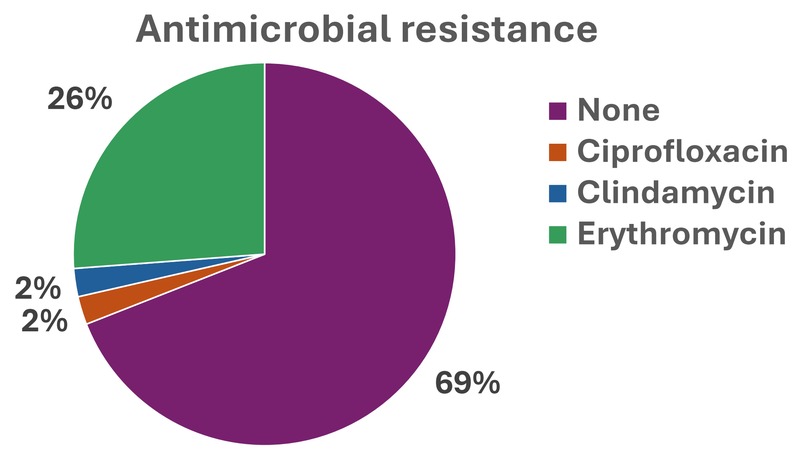

Table 3: Antimicrobial resistance pattern of the isolates

Conclusions

The frequent detection of *B. cereus* strains carrying multiple virulence factors highlights a potential safety concern. These findings contribute to addressing the limited availability of data on *B. cereus* in powdered formulae. Particular attention should be paid to ces-positive strains, considering the recent outbreak due to cereulide contamination.

Continuous monitoring of raw materials and finished products, together with virulence profiling of isolates, may improve risk assessment. Proper hygiene and handling of reconstituted formula remain crucial to protect vulnerable consumers.

Future studies will evaluate the influence of different reconstitution practices on spore germination, growth potential and cereulide production of selected *B. cereus* isolates in reconstituted formula.

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