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Molecular surveillance of antimicrobial resistance in *Bacillus cereus sensu lato*: A proposed conceptual framework in the One Health context

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Abstract

Bacillus cereus sensu lato (s.l.) is a One Health-relevant bacterial group linking human and veterinary medicine with food safety and the environment. Besides causing foodborne intoxications and opportunistic extraintestinal infections, *B. cereus* shows inherent non-susceptibility to a number of β-lactams and carries a range of acquired antimicrobial resistance (AMR) determinants. This review summarises knowledge on AMR mechanisms in *B. cereus* s.l., critically evaluates phenotypic and molecular approaches for detection, and proposes a conceptual framework for molecular AMR monitoring. Phenotypic testing of antimicrobial susceptibility remains the reference standard because it directly measures expressed resistance. PCR-based methods, particularly quantitative real-time PCR (qPCR), support these assays by enabling rapid, target-specific screening for predefined resistance genes in clinical, food and environmental samples and, when appropriately calibrated, by quantifying copies of AMR genes. Within this context, we outline a molecular monitoring framework that organises key AMR targets into two sector-specific panels: (i) a clinical panel focused on detecting determinants with direct therapeutic relevance and sentinel genes affecting last-resort agents, and (ii) a One Health panel designed for monitoring AMR reservoirs and selection pressure in the food chain and the environment. Because the limited multiplexing capacity of standard qPCR platforms requires running several separate reactions to cover all relevant targets, we discuss high-multiplex ligation-based technologies, exemplified by Multiple Oligonucleotide Ligation PCR (MOL-PCR). Drawing on validated applications of MOL-PCR in other bacterial species, we propose its use as a strategic, yet currently conceptual, high-multiplex tool to support integrated molecular AMR surveillance for *B. cereus* s.l. across One Health sectors.

Food safety, genotyping, MOL-PCR, antimicrobial resistance genes

The *Bacillus cereus sensu lato* (*B. cereus* s.l.) complex comprises phylogenetically closely related, spore-forming, Gram-positive bacteria. The most studied species within this group are *Bacillus anthracis*, the aetiological agent of anthrax, *Bacillus cereus sensu stricto* (hereafter referred to as *B. cereus*), a foodborne pathogen, and *Bacillus thuringiensis*, an entomopathogenic species used as a biopesticide (Fayad et al. 2019; Bianco et al. 2021). In *B. cereus* s.l., phenotypic diversity and pathogenicity are strongly determined by large virulence plasmids and other mobile genetic elements (Zheng et al. 2015; Fayad et al. 2019). *Bacillus cereus* is a widespread environmental saprophyte occurring in soil, water, and food, and is also an important opportunistic human pathogen (Farina et al. 2024). Spores of *B. cereus* can survive dehydration and pasteurisation processes and are highly resistant to heat and to multiple chemical and physical treatments (Fraccalvieri et al. 2022; Tirloni et al. 2022). Furthermore, *B. cereus* strains readily form biofilms on food-processing surfaces, which complicates sanitation and likely contributes to their frequent occurrence in meat, dairy products, and foods of plant origin, including cereal products (Dorotíková et al. 2022; Tirloni et al. 2022; Algammal et al. 2024).

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Clinically, *B. cereus* food poisoning presents as two gastrointestinal syndromes: emetic and diarrhoeal (Dušková et al. 2025). The emetic form is caused by cereulide, a stable cyclic dodecadepsipeptide toxin produced by *B. cereus*. It is pre-formed in contaminated food and induces nausea and vomiting upon ingestion (Messelhäußer and Ehling-Schulz 2018; Jessberger et al. 2020). In contrast, the diarrhoeal syndrome occurs after ingestion of food contaminated with viable *B. cereus* cells, as the diarrhoea-associated enterotoxins haemolysin BL (Hbl), non-haemolytic enterotoxin (Nhe), and cytotoxin K (CytK) are not produced in the food matrix itself but only in the intestine (Messelhäußer and Ehling-Schulz 2018). Although both clinical forms are usually mild and self-limiting, severe and sometimes fatal cases have been reported, including acute liver failure and multi-organ dysfunction or failure (Jessberger et al. 2020; Thery et al. 2022).

Beyond foodborne intoxications, *B. cereus* can cause a broad spectrum of local and systemic infections in humans. Endophthalmitis, pneumonia, sepsis and meningitis have been described in neonates and immunosuppressed patients, often associated with indwelling catheters and other biomedical devices (Bianco et al. 2021; Schmid et al. 2024). In some cases, infections have been linked to strains closely related to *B. anthracis* that harbour *B. anthracis* virulence genes or virulence plasmids and cause anthrax-like disease (Jessberger et al. 2020). These observations highlight that *B. cereus* is not only ubiquitous in the environment and food chain but also an under-recognised cause of severe extraintestinal infection (Messelhäußer and Ehling-Schulz 2018; Schmid et al. 2024).

From an ecological viewpoint, *B. cereus* occupies overlapping niches in environmental, food-processing, and clinical settings. It is found in soils irrigated with wastewater (Anjum and Krakat 2016) and in foods of animal and plant origin (Algammal et al. 2024), and its ability to form spores and biofilms enables long-term persistence in soils and food-processing environments (Anjum and Krakat 2016; Tirloni et al. 2022; Algammal et al. 2024). Although *B. cereus* is primarily recognised as a foodborne pathogen, several studies indicate that it can function as an environmental reservoir of antimicrobial resistance (AMR) genes, including mobile elements capable of horizontal transfer (Agersø et al. 2002; Zhai et al. 2023; Farina et al. 2024). The One Health concept recognises the close interconnection between human, animal, and environmental health and promotes a collaborative, cross-sectoral approach to health threats, including antimicrobial resistance. Taken together, ecological and clinical data demonstrate that *B. cereus* links environmental persistence with clinically relevant pathogenicity and underscore its relevance as a One Health pathogen at the interface of food, environment, and human health (Bogaerts et al. 2023; Farina et al. 2024; Schmid et al. 2024).

European surveillance data indicate that *B. cereus* is a recurrent cause of foodborne outbreaks. In 2023, the EU One Health Zoonoses report stated that *B. cereus* toxins were the second most frequently reported known causative agents of foodborne outbreaks, with 474 outbreaks recorded across EU Member States (EFSA 2024). In China, 419 outbreaks resulted in 7,892 reported cases, 2,786 hospitalisations and 5 deaths between 2010–2020, with most outbreaks occurring between May and September (Duan et al. 2023). Similar patterns are observed in U.S. surveillance, where *B. cereus* is consistently documented among the bacterial agents responsible for foodborne illness (Bennett et al. 2013; White et al. 2022). Collectively, these data confirm the global distribution of *B. cereus* foodborne disease, but the actual burden is likely underestimated because illnesses are often mild and self-limiting and clinical or food specimens are not routinely tested for *B. cereus* or its toxins (Bennett et al. 2013; Duan et al. 2023; Rahnama et al. 2023; EFSA 2024; Li et al. 2025). Overall, *B. cereus* emerges as an important reservoir and potential vector of AMR genes at the food–environment–clinical interface, providing a clear rationale for examining its antimicrobial resistance within a One Health framework in this review.

Antimicrobial resistance in *Bacillus cereus* from a One Health perspective

Bacillus cereus exhibits a complex AMR profile shaped by intrinsic determinants and horizontally acquired genes. A key feature is intrinsic non-susceptibility to many β-lactam antibiotics, particularly to penicillins and early-generation cephalosporins, largely mediated by the class A serine β-lactamase BcI and the zinc-dependent metallo-β-lactamase BcII, as well as additional β-lactamases encoded by *bla* genes (e.g., *bla1* and *bla2*) (Mills et al. 2022; Farina et al. 2024). Consequently, penicillin-class β-lactams are generally unsuitable for severe *B. cereus* infections. Therapy instead relies on agents to which isolates usually remain susceptible, including vancomycin, gentamicin, and fluoroquinolones. However, reduced susceptibility to meropenem and vancomycin has already been reported in food and environmental isolates (Mills et al. 2022; Zhai et al. 2023; Farina et al. 2024; Zheng et al. 2024).

Acquired resistance determinants described in *B. cereus* include macrolide resistance genes (*mphB* and *erm*), tetracycline genes [*tet(M), tet(L), tet(45)*], aminoglycoside-modifying enzymes [*aph*, ant(6), *aadA* family], quinolone resistance mutations in *gyrA* and *parC*, cat-family chloramphenicol acetyltransferases and *van*-like glycopeptide loci (Agersø et al. 2002; Glenwright et al. 2017; Fiedler et al. 2019; Bianco et al. 2021; Mills et al. 2022; Zhai et al. 2023; Sayem et al. 2025). The distribution of these determinants varies among clinical, food, and environmental isolates. Phenotypic surveys of foodborne strains document heterogeneous but sometimes multidrug-resistant profiles, particularly to β-lactams, tetracycline, erythromycin and aminoglycosides (Fiedler et al. 2019; Cha et al. 2023; Torki Baghbadorani et al. 2023).

Whole-genome sequencing (WGS) has further expanded understanding of the *B. cereus* resistome and its ecological context. Across WGS datasets from blood cultures, foods (including ice cream, vegetables and pasteurised milk) and environmental matrices, *B. cereus* group isolates consistently carry a core set of AMR genes, notably, genes encoding the class A serine β-lactamase BcI and the zinc-dependent metallo-β-lactamase BcII, along with additional β-lactamase genes (*bla1*, *blaZ*, occasionally *blaTEM*) and *fosB*, with variable co-occurrence of macrolide, tetracycline, aminoglycoside-modifying and *van*-like loci (Parulekar and Sonawane 2018; Fiedler et al. 2019; Fraccalvieri et al. 2022; Farina et al. 2024). These determinants are mostly chromosomal, but plasmid-borne genes such as *tetL* in *B. cereus* from pasteurised milk illustrate the potential for horizontal transfer (Zhai et al. 2023). Multidrug-resistant isolates from hospital wastewater carrying multiple AMR genes together with prophages, CRISPR–Cas systems, and transposable elements further highlight the potential for horizontal gene transfer at the clinical–environmental interface (Fraccalvieri et al. 2022; Sayem et al. 2025). Combined with the detection of multidrug-resistant *B. cereus* in meat and meat products (Algammal et al. 2024), these findings support the role of *B. cereus* as an ecological reservoir and disseminator of AMR genes across environmental, food, and clinical compartments. The principal AMR determinants and their mechanisms are summarised in Table 1.

The repeated detection of overlapping AMR determinants across food, clinical, and environmental isolates highlights the interconnection of these sectors and supports a One Health approach. Understanding these shared resistance mechanisms provides a foundation for targeted detection strategies relevant to both clinical diagnostics and integrated AMR surveillance systems.

Phenotypic detection of antibiotic resistance

Phenotypic antimicrobial susceptibility testing (AST) remains the reference standard for confirming resistance, as it directly measures growth inhibition in the presence of defined antibiotic concentrations. For *B. cereus*, phenotypic assays are indispensable but technically

challenging: diffusion-based methods are prone to artefacts due to sliding motility on agar, which can distort inhibition-zone boundaries and generate pseudo-resistance, particularly for glycopeptides, whereas broth microdilution provides more reliable minimum inhibitory concentration data and is recommended as the reference method (Mills et al. 2022; Schmid et al. 2024).

Table 1. Main antibiotic resistance genes and their mechanisms in *B. cereus*.

Antibiotic class	Gene	Mechanism of resistance	Verified source
β-lactams	*BcI* *BcII* *bla1* *bla2*	Metallo-β-lactamases (BcII) and class A β-lactamases (BcI) hydrolyzing penicillins and many cephalosporins; reduced susceptibility to some carbapenems	Farina et al. 2024; Mills et al. 2022; Zheng et al. 2024
Macrolides Lincosamides Streptogramins B (MLSB)	*mphB* *erm*	Macrolide 2′-phosphotransferase (drug inactivation) and 23S rRNA methylation (MLSB resistance)	Jensen et al. 2002; Fiedler et al. 2019; Bianco et al. 2021; Mills et al. 2022
Tetracyclines	*tet(L)* *tet(45)*	Active antibiotic efflux via membrane pumps	Fiedler et al. 2019; Mills et al. 2022; Zhai et al. 2023
	tet(M)	Ribosomal protection from tetracycline binding	Agersø et al. 2002; Glenwright et al. 2017; Zhai et al. 2023
	aph	Phosphorylation (aminoglycoside phosphotransferase)	Parulekar and Sonawane 2018; Cha et al. 2023
Aminoglycosides	*ant(6)* *aadA1* *aadA31*	Adenylation (aminoglycoside adenylyltransferases)	Cha et al. 2023
Fluoroquinolones	*gyrA*	Amino acid substitutions in DNA gyrase → reduced fluoroquinolone binding	Antwerpen et al. 2007
	parC	Amino acid substitutions in topoisomerase IV → reduced fluoroquinolone binding	Antwerpen et al. 2007
Glycopeptide	*van*-like genes (partial clusters)	Incomplete glycopeptide-associated loci; generally not associated with elevated vancomycin MICs in *B. cereus*	Bianco et al. 2021; Mills et al. 2022; Sayem et al. 2025
Phenicols	*catQ* *catA*	Chloramphenicol O-acetyltransferases (drug inactivation)	Glenwright et al. 2017 Fiedler et al. 2019; Farina et al. 2024
Fosfomycin	*fosB*	Drug inactivation via thiol transfer (FosB)	Fraccalvieri et al. 2022; Sayem et al. 2025
Bacitracin	*bcrA* *bcrB* *bcrABC* operon	Active efflux reducing intracellular bacitracin	Anjum and Krakat 2016; Sayem et al. 2025
Multidrug resistance	*ykkC* *ykkCD*	Broad-spectrum efflux pumps	Fiedler et al. 2019; Algammal et al. 2024; Sayem et al. 2025

Phenotypic validation is also crucial for interpreting molecular data. Studies in the *B. cereus* group have highlighted recurrent discordance between the presence of *van*-like loci, macrolide or tetracycline genes and the expressed phenotype, underscoring that gene detection alone does not equate to clinically relevant resistance (Glenwright et al. 2017; Mills et al. 2022; Farina et al. 2024; Schmid et al. 2024). The expression and phenotypic impact of such genes can be influenced by regulatory architecture (including weak or inducible promoters), environmental conditions and additional mutations that modulate antibiotic-target interactions (Glenwright et al. 2017; Gil-Gil et al. 2021; Farina et al. 2024). While phenotypic assays define expressed resistance, they do not capture the full resistome or identify latent, horizontally transferable determinants. Molecular approaches, therefore, provide a complementary dimension by enabling rapid, target-specific detection of key AMR genes irrespective of their current expression state and by revealing genetic capacities that may become clinically relevant under selective pressure (Gil-Gil et al. 2021; Farina et al. 2024; Hattab et al. 2024). These synergistic strengths underpin the molecular detection strategies discussed in the following section.

Molecular detection of antibiotic resistance: qPCR and multiplex qPCR

In *B. cereus*, molecular methods enrich phenotypic susceptibility testing by enabling rapid, target-specific detection of AMR genes. This is particularly relevant because several resistance determinants exhibit inducible or stress-dependent expression and may remain undetectable under standard phenotypic testing conditions (Glenwright et al. 2017; Gil-Gil et al. 2021; Mills et al. 2022; Farina et al. 2024). Conventional PCR is widely applied to detect key AMR genes in *B. cereus*, including *bla*, *erm*, *tet* and *van* loci, depending on the panel used in individual studies (Agersø et al. 2002; Abdelaziz et al. 2024; Algammal et al. 2024). Quantitative PCR (qPCR) offers higher analytical sensitivity and specificity and can be applied to clinical, food, and environmental samples (Antwerpen et al. 2007; Kralik and Ricchi 2017). It also permits the absolute or relative quantification of target gene copy numbers, enabling the comparison of gene abundance between samples or conditions and providing insight into the dynamics of AMR gene carriage in *B. cereus* populations (Turgeon et al. 2008; Kralik and Ricchi 2017; Frentzel et al. 2018).

Multiplex qPCR extends qPCR-based detection by enabling simultaneous amplification of several genetic targets within a single reaction using fluorophore-labelled primer–probe sets. This format increases throughput and reduces reagent consumption, making it particularly suitable for high-volume AMR monitoring in One Health settings. Although multiplex qPCR assays have been developed for parallel detection and differentiation of *B. cereus* group species (Frentzel et al. 2018), AMR detection in *B. cereus* still relies predominantly on single-target PCR assays. Nevertheless, the performance advantages demonstrated by multiplex systems in broader AMR surveillance underscore their potential applicability to *B. cereus* (Galhano et al. 2021; Yamin et al. 2023).

Despite their speed and sensitivity, qPCR-based assays remain confined to predefined genetic targets and cannot detect novel resistance variants or directly assess functional gene expression. Phenotypic testing, in turn, does not capture latent or inducible resistance determinants that are genetically present but not expressed under standard testing conditions. Consequently, combined genotypic–phenotypic approaches provide the most robust assessment of AMR: qPCR enables rapid screening and high-throughput surveillance across clinical, food, and environmental settings, whereas phenotypic methods confirm functional resistance profiles (Kralik and Ricchi 2017; Gil-Gil et al. 2021; Yamin et al. 2023; Hattab et al. 2024).

Proposed conceptual framework for molecular surveillance of antibiotic resistance in *Bacillus cereus* s.l.

In the context of *B. cereus* s.l., AMR surveillance must address both severe clinical infections and the role of *B. cereus* as an environmental reservoir and vector of AMR genes (Farina et al. 2024; Maalouf et al. 2025). Molecular methods, particularly PCR-based assays including qPCR and multiplex qPCR, bring a rapid and sensitive platform for detecting known resistance determinants, thereby serving as an essential complement to phenotypic testing (Gao et al. 2018; Fiedler et al. 2019). Within this framework, these approaches enable rapid screening for the presence of established AMR loci.

However, effective AMR monitoring requires diagnostic strategies tailored to specific contexts rather than a universal panel, because interpretive needs differ substantially between sectors:

- Clinical diagnostics: prioritise the rapid identification of resistance-associated genes linked to potential treatment failure and high-risk determinants, enabling timely adjustment of treatment
- One Health surveillance (food, feed, and environment): requires broader monitoring to assess ecological distribution, track temporal trends and identify selective pressures arising from agricultural or environmental antibiotic exposure.

Based on published data and our evaluation of current methodologies, we propose the following conceptual framework for molecular AMR surveillance in *B. cereus* s.l. This framework organises target genes into two sector-specific panels (clinical and One Health), each further stratified into Class 1 and Class 2 markers based on diagnostic relevance, analytical feasibility and practical applicability.

Panel 1: Clinical panel (for human systemic infections)

The clinical panel is designed to provide rapid and clinically relevant information to guide antibiotic treatment in patients with severe systemic infections caused by *B. cereus*. This panel focuses on first-line drugs, key alternatives and critical last-resort agents and is summarised in Table 2.

Table 2. Proposed clinical AMR panel for *B. cereus*.

Class	Gene	Significance
1	*erm(B), erm(C)*	Major mechanism of MLS_B resistance (macrolides, lincosamides)
1	*gyrA*	Key determinant of fluoroquinolone resistance
1	*cfr, optrA*	Linezolid/phenicol resistance potential; high-risk transferable elements currently described in other Gram-positive bacteria (including *Bacillus* spp.) but not yet reported in *B. cereus*; included as sentinel markers
2	*BcI*, *BcII* *bla1, bla2*	Intrinsic β-lactamases that explain non-susceptibility to penicillins and many cephalosporins; mainly confirmatory markers
2	*lsa(B)*	Alternative mechanism of MLS_B resistance, including clindamycin; documented in *B. cereus* group
2	*parC*	Secondary marker increasing accuracy of fluoroquinolone resistance prediction
2	*tet(L)*, *tet(M)*	Ribosomal protection or efflux; tetracycline resistance (not clinically first-line, but epidemiologically relevant)
2	*aph*, *aad*, *aac(3)-IV*, *ant(6)*	Aminoglycoside-modifying enzymes; impact on combination therapy
2	*catQ*	Chloramphenicol acetyltransferase; contributes to phenicol resistance (CNS penetration considered)

Class 1 includes gene targets that are directly relevant to urgent clinical decision-making. These markers provide information essential for identifying resistance to key therapeutic options (for example, fluoroquinolones via *gyrA* mutations or macrolide/lincosamide resistance via *erm* genes), as well as for detecting high-risk, potentially transferable determinants such as *cfr* and *optrA*. Although *cfr* and *optrA* have not yet been identified in *B. cereus* isolates in the studies reviewed here, their inclusion as sentinel markers is justified by their role in compromising last-resort drugs in other Gram-positive pathogens (Gao et al. 2012; Schwarz et al. 2021). Together, these targets allow the clinician to rapidly exclude ineffective therapies and adjust treatment in life-threatening systemic infections. Class 2 includes supplementary markers that broaden diagnostic depth. These encompass genes that refine mechanistic interpretation of resistance (e.g. *parC* alongside *gyrA*), determinants relevant for combination therapy or secondary options (tetracycline and aminoglycoside resistance genes, *catQ*), and intrinsic β-lactamases (BcI/BcII, *bla1*, *bla2*), which primarily confirm the expected non-susceptibility of *B. cereus* to penicillin-class antibiotics. The inclusion of intrinsic β-lactamase genes in Class 2 emphasises their confirmatory rather than decision-driving role in clinical practice. Therefore, these Class 2 markers enhance both antimicrobial stewardship and the understanding of resistance patterns within *B. cereus* clinical isolates (Mills et al. 2022; Farina et al. 2024).

Panel 2: Food and feed safety panel (One Health)

The One Health panel (Table 3) is intended to monitor and assess the spread of antibiotic resistance genes in the food chain and the environment. It aims to identify reservoirs of multidrug-resistant strains, track the selection pressure from antibiotics used in agriculture, and provide data for risk assessment within the One Health framework (Fiedler et al. 2019; Farina et al. 2024). The proposed targets and their classification are listed in Table 3.

Table 3. Proposed One Health AMR panel for *B. cereus*.

Class	Gene	Significance
1	*tet(L), tet(M), tet(45)*	Common tetracycline resistance markers under agricultural selection pressure
1	*erm(B), erm(C)*	Markers of macrolide resistance frequently present in food/environmental *B. cereus*
1	*BcI*, *BcII, bla1, bla2*	Baseline β-lactam resistance; confirms species-level intrinsic profile
1	*aph*	Aminoglycoside resistance marker commonly detected in environmental isolates
1	*fosB*	Major fosfomycin resistance determinant widely distributed in *B. cereus*
1	*bcrA*, *bcrB*	Bacitracin efflux genes; key environmental AMR markers
2	*gyrA*, *parC*	Indicators of fluoroquinolone selection pressure (e.g., enrofloxacin use)
2	*lsa(B)*	Horizontal transfer MLS_B determinant; LAB ↔ *B. cereus* interactions
2	*aac*	Additional aminoglycoside-modifying enzymes (epidemiological value)
2	*catQ*	Phenicol resistance determinant in food isolates
2	*cfr*, *optrA*	Early warning indicators for potential transfer of linezolid resistance
2	*sul1*, *sul2*	Broad environmental AMR indicators
2	*van*-like genes *(vanW*, *vanZ*, *vanY,* vanXY-type)	Environmental glycopeptide-associated loci; non-clinical early warning
2	*mphB*	Macrolide 2′-phosphotransferase; additional marker of macrolide selection pressure in food and environmental isolates

Class 1 includes gene targets with the highest epidemiological relevance in the agri-food sector, particularly tetracycline resistance genes [*tet(L), tet(M), tet(45)*], macrolide resistance genes [*erm(B), erm(C)*] and aminoglycoside resistance markers (*aph*), as well as widely distributed intrinsic determinants such as *fosB* and the bacitracin efflux genes *bcrA*/*bcrB*. These determinants are consistently reported in food and environmental *B. cereus* isolates and reliably reflect agricultural selection pressure and baseline resistance characteristics of the species (Yu et al. 2020; Algammal et al. 2024; Farina et al. 2024). Class 2 broadens the surveillance scope by incorporating additional and sentinel markers, including phenicol resistance (*catQ*), transferable oxazolidinone resistance determinants (*cfr*, *optrA*), QRDR mutations (*gyrA*, *parC*), *van*-like glycopeptide-associated loci and aminoglycoside-modifying enzymes (*aac*). The inclusion of *tet(45)* in Class 1 and *mph(B)* in Class 2 extends coverage of tetracycline and macrolide resistance beyond the classical *tet(M)*, *tet(L)*, and *erm* loci, reflecting their documented presence and mobility in *B. cereus* s.l. and related Gram-positive bacteria (Glenwright et al. 2017; Kowalewicz et al. 2023). Although *van*-like clusters in *B. cereus* do not confer vancomycin resistance, their monitoring is epidemiologically relevant because they trace the environmental circulation of glycopeptide-associated genes typical of enterococci (Bianco et al. 2021). Broad environmental ARG indicators such as *sul(1)* and *sul(2)* can be used to characterise overall sulphonamide resistance pressure in complex matrices (Yamin et al. 2023).

A particularly relevant application of the One Health panel is the analysis of complex fermented food matrices (e.g., cheeses, dry fermented meat products). These ecosystems contain intentionally added starter cultures, typically lactic acid bacteria (LAB), coexisting with incidental microbiota, among which *B. cereus* is a recurrent contaminant. Such close microbial coexistence creates favourable conditions for horizontal gene transfer, especially when mobile AMR genes are carried on conjugative plasmids or transposons (Jensen et al. 2002; Parulekar and Sonawane 2018). In principle, two bidirectional risks may occur: (1) LAB used as starter cultures may harbour transferable AMR genes [e.g., *tet(M)* on Tn916-type elements], which in principle could be acquired by *B. cereus* under suitable conditions, although such transfer has not yet been documented in food matrices; and (2) *B. cereus* strains present in the product may potentially acquire mobile determinants conferring resistance to critically important antimicrobials (e.g., the linezolid-resistance gene *cfr* described on plasmids in *Bacillus* spp.), which could in principle be transferred to LAB and contribute to the emergence of AMR reservoirs. Because of this risk, regulatory authorities, including the European Food Safety Authority (EFSA), emphasise that starter and probiotic strains, including those considered within the concept of Qualified Presumption of Safety list, must be assessed for the presence of transferable AMR genes and that strains carrying such determinants should not be used as food or feed cultures (Yang and Yu 2019; Stefańska et al. 2021). The proposed One Health panel is therefore applicable not only for monitoring *B. cereus* but also for evaluating the safety of functional cultures and modelling the horizontal gene transfer risk in multispecies food ecosystems.

As detailed in the previous sections, the clinical and One Health panels share many AMR gene targets, but their categorisation into Class 1 or Class 2 differs according to context. Genes such as *tet(L)*, *tet(M)*, *tet(45)* or aminoglycoside-modifying enzymes (*aph*, *aac*) are placed in Class 2 of the clinical panel because tetracyclines and aminoglycosides are not first-line therapeutic options for systemic *B. cereus* infections. In the One Health panel, however, they belong to Class 1 due to their high prevalence in food and environmental isolates and their reliability as indicators of agricultural selection pressure (Algammal et al. 2024). Conversely, sentinel determinants such as *cfr* or *optrA* are included in Class 1 of the clinical panel because their detection would have immediate therapeutic consequences, particularly regarding linezolid efficacy. Whereas, in the One Health panel, they belong to

Class 2 as high-risk markers used for early epidemiological warning rather than routine screening (Rodríguez-Lucas et al. 2022; Kowalewicz et al. 2023).

Thus, the distinction between the clinical and One Health panels represents an interpretive rather than a genetic differentiation: both panels draw from the same validated gene set, but their prioritisation reflects specific diagnostic or surveillance objectives. Depending on the sample origin (clinical material vs food or environmental sample), the appropriate interpretive filter ensures that results are actionable and sector-specific.

However, implementing the complete framework faces practical and technological constraints. A comprehensive panel covering all relevant AMR targets in *B. cereus* would require simultaneous detection of approximately 18–25 gene determinants. Most routine real-time PCR platforms offer only a limited number of fluorescence channels per run (typically 4–6), meaning that real-world implementation of the complete panel would require multiple parallel qPCR reactions for each sample (Reslova et al. 2019). Performing multiple parallel tests for one sample increases labour, time and cost, forming a bottleneck for high-throughput surveillance. As a result, laboratories often restrict testing to a small subset of markers (e.g. Class 1), leading to fragmentation of AMR data and limited comparability between sectors. These limitations call for the need for next-generation molecular technologies capable of high-multiplex detection. Methods with expanded target capacity capable of robustly detecting dozens of AMR genes in a single reaction represent a promising strategy for the full and cost-effective implementation of the proposed integrated surveillance panels, as discussed in the following chapter.

MOL-PCR: Principle, advantages, limitations, and application for antibiotic resistance detection in *Bacillus cereus*

The Multiplex Oligonucleotide Ligation-PCR (MOL-PCR) method is a molecular diagnostic platform designed to address key limitations of traditional multiplex PCR assays. It combines three principles: enzymatic ligation of specific probes (so-called MOLigos), exponential amplification by PCR, and fluorescent detection on a microsphere-based (bead-based) xMAP (extended multi-analyte profiling platform such as Luminex) (Reslova et al. 2019; Hrdy et al. 2021). Multiplexing is shifted from parallel PCR, where competing primer sets can generate amplification bias, to the ligation step. The subsequent PCR is run as a singleplex reaction with a single pair of universal primers, largely avoiding the amplification bias and cross-hybridisation typical of conventional multiplex PCR (Reslova et al. 2019).

Although MOL-PCR is only a semi-quantitative reaction and requires multiple post-PCR handling steps rather than fully closed-tube detection (Hrdy et al. 2021), it offers several advantages over multiplex qPCR. In combination with the MAGPIX platform (a compact, bead-based multiplex analyser that uses LED-induced fluorescence to detect targets bound to xMAP microspheres), it enables the robust detection of up to 50 markers in a single reaction, including single nucleotide polymorphisms (SNPs). Moreover, the assay panel can be extended by designing additional MOLigo probe pairs for new targets (Reslova et al. 2019). These features make MOL-PCR an attractive option for high-throughput AMR surveillance that requires the simultaneous detection of multiple resistance determinants, which exceeds the multiplexing capacity of qPCR.

Applications in different bacterial species illustrate the flexibility of MOL-PCR analysis. For example, Song et al. (2010) used MOL-PCR for simultaneous detection of high-risk biothreat pathogens, including SNPs linked to fluoroquinolone and tetracycline resistance, and Kowalewicz et al. (2023) developed a 45-plex ligase-based bead array targeting 41 AMR genes across nine antimicrobial classes in Gram-positive livestock-associated bacteria. Similar xMAP-based MOL-PCR assays have been reported for β-lactamase

genotyping and *Salmonella* subtyping (Wuyts et al. 2015; Ceyssens et al. 2016). These studies demonstrate that MOL-PCR is a reliable and flexible method for detecting a broad spectrum of antimicrobial resistance determinants and other clinically relevant genetic markers. Given the breadth of the resistance and toxigenic repertoire of *B. cereus*, including the three-component enterotoxins Hbl and Nhe, cytotoxin K (CytK) and the genes for synthesis of the emetic peptide cereulide (*ces*), MOL-PCR is a promising platform for their simultaneous detection together with AMR genes in a single multiplex assay. Such a combined panel would enable a more comprehensive assessment of the resistance and toxigenic potential of isolates and could strengthen epidemiological analysis within the One Health concept. However, to our knowledge, no validated MOL-PCR panel covering a similarly broad spectrum of AMR genes specifically for *B. cereus* has been published yet. Thus, our proposal remains conceptual, as it is based on analogous applications in other bacterial species.

Interpretation, pitfalls, and integration into practice

The development of molecular methods has fundamentally changed approaches to AMR surveillance across One Health sectors. High-throughput genotyping now enables simultaneous detection of multiple resistance and virulence genes in clinical, veterinary and environmental settings. However, as underlined by data on *B. cereus* s.l., a central interpretive principle remains unchanged: genotype is not equivalent to phenotype. The mere presence of a resistance gene does not guarantee its expression or clinical relevance. On the other hand, reduced phenotypic susceptibility may occasionally arise from regulatory or physiological adaptations that are not captured by simple gene presence/absence assays (Gil-Gil et al. 2021; Farina et al. 2024).

This discordance has been documented in the *B. cereus* group for *van*-like loci, macrolide and tetracycline resistance genes and various efflux systems, where phenotypic susceptibility often persists despite the presence of detectable AMR determinants (Mills et al. 2022). In addition, the sliding motility on solid media can generate pseudo-resistance, particularly for glycopeptides, which is resolved when isolates are tested by broth microdilution (Schmid et al. 2024). Taken together, these findings illustrate that molecular detection provides an important predictive layer but cannot replace phenotypic antimicrobial susceptibility testing, which remains indispensable for defining expressed resistance and guiding therapy in severe infections.

Within this context, our proposed clinical and One Health panels should be viewed as tools for structured genotypic screening rather than standalone diagnostic solutions. In clinical practice, panel-based detection of key determinants such as *gyrA* mutations, *erm* genes, or sentinel markers like *cfr* and *optrA* can rapidly identify high-risk resistance profiles and support early optimisation of therapy. Nevertheless, the phenotypic confirmation of critical results remains mandatory. In the One Health sector, the same gene set, re-prioritised according to the context, provides a framework for mapping AMR reservoirs, selection pressure, and potential routes of horizontal gene transfer across the food–environment–clinical interface (Fiedler et al. 2019; Fraccalvieri et al. 2022; Farina et al. 2024).

A key methodological issue that arises from this framework is the balance between multiplex coverage and quantitative resolution. Real-time qPCR allows both sensitive detection and, when properly calibrated, also the absolute or relative quantification of AMR gene copy numbers, which is highly valuable for environmental surveillance and longitudinal trend analysis (Kralik and Ricchi 2017). In contrast, MOL-PCR and related ligation-based bead assays typically result in semi-quantitative detection of targets and their approximate abundance, but do not provide direct copy-number estimation (Reslova et al. 2019; Hrdy et al. 2021). If MOL-PCR were to replace qPCR entirely, this would inevitably reduce the quantitative information available for panel 2 (One Health) applications.

Whole-genome sequencing represents another valuable option for AMR surveillance, providing comprehensive resistome data and high-resolution phylogenetic context in a single assay. However, WGS currently remains more resource-intensive than targeted PCR-based methods, requiring higher per-sample costs, longer turnaround times and specialised bioinformatic analysis (Bianco et al. 2021; Yamin et al. 2023; Farina et al. 2024). For the routine screening of large sample sets or for laboratories without extensive sequencing infrastructure, high-multiplex panels such as MOL-PCR can therefore offer a more practical first-line approach. Screening by WGS can be reserved for selected isolates where detailed characterisation or outbreak investigation is required.

We do not propose MOL-PCR as a wholesale substitute for qPCR or WGS, but as a high-multiplex screening layer that complements existing quantitative and genome-wide approaches. For large sample sets where broad gene coverage is the primary objective, such as baseline mapping of AMR determinants in foods, feed, or wastewater, MOL-PCR can cost-effectively screen dozens of resistance and toxin genes in parallel, identifying which markers are present and which matrices or locations warrant closer attention (Song et al. 2010; Kowalewicz et al. 2023). For a subset of epidemiologically or clinically critical targets (e.g. selected tetracycline, macrolide or sulphonamide resistance genes, or specific hotspots in the food chain), qPCR can be applied in a second step to determine copy numbers and monitor temporal dynamics. Subsequently, WGS can provide in-depth resistome and phylogenetic data for representative isolates. The inclusion of rare but high-risk determinants such as *cfr* and *optrA* as sentinel markers is particularly well suited to this multiplex screening layer, as their presence can be monitored at minimal additional cost or assay complexity (Wuyts et al. 2015; Ceyssens et al. 2016).

Reliable implementation of such a system requires robust validation and quality assurance. Each MOL-PCR panel should incorporate internal amplification controls, be evaluated against reference strains and well-characterised field isolates and have clearly defined analytical sensitivity and specificity for each target. Harmonised data interpretation and reporting are equally important. Linking panel design and results to curated databases, such as those cataloguing AMR genes and virulence factors, would facilitate consistent annotation and improve interoperability between laboratories and sectors (Yamin et al. 2023; Hattab et al. 2024). In the long term, integrating MOL-PCR outputs with metagenomic and epidemiological datasets could help bridge the gap between targeted genotyping and broader surveillance of the resistome.

Using *B. cereus* as an example highlights both the potential and the limitations of this approach. The species complex combines an extensive toxigenic repertoire with a heterogeneous but increasingly documented AMR profile (Farina et al. 2024). It occupies a central position at the intersection of food production, the environment, and human health. In this setting, a structured molecular surveillance framework anchored in phenotypic testing, supported by qPCR-based quantification where needed and extended by high-multiplex technologies such as MOL-PCR, with WGS reserved for in-depth characterisation, offers a rational path towards more integrated AMR monitoring.

In summary, *B. cereus* s.l. should be regarded not only as a cause of foodborne intoxications and opportunistic infections, but also as a resilient environmental reservoir and potential vector of antimicrobial resistance genes within the One Health continuum. Its intrinsic β-lactam non-susceptibility, diverse acquired resistance determinants, and ability to persist in food and environmental matrices underline the need for coordinated surveillance across clinical, food, and environmental sectors (Mills et al. 2022; Rahnama et al. 2023; EFSA and ECDC 2024). By summarising current knowledge on AMR mechanisms, evaluating phenotypic and molecular detection methods and proposing sector-specific clinical and One Health gene panels, this review provides a conceptual basis for molecular AMR surveillance in *B. cereus* s.l. High-multiplex platforms, such as MOL-PCR, have

the potential to operationalise these panels in practice, provided that their semi-quantitative nature is recognised and complemented by targeted quantitative PCR, robust phenotypic testing and, where appropriate, WGS-based characterisation (Reslova et al. 2019; Hrdy et al. 2021). Future work should focus on panel refinement, technical validation and the integration of these tools into harmonised surveillance programmes, enabling more effective and interoperable monitoring of *B. cereus*-associated AMR within the One Health framework.

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