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**Extraction of the epidemiological and microbiological data from a
set of *Bacillus cereus* literature**

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Declaration of independence

I hereby declare that I have written this work independently and have not used any sources or resources other than those indicated. All text taken from external sources has been acknowledged.

I performed the critical work myself, and acknowledged all contributors and their contributions to this work.

This work has not been submitted or published elsewhere.

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Abstract

Bacillus cereus (*B. cereus*) is a significant cause of foodborne illness worldwide. However, discrepancies between reporting systems and underreporting complicate accurate estimates of infection incidence. This diploma thesis presents a systematic evaluation of foodborne outbreaks caused by *B. cereus* with a focus on sources, food matrices, pathogenicity, toxicity, and diagnostic approaches. Epidemiological and microbiological data were extracted and analyzed from 39 reviews and 40 surveillance reports to identify patterns in outbreak occurrence, detection methods, and gaps or inconsistencies in the existing literature.

Analysis of the reviews revealed that temperature misuse and hygiene measures were frequently emphasized in most studies. Conventional identification methods include culture-based techniques, biochemical assays, and polymerase chain reaction (PCR). Most reviews concentrated on epidemiological aspects, while fewer addressed food safety or microbiological characterization. Rice-based products were the most frequently implicated food vehicle, with proteinaceous foods linked to diarrheal illness, and farinaceous foods to emetic syndrome. Vulnerable populations, including immunocompromised individuals, showed higher mortality. Comparisons with AI-assisted data extraction highlighted limitations in automated analysis and underscored the need for further development in this area, particularly in terms of outbreak details and microbiological characterization.

Analysis of surveillance reports confirmed fried rice as the primary outbreak vehicle. Additionally, meat products, dairy products, and certain "Chinese foods" were frequently cited as significant vehicles of transmission. Food service establishments, particularly restaurants, were the most common settings, and contributing factors included improper holding temperatures and inadequate cooking. Geographic coverage was uneven, with Europe and North America most represented, while Africa, Antarctica, and much of Asia and Oceania were largely unreported.

Overall, the study highlights the underreporting of *B. cereus* outbreaks and identifies existing gaps in epidemiological and microbiological data. To enhance outbreak surveillance and public health strategies, it is essential to address these data gaps. The following measures would be of utmost importance to close the existing gaps: standardizing reporting practices, strengthening laboratory capacity in low-income regions, and introducing innovative detection methods.

Zusammenfassung

Bacillus cereus (*B. cereus*) ist ein weltweit bedeutender Erreger lebensmittelbedingter Erkrankungen. Abweichungen zwischen Meldesystemen und Untererfassung erschweren jedoch eine präzise Schätzung der Infektionszahlen. Diese Diplomarbeit präsentiert eine systematische Auswertung lebensmittelbedingter Ausbrüche durch *B. cereus*, mit Fokus auf Quellen, Lebensmittelmatrizes, Pathogenität, Toxizität und diagnostische Methoden. Es wurden epidemiologische und mikrobiologische Daten aus 39 systematischen Reviews und 40 Überwachungsberichte extrahiert, um Ausbruchsmuster, Erkennungsmethoden sowie Lücken und Inkonsistenzen in der Literatur zu identifizieren.

Die Analyse der Reviews ergab, dass in den meisten Studien vor allem Temperaturmissbrauch und Hygienemaßnahmen thematisiert wurden. Gängige Identifikationsmethoden umfassen kulturbasierte Verfahren, biochemische Tests und die Polymerase-Kettenreaktion (PCR). Die meisten Reviews konzentrierten sich auf epidemiologische Aspekte, während Lebensmittelsicherheit und mikrobiologische Charakterisierung seltener im Mittelpunkt standen. Reisbasierte Produkte wurden am häufigsten als Übertragungsweg identifiziert. Proteinreiche Lebensmittel wurden vor allem mit dem diarrhöischen Syndrom assoziiert, während das emetische Syndrom überwiegend durch stärkehaltige Produkte ausgelöst wurde. Besonders gefährdete Personengruppen, wie immungeschwächte Individuen, wiesen eine höhere Sterblichkeit auf. Vergleiche mit KI-gestützter Datenauswertung zeigten Einschränkungen automatisierter Analysen und die Notwendigkeit an Weiterentwicklungen in diesem Bereich, insbesondere bei Details zu einzelnen Ausbrüchen und der mikrobiologischen Charakterisierung.

Die Analyse der Überwachungsberichte bestätigte, dass *B. cereus* primär in Reisgerichten vorkommt. Fleischprodukte, Milchprodukte und bestimmte asiatische Gerichte wurden ebenfalls häufig als relevante Übertragungswege genannt. Lebensmittelbetriebe, insbesondere Restaurants, waren die häufigsten Ausbruchsorte, wobei falsche Lagertemperaturen und unzureichendes Erhitzen als beitragende Faktoren identifiziert wurden. Die geografische Abdeckung der Ausbrüche war ungleichmäßig: Während Europa und Nordamerika stark vertreten waren, wurden Ausbrüche in Afrika, weiten Teilen Asiens, Ozeanien und der Antarktis nur selten dokumentiert.

Insgesamt unterstreicht die Arbeit die Untererfassung von *B. cereus*-Ausbrüchen und identifiziert bestehende Lücken in epidemiologischen und mikrobiologischen Daten. Die Ergebnisse liefern eine Grundlage für die Optimierung von Überwachungsstrategien und präventiven Maßnahmen im Bereich der öffentlichen Gesundheit.

Table of contents

1.	INTRODUCTION.....	8
1.1.	OUTBREAK CRITERIA AND THE SIGNIFICANCE OF <i>BACILLUS CEREUS</i>	8
1.1.1.	Outbreak definition and investigation	8
1.1.2.	<i>Bacillus cereus</i> : an important foodborne pathogen.....	9
1.2.	TAXONOMY AND GENOMIC DIVERSITY	10
1.3.	TYPES OF FOOD POISONING CAUSED BY <i>BACILLUS CEREUS</i>	12
1.3.1.	Emetic syndrome	12
1.3.2.	Diarrheal syndrome	12
1.4.	THE ROLE AND RELEVANCE OF EPIDEMIOLOGY IN PUBLIC HEALTH	13
1.5.	DETECTION OF <i>BACILLUS CEREUS</i>	13
1.5.1.	Culture-based methods	13
1.5.2.	Molecular methods	14
1.5.2.1.	PCR-based methods.....	14
1.5.2.2.	Gene-targeted typing approaches.....	15
1.5.2.3.	Whole-genome approaches	16
1.5.2.4.	Protein-based identification approaches	16
1.5.2.5.	Plasmid profiling and horizontal gene transfer	17
1.5.2.6.	Omics approaches	17
1.5.3.	Biosensor approaches.....	18
1.5.4.	Toxin detection	19
1.5.4.1.	Cytotoxicity and virulence phenotyping	19
1.5.4.2.	Cereulide detection	19
1.5.4.3.	Enterotoxin detection	20
1.6.	ANTIMICROBIAL RESISTANCE	20
1.7.	AIM OF THIS STUDY	21
2.	MATERIALS AND METHODS	22

2.1.	STUDY IDENTIFICATION	22
2.1.1.	Search strategy	22
2.1.2.	Study selection	24
2.2.	DATA EXTRACTION.....	26
2.2.1.	Reviews	26
2.2.2.	Surveillance reports.....	26
2.3.	INFORMATION SYNTHESIS.....	27
2.3.1.	Reviews	27
2.3.2.	Surveillance reports.....	27
3.	RESULTS.....	28
3.1.	REVIEWS	28
3.1.1.	Key epidemiological insights and focus areas of the reviews	28
3.1.2.	Temporal trends	32
3.1.3.	Outbreak trends.....	35
3.1.4.	Inconsistencies or contradictions in the literature	45
3.1.5.	Comparison with Artificial Intelligence (AI)	45
3.2.	SURVEILLANCE REPORTS	46
3.2.1.	Outbreak trends and patterns.....	46
3.2.2.	Inconsistencies and gaps in surveillance materials	49
4.	DISCUSSION	51
4.1.	TRENDS IN <i>BACILLUS CEREUS</i> OUTBREAK REPORTING	51
4.2.	DISCREPANCIES BETWEEN SURVEILLANCE DATA AND PUBLISHED LITERATURE.....	51
4.3.	REGIONAL DISPARITIES IN OUTBREAK DATA	52
4.4.	PATTERNS AND CHARACTERISTICS OF <i>BACILLUS CEREUS</i> OUTBREAKS.....	53
4.5.	METHODOLOGICAL CONSIDERATIONS.....	53
4.6.	LIMITATIONS OF THIS STUDY	54
4.7.	FUTURE DIRECTIONS.....	54
5.	REFERENCES.....	55

6.	APPENDIX	66
6.1.	LIST OF ABBREVIATIONS	66
6.2.	SUPPLEMENTARY MATERIALS	67
6.2.1.	Supplementary tables	67
6.2.2.	ChatGPT protocol for data extraction	81
6.2.3.	Supplementary figures	83
7.	LIST OF FIGURES AND TABLES	85

1. Introduction

1.1. Outbreak criteria and the significance of *Bacillus cereus*

1.1.1. Outbreak definition and investigation

In epidemiological terms, an "outbreak" is typically defined as the occurrence of a number of disease cases that exceeds the expected baseline within a specified geographic area over a defined time period (1). However, an outbreak may also be recognized through the identification of a cluster of cases, commonly two or more, that are epidemiologically linked by a shared exposure or risk factor, even when the overall incidence does not surpass expected levels. A defining characteristic of an outbreak is its abrupt onset, which typically necessitates the prompt implementation of public health interventions (1).

A systematically conducted outbreak investigation serves multiple purposes. Foremost, it aims to identify and eliminate the source of the outbreak while providing post-exposure prophylaxis to individuals at risk. Additionally, such investigations frequently contribute to the detection of previously unrecognized pathogens, thereby expanding the existing knowledge base. The rarity of (re)emerging pathogen outbreaks makes them valuable opportunities to conduct basic, translational, clinical, and epidemiological studies that can inform the development of next-generation medical countermeasures (1). Outbreak response research occurs in settings marked by stress and apprehension in affected communities and their health systems. To address the concerns of both community members and policy makers while maintaining methodological rigor, such studies must be designed and implemented with urgency (2). Measurement error is a common challenge in epidemiology, particularly for complex exposures such as diet and environmental contaminants. Practical measurement methods are prone to error, often resulting in random deviations, including short-term fluctuations or assay errors, as well as systematic bias from inaccurate self-reporting (3). The choice of research designs and methods should be determined by the specific knowledge gaps and by the feasibility of the research, considering what is both acceptable and practicable within the outbreak context (4). When selecting a testing method, it is recommended to consult relevant stakeholders and inspect the existing literature to gain an overview of available options. The final choice should consider budget and resource limitations, the test's sensitivity and specificity, the characteristics of the target population, and the surveillance framework in use (4).

Regardless of the disease in question, fundamental information is required to accurately characterize the overall pattern of disease occurrence within the at-risk population. This includes case identification, such as name, address, and other relevant identifiers. In addition, demographic characteristics, such as age and sex, must be documented. Clinical data are also essential and should contain the disease onset, the time of exposure to the infectious agent, clinical manifestations, and laboratory test results. Finally, information on potential risk factors, including underlying conditions that may influence an individual's susceptibility to the disease, is crucial for understanding the disease transmission and implementing control measures (1).

1.1.2. *Bacillus cereus*: an important foodborne pathogen

According to the European Union One Health 2023 Zoonoses report published by the European Food Safety Authority (EFSA) and the European Centre for Disease Prevention and Control (ECDC), bacterial toxins were implicated in approximately 15.7% of all reported foodborne outbreaks (5). Within this category, *B. cereus* toxins were the most frequently identified agents, accounting for 8.3% of total outbreaks. Notably, *B. cereus* toxins ranked as the second most commonly reported known causative agents of foodborne outbreaks in 2023, surpassed only by *Salmonella* (5). In comparison, approximately 14% of foodborne illnesses reported annually in the United States are attributed to *B. cereus*, *Clostridium perfringens* (*C. perfringens*), and *Staphylococcus aureus* (*S. aureus*) (6). Furthermore, between 1998 and 2008, nearly one in ten foodborne disease outbreaks reported to the Foodborne Disease Outbreak Surveillance System (FDOSS), a program of the Centers for Disease Control and Prevention (CDC) that collects and reports data about foodborne disease outbreaks in the United States, were caused by *B. cereus*, *C. perfringens*, or *S. aureus*. Among these, *B. cereus* accounted for 19% of the confirmed outbreaks (6). By contrast, a report by the Chinese Center for Disease Control and Prevention (China CDC) indicates that *B. cereus* accounted for only 1.89% of all foodborne disease outbreaks reported in catering facilities in China between 2010 and 2020 (7). Although global reports underscore the significance of *B. cereus* as a causative agent of foodborne illnesses, it is important to recognize that the reported percentages likely underestimate its true prevalence (8). Food poisoning caused by *B. cereus* is often underreported due to the typically mild symptoms and short duration of illness. Consequently, only a minority of affected individuals seek medical attention (9). Owing to its widespread prevalence, *B. cereus* readily

contaminates the food supply chain and is implicated in the etiology of foodborne illnesses (10).

1.2. Taxonomy and genomic diversity

Given its epidemiological significance, it is essential to understand the characteristics of *B. cereus*. *B. cereus* is a Gram-positive, facultative anaerobic, endospore-forming rod-shaped bacterium, commonly found in the environment (9).

B. cereus sensu stricto (s.s.) is a part of the so-called *Bacillus cereus* group, also known as *Bacillus cereus sensu lato* (*B. cereus* s.l.). The *B. cereus* group consists of genetically closely related bacteria, notably *Bacillus anthracis* (*B. anthracis*), *B. cereus* s.s., *Bacillus thuringiensis* (*B. thuringiensis*, Bt), and *Bacillus cytotoxicus* (*B. cytotoxicus*) (11–13).

Anthrax, caused by *B. anthracis*, is a zoonotic disease traditionally classified into cutaneous, gastrointestinal, and inhalational clinical syndromes (14, 15). Additionally, a fourth form, associated with severe soft tissue infection in injection drug users, has more recently been recognized. Cutaneous anthrax accounts for about 95% of reported cases, mainly in regions of Africa, Asia, and Eastern Europe with limited vaccination coverage (15). Untreated infections have a mortality of 5 to 20%, though the condition often resolves spontaneously. Infection occurs when spores enter skin breaks, germinating locally or in regional lymph nodes, with an incubation period of 1 to 12 days (15). Lesions initially form as painless papules with marked edema, progressing to vesicles and eventually ulcerating. Fever and regional lymphadenopathy may occur and facial or neck involvement may result in severe edema and airway compromise. Gastrointestinal anthrax, typically resulting from ingestion of contaminated meat, can reach mortality rates up to 60% and is often complicated by shock. Inhalational anthrax arises when inhaled spores reach mediastinal lymph nodes, potentially causing hemorrhagic mediastinitis (15). However, *B. anthracis* is not a central focus of this diploma thesis.

B. thuringiensis is naturally found in the gut of caterpillars of various moth and butterfly species, as well as on plant surfaces, in aquatic habitats, animal feces, and in facilities such as flour mills (16). In addition to spore formation, the species *B. thuringiensis* produces crystal inclusions containing insecticidal proteins, primarily encoded by *cry* (crystal) genes and, in certain strains such as *B. thuringiensis* subsp. *israelensis*, *cyt* (cytolytic) genes (16, 17). Certain strains, including *B. thuringiensis* subsp. *aizawai* ABTS-1857 and GC-91, display insecticidal properties through the production of δ -endotoxins (17). Commercial formulations

of Bt are typically produced as powders containing a combination of dried spores and toxin crystals. *B. thuringiensis* has been successfully applied in the management of insect pests in agriculture. Agricultural uses represent over 60% of the global market for Bt bioinsecticides (16). Although generally regarded as a safe and environmentally friendly microbial pesticide, *B. thuringiensis* cannot be distinguished from *B. cereus* at the chromosomal level (17). The majority of *B. cereus* strains carry the non-hemolytic enterotoxin complex (*nhe*) genes, while the hemolysin BL complex (*hbl*), and cytotoxin K (*cytK*) are present in 30 to 70% of isolates. These potential enterotoxins are also commonly detected in other members of the *B. cereus* group, such as *B. thuringiensis*. Consequently, levels of enterotoxigenic *B. cereus* that pose a risk to consumers likely apply to *B. thuringiensis* (16). Hitherto, there is no evidence that *B. thuringiensis* harbors the genetic determinants for the emetic toxin cereulide (18).

Comprehensive molecular characterization of *B. cereus* group strains is crucial for distinguishing *B. thuringiensis* from *B. cereus* and identifying strains associated with commercial biopesticides (17). Outbreak strains should be preserved in reference laboratories. Research on dose–response relationships, strain behavior, and environmental-to-food transfer pathways, as well as field studies following *B. thuringiensis* application, could support risk assessment and guide the establishment of pre-harvest intervals (16).

Even though *B. cytotoxicus* has been isolated from insect-related products and dried food products, it is challenging to estimate its impact as a foodborne pathogen. CytK, which was mentioned in the previous paragraph, exists in two variants sharing over 80% amino acid sequence identity. CytK-1 exhibits stronger hemolytic and cytotoxic effects on human intestinal cells than CytK-2. All *B. cytotoxicus* strains carry the *cytK-1* gene, whereas *B. cereus* strains possess the *cytK-2* gene variant. The cytotoxic potential of *B. cytotoxicus* warrants further study, alongside broader surveillance in food items and other environments. With its detection in edible insects, research on the effects of heating and other processing steps is needed to better assess its risk as a foodborne pathogen (19).

International standards (ISO 7932:2005-03) merely require the detection of “presumptive *B. cereus*” in routine examinations. Therefore, there is rarely discrimination between the members of the *B. cereus* group (11). Recently, emerging *B. cereus* strains have been linked to anthrax-like disease in humans and animals. These strains combine traits of *B. cereus* and *B. anthracis*, cause similar symptoms, and remain susceptible to standard anthrax antibiotics. Furthermore, vaccines provide protection in animal models. Their emergence highlights ongoing genetic exchange within the *Bacillus* genus and underscores the evolving

complexity of the *B. cereus* s.l. group, emphasizing the need for continued research in medical microbiology (20).

1.3. Types of food poisoning caused by *Bacillus cereus*

1.3.1. Emetic syndrome

Two distinct forms of foodborne illness caused by *B. cereus* s.s. have been described (21). The emetic syndrome, colloquially referred to as the “Fried Rice Syndrome” or “Chinese Restaurant Syndrome” (22, 12), results from intoxication with preformed cereulide, which is a toxic, ring-shaped, heat-resistant dodecadepsipeptide (23). The emetic toxin cereulide exhibits close chemical similarity to the potassium ionophore valinomycin. Cereulide is synthesized by a non-ribosomal peptide synthetase (NRPS) complex during the late logarithmic phase (24). The enzymes responsible for its biosynthesis are encoded within the cereulide synthetase (*ces*) gene cluster, which is located on a megaplasmid known as pCER270 (25). The emetic type of *B. cereus* food poisoning is characterized by acute nausea and vomiting, typically occurring 30 minutes to 6 hours after food consumption (21). The emetic syndrome gained attention in the United Kingdom during the 1970s after several incidences involving fried rice were reported (14, 21). In addition to rice, other starchy food products, such as pasta and noodles, have also been implicated as common vehicles for the transmission of cereulide. Although the emetic form of *B. cereus* foodborne illness typically manifests as a mild, self-limiting condition, exposure to elevated doses of cereulide has been associated with hepatotoxicity and neurotoxicity, particularly in immunocompromised individuals (10, 26, 27).

1.3.2. Diarrheal syndrome

In contrast to the emetic form of *B. cereus* illness, the diarrheal syndrome results from ingestion of enterotoxigenic *B. cereus* spores or vegetative cells (12). Within the host’s small intestine, several protein enterotoxins may be produced, including the tripartite non-hemolytic enterotoxin (Nhe), the tripartite hemolysin BL (Hbl), and the single-component Cytotoxin K (CytK). These enterotoxins damage the intestinal epithelial cells through their ability to form pores (27). Clinical symptoms of the diarrheal syndrome, such as nausea, diarrhea, and abdominal pain, typically manifest approximately 12 hours after the consumption of the contaminated food product. An incubation period of 8 to 16 hours has been described. The diarrheal form of *B. cereus* foodborne illness was first documented in Norway during the 1950s, in connection with cases of diarrhea following the consumption of vanilla sauce (14).

Enteropathogenic strains are typically associated with a wide range of foods, such as dairy products, meat products, vegetables, and spices. Similar to the emetic syndrome, the diarrheal syndrome typically presents a mild, self-limiting, self-treating disease progression (10).

1.4. The role and relevance of epidemiology in public health

While microbiological and genomic studies explain how an organism can cause the intoxication, epidemiology can reveal the scale of the impact by documenting the outbreaks, identifying vulnerable populations, and tracing sources of contamination in food supply chains. Epidemiology examines how diseases are distributed, the underlying causes and contributing factors, and strategies for their prevention and control. This field requires insight into the ways political, social, and scientific contexts interact to shape disease risk, which distinguishes it as a unique scientific discipline. However, some critics consider epidemiology imprecise, viewing it as a set of methods applied by other fields, and argue that its heavy reliance on observational data can make it resemble investigative reporting rather than a rigorous scientific enterprise (28).

Epidemiological research has resulted in measurable benefits, saving millions of lives from infectious diseases through interventions and prevention programs employed by study findings. Despite uncertainties and imperfect data, epidemiology remains central to protecting public health. It plays a crucial role in forecasting epidemics and pandemics, identifying diseases with outbreak potential, and guiding forward-planning measures to minimize fatalities. Furthermore, epidemiological research plays an increasingly vital role in assessing the impact of climate change on disease burden, encompassing the effects of temperature and other environmental factors, as well as the shifting geographic ranges of disease vectors (28). In this context, it remains essential for guiding public health policy in the foreseeable future.

1.5. Detection of *Bacillus cereus*

1.5.1. Culture-based methods

A variety of methodologies have been applied for the detection of *B. cereus*. However, the close genetic relatedness within the *B. cereus* group poses a challenge to the reliable identification of individual species and routine analysis typically reports presumptive *B. cereus* rather than definitive species identification (29). Culture-based methods rely on the bacterium's ability to grow on selective and differential media under specific conditions,

which targets the characteristic and biochemical properties of the organism to distinguish it from other microorganisms in a complex food matrix. The most commonly used media for enumeration are mannitol–egg yolk–polymyxin (MYP), as well as the same agar modified with pyruvate and bromothymol blue. Unlike other *Bacillus* species, *B. cereus* is typically mannitol negative and lecithinase (phosphatidylcholine-preferring phospholipase C) positive (14). However, the use of lecithinase production as a differential factor has been reported as unreliable in several studies (12). Conventional detection methods are time-consuming, resource-intensive, and require trained staff (29).

1.5.2. Molecular methods

1.5.2.1. PCR-based methods

While classical microbiological techniques have long been used for the detection of *B. cereus*, molecular methods have increasingly become the preferred approach due to their ability to distinguish *B. cereus* from closely related species, reduced time consumption, and lower resource requirements (29).

Conventional PCR enables the selective amplification of defined DNA regions through the use of sequence-specific primers, template DNA, the thermostable DNA polymerase Taq, deoxynucleoside triphosphates (dNTPs), and an appropriate reaction buffer (30).

Amplification is typically conducted in a DNA thermal cycler and the resulting products are commonly analyzed by agarose gel electrophoresis, which enables reliable size-based separation of DNA fragments (30, 31). For the detection of *B. cereus* group cells, a PCR assay targeting the 16S rDNA has been developed, employing group-specific primers alongside universal 16S rDNA primers as an internal control to ensure assay reliability (32).

Building upon conventional PCR, multiplex PCR allows the simultaneous amplification of multiple target sequences within a single reaction. The observation that amplicons of varying lengths, defined by distinct primer pairs, can be generated in one PCR tube represented a significant milestone in PCR diagnostics and was initially applied for the detection of deletions in the dystrophin gene (DMD), one of the largest human genes (33).

Over the past decades, numerous PCR-based techniques have been developed for the identification of *B. cereus*, including real-time PCR (qPCR), which enables real-time monitoring and quantification of target DNA (29, 34). In contrast, fingerprinting techniques such as randomly amplified polymorphic DNA PCR (RAPD PCR) and pulsed-field gel electrophoresis (PFGE) generate distinctive genomic profiles that facilitate strain-level

differentiation rather than gene-specific identification. RAPD PCR employs short, arbitrary primers to amplify random DNA segments, producing unique banding patterns for strain identification, whereas PFGE separates large DNA fragments under an alternating electric field, generating high-resolution patterns widely used in molecular epidemiology (35, 36). More recent studies have proposed an approach to accelerate the post-PCR phase by replacing the labor-intensive electrophoretic detection of PCR products with a rapid visualization system using gold nanoparticles (AuNPs) (29). Additionally, propidium monoazide (PMA) has been applied to selectively detect viable *B. cereus* cells, as PMA can only bind to the DNA of dead cells (29).

Despite these advances, PCR-based methods frequently fall short of enabling rapid, point-of-care detection, particularly due to their reliance on thermal cyclers, which complicates integration into farm-to-table safety testing (29).

1.5.2.2. Gene-targeted typing approaches

A variety of gene-targeted molecular techniques have been applied for bacterial identification, including 16S rRNA gene sequencing, amplified fragment length polymorphism (AFLP), and DNA-DNA hybridization (DDH) (37). The 16S rRNA gene is commonly used for microbial identification and taxonomic classification. However, its effectiveness for distinguishing members of the *B. cereus* group is limited, and its genomic distribution within this group has not been fully elucidated. Consistent with previous studies, the 16S rRNA gene does not provide sufficient resolution to reliably differentiate closely related *B. cereus* species (38). AFLP is particularly suited for high-throughput analysis, as it avoids labor-intensive sequencing. To facilitate the assignment of strains to the seven major genetic clusters (I-VII), the Sym'Previus *Bacillus cereus* typing tool has been developed (39). These molecular techniques served as essential tools for distinguishing among members of the *B. cereus* group, however, DDH is rarely used for routine typing given its complexity (38).

Advances in sequencing technologies and the discovery of novel species have led to increased application of gene-targeted approaches, including single-locus (SLST) and multi-locus sequence typing (MLST). These methods have become essential for the accurate identification and characterization of *B. cereus* group members (37, 40).

The pantoate- β -alanine ligase (*panC*) gene is commonly used in SLST to classify *B. cereus* group strains. Initially divided into seven phylogenetic clusters (Groups I–VII), which provided higher resolution than the 16S rRNA (41). The scheme was later updated to eight groups (I–

VIII) with representative species names (37). Over the past decade, multiple MLST schemes have been established for the typing of *B. cereus* strains. These approaches have been effectively used to investigate the genetic relatedness of isolates originating from diverse sources, including soil, food products, and clinical samples. While the schemes differ in the housekeeping genes they employ, they consistently identify three major phylogenetic clades. However, MLST is limited by its labor-intensive workflow, reducing its suitability for large-scale epidemiological studies (39).

1.5.2.3. Whole-genome approaches

Beyond targeted loci, whole-genome sequence (WGS) analyses offer higher resolution for strain differentiation, as they involve sequencing, analyzing, and comparing the entire DNA content of *B. cereus* isolates. Pan-genome analysis offers a comprehensive overview of the genetic content of bacterial strains, revealing insights into their environmental adaptability and metabolic potential. By comparison, whole-genome single nucleotide polymorphism (wgSNP) analysis enables high-resolution differentiation of closely related strains, detecting subtle genetic variations. Single nucleotide polymorphisms (SNPs) serve as evolutionarily stable markers that are useful for resolving deep phylogenetic relationships across global strain populations (38).

Digital DNA-DNA hybridization (dDDH) is a rapid and accurate alternative to traditional DDH. Based on the Genome-Blast Distance Phylogeny (GBDP) method, it calculates intergenomic distances from whole-genome sequences to estimate species relatedness. dDDH values, obtained via the Genome-to-Genome Distance Calculator (GGDC), are highly reliable for species delimitation and allow simultaneous construction of phylogenies with statistical support (38).

1.5.2.4. Protein-based identification approaches

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) has been routinely adopted as a first-line identification method in laboratories over the past 15 years. This technique offers several advantages over conventional microbiological and molecular methods for species-level identification, including reliability and rapid results, allowing microbial identification within minutes (42, 43). Complementary to this, spectroscopic approaches such as Fourier Transform Infrared (FTIR) spectroscopy provide rapid strain typing, measuring absorption of specific infrared frequencies that correspond to vibrational motions of chemical bonds (44). The resulting absorption spectrum generates a

characteristic profile that reflects the functional groups and molecular structures within a sample. Both MALDI-TOF and FTIR spectroscopy complement traditional molecular methods by enabling fast, accurate, and cost-effective identification of bacterial strains (43, 44).

Immunological assays, such as enzyme-linked immunosorbent assays (ELISA) and lateral flow devices (LFDs), are suitable methodologies for the rapid visual detection of *B. cereus*. While these methods display lower sensitivity compared to PCR-based techniques, they provide prompt results and on-site applicability (12).

Moreover, other protein-based molecular methods enable more detailed characterization of specific proteins (45, 46). Western blotting (WB), also called immunoblotting, is a widely used technique for detecting and analyzing individual proteins based on their molecular weight and charge (46). In contrast, proteomics approaches, such as liquid chromatography–mass spectrometry (LC-MS/MS), are widely employed to characterize pathogen structures and components associated with virulence. These methods facilitate the analysis of both bacterial and viral pathogens, aiming not only to identify structural and non-structural proteins involved in pathogenicity but also to investigate underlying metabolic and physiological factors (46).

1.5.2.5. Plasmid profiling and horizontal gene transfer

Plasmid profiling characterizes extrachromosomal DNA elements, many of which are involved in horizontal gene transfer (HGT). HGT plays a central role in the evolution of bacterial populations. Key contributors to HGT include conjugation-like mechanisms and mobile plasmids. In the *B. cereus* group, conjugative plasmids facilitate both self-transfer and mobilization of co-resident plasmids. Since plasmids often carry virulence or resistance determinants, their identification provides valuable insights into genetic exchange and the evolutionary dynamics of *B. cereus* group members (47). Recent advances in long-read whole-genome sequencing (LR-WGS) now often replace classical plasmid profiling, enabling the generation of complete plasmid sequences and more comprehensive analyses of plasmid-mediated gene transfer (48, 49).

1.5.2.6. Omics approaches

Since the initiation of the human genome project, advances in high-throughput technologies have enabled the global measurement of biological molecules within cells or tissues, providing detailed insights into cellular processes. The study of such molecules at a system-wide level is broadly referred to as “omics” and includes fields such as genomics (genes), transcriptomics (RNA), proteomics (proteins), and metabolomics (metabolites) (50).

In the context of *B. cereus*, transcriptomics approaches have increasingly been applied to investigate gene expression under varying environmental conditions and during host-pathogen interactions. The transcriptome encompasses all RNA molecules in a cell, including ribosomal RNA (rRNA), messenger RNA (mRNA), transfer RNA (tRNA), and non-coding RNAs (ncRNAs). Transcriptome profiling is commonly performed using microarrays or RNA sequencing (RNA-seq). These analyses provide insights into regulatory mechanisms and virulence gene expression, linking genetic potential to phenotypic traits (50, 51). Complementary to transcriptomics, proteomics involves the comprehensive analysis of protein structure and function. It enables the analysis of protein expression at multiple levels, assessing both quantitative and qualitative cellular responses associated with specific proteins (52). Metabolomics allows the detection of endogenous and exogenous metabolic end products and is particularly valuable for identifying subtle metabolic changes under environmental stress that are not detectable through conventional phenotypic assays. By integrating metabolomics with transcriptomics and proteomics, previously uncharacterized genes, proteins, and metabolic pathways involved in stress responses can be elucidated, providing a systems-level understanding of *B. cereus* and its adaptive mechanisms (53).

1.5.3. Biosensor approaches

Over the past few years, biosensors have gained recognition as promising tools for the identification of foodborne pathogens. These miniaturized analytical devices provide quick results and are suitable for point-of-care diagnostics. Additionally, biosensors turn out to be cost-efficient, reliable, and require minimal sample volumes. In particular, DNA-based biosensors have proven to be efficient due to their ability to selectively identify distinct *B. cereus* strains (29). Monoclonal or polyclonal antibodies directed against *B. cereus* cells represent an alternative to DNA-based probes as recognition elements in biosensor systems. A variety of antibody-based strategies have been adopted for the specific detection of *B. cereus*. However, the elevated cost and relatively low affinity of commercially available anti-*B. cereus* antibodies limit the applicability of this detection method (29). Bacteriophages and their derived proteins represent viable alternatives to monoclonal or polyclonal antibodies due to their high host specificity, strong binding affinity, and notable resistance to chemical substances as well as environmental fluctuations in temperature and pH (29). Bacteriophages can be effectively employed as biorecognition elements in biosensor systems designed for the detection of bacterial pathogens. Notably, a *B. cereus*-specific endolysin-derived cell wall-binding domain (CBD) has been utilized to develop a surface

plasmon resonance (SPR) biosensor, which demonstrates high specificity and sensitivity toward *B. cereus* (29, 12).

1.5.4. Toxin detection

1.5.4.1. Cytotoxicity and virulence phenotyping

Cytotoxicity assays are employed to assess the functional activity of *B. cereus* toxins and overall virulence potential. These assays typically involve exposing cultured mammalian cells, such as Vero, HEp-2, or Caco-2 cells, to bacterial supernatants or purified toxins, followed by measurement of cell viability, membrane integrity, or metabolic activity (54, 55). By linking the presence of toxin genes or proteins to their actual effects on cells, cytotoxicity assays complement genetic analyses, offering a more complete assessment of a strain's virulence (56).

1.5.4.2. Cereulide detection

Various methods have been employed for the detection of cereulide. In the early stages of investigation, monkey feeding trials were conducted due to the absence of more suitable analytical techniques (57). Owing to cereulide's ability to induce vacuolization in human larynx carcinoma (HEp-2) cells, multiple variants of cell culture assays were subsequently developed (57). The boar sperm bioassay, which serves as a practical alternative to more recent methods for the screening of *B. cereus*, utilizes the mitochondria-damaging properties of cereulide, resulting in impaired sperm motility (9). Another approach for cereulide detection involves the application of a quantitative assay using rat liver mitochondria. This method is based on cereulide's capacity to interfere with mitochondrial respiratory activity (57). In recent years, PCR-based detection of the cereulide synthetase (*ces*) gene has frequently been employed to detect emetic *B. cereus* strains (24). While the presence of the *ces* gene does not definitively confirm the cereulide production, it indicates the capacity of a strain to synthesize the toxin under favorable environmental conditions. Analytical techniques such as LC-MS and MALDI-TOF MS are widely employed for the quantification of cereulide due to their high specificity and low cross-reactivity. However, these methods require the use of an appropriate chemical standard (29). Valinomycin, a commercially available compound with structural similarity to cereulide, has frequently been utilized for this purpose. Nonetheless, quantifying cereulide concentrations as valinomycin equivalents has been the subject of critical discussion, given the noteworthy chemical and biological differences. To address this limitation, several studies have investigated the application of isotope-labelled

cereulide as a more suitable reference standard (58, 59). At present, definitive confirmation and/or quantification of the emetic toxin requires time-consuming and costly high-performance liquid chromatography coupled with mass spectrometry (HPLC-MS) or LC-MS/MS (27).

1.5.4.3. Enterotoxin detection

The principal virulence factors implicated in the diarrheal form of *B. cereus* foodborne disease are the cytotoxins Hbl, Nhe, and CytK (9). Early detection of toxicity relied on nonspecific methods, including the observation of fluid accumulation in ligated ileal loops of rabbits and the use of tissue culture assays. Antibody-based detection kits targeting the enterotoxins are commercially available for three-component toxins Nhe and Hbl. These immunoassay kits utilize lateral flow immunological detection to enable rapid analysis (27). However, no comparable diagnostic tools are currently available for the single-component cytotoxin CytK. As the commercial detection kits target merely one component of each tripartite toxin, the presence of a fully functional toxin complex cannot be conclusively confirmed (57). Nevertheless, results obtained from tissue culture assays show a good correlation with those obtained from the immunoassays (27). Another approach involves the application of PCR-based methods for the detection of enterotoxin-encoding genes (*hbl*, *nhe*, and *cytK*) (57). As with the PCR-based detection of cereulide, these methods are subject to limitations, as the mere presence of such genes does not necessarily indicate that the strain exhibits toxigenic activity (60, 29). Although the use of MALDI-TOF MS for the detection of diarrheal toxins demonstrates high specificity and sensitivity, this method is labor-intensive and requires extensive maintenance (29).

1.6. Antimicrobial resistance

Infections caused by *B. cereus* are commonly managed with antibiotic therapy. However, the disproportionate use of these antimicrobial agents has contributed to the development of antibiotic resistance (61). This phenomenon represents a significant public health concern, as infections caused by resistant bacterial strains are markedly more difficult to treat than those involving susceptible organisms. Consequently, antibiotic resistance is associated with increased healthcare costs and higher mortality rates. Bacterial resistance to antibiotics can be classified as either intrinsic or acquired. Intrinsic resistance is inherently encoded in the bacterial genome and is not influenced by exposure to antimicrobial agents. In contrast, acquired resistance develops through the transfer of external genetic material, typically

mediated by mobile genetic elements such as plasmids, integrons, transposons, and bacteriophages (62). *B. cereus* strains are characteristically resistant to β -lactam antibiotics, such as penicillin G and cefotaxime (61). However, they generally remain susceptible to a variety of other antimicrobial agents, including chloramphenicol, clindamycin, vancomycin, ciprofloxacin, erythromycin, gentamicin, doxycycline, and tetracycline (61). The underlying mechanisms responsible for antibiotic resistance in *B. cereus* remain unclear, primarily due to the limited number of studies dedicated to this topic (62).

1.7. Aim of this study

The aim of the study is to conduct a comprehensive and systematic evaluation of published reviews and surveillance reports on foodborne outbreaks caused by *B. cereus* with particular attention to sources, food matrices, pathogenicity, toxicity, and diagnostic approaches in all geographic regions. By exclusively focusing on peer-reviewed reviews and published surveillance reports obtained from searches of the peer-reviewed literature databases, this study seeks to recognize the current stage of the *B. cereus* outbreak documentation, pinpoint the methodological and reporting shortcomings, identify existing gaps, and complement the current state of knowledge. This knowledge can enhance the accuracy and utility of future epidemiological and methodological assessments.

2. Materials and methods

2.1. Study identification

This diploma thesis contributes to a broader project conducted by Masoumeh Alinaghi, PhD. The project aims to use a scoping review approach to compile literature on *B. cereus* outbreaks worldwide and to identify gaps in epidemiological and analytical characterization for further research.

2.1.1. Search strategy

The databases used for the literature search were PubMed, Web of Science Core Collection, CABI: CAB Abstracts, and Food Science and Technology Abstracts (FSTA). Solely papers in English were included, and the search terms were combinations of keywords like "*Bacillus*" and "outbreak" as listed below (Fig. 1).

```
("outbreak"[Title/Abstract] AND "english"[Language] AND (("disease outbreaks"[MeSH Terms]
OR "epidemiology"[MeSH Subheading]) AND "english"[Language]) AND "english"[Language]
AND (("Bacillus"[Title/Abstract] OR "Cereus"[Title/Abstract] OR "kurstaki"[Title/Abstract] OR
"Aizawai"[Title/Abstract] OR "thurinigiensis"[Title/Abstract] OR "thuringensis"[Title/Abstract]
OR "turingiensis"[Title/Abstract] OR OR "weihenstephanensis"[Title/Abstract] OR
"cytotoxicus"[Title/Abstract]) AND "english"[Language])) AND (english[Filter])
```

Fig. 1: Search terms used during the literature search.

The eligibility criteria, including study characteristics, geographic regions, and time periods, are shown in Table 1. The retrieved papers were subsequently imported into the reference management tool "EndNote", where references from multiple databases were merged and the databases were organized.

Table 1: Eligibility criteria for the study.

Study characteristics	• Primary research studies, including epidemiological reports, outbreak investigations, and surveillance studies. • Secondary research studies, including narrative reviews and systematic reviews (ensuring primary studies cited are deduplicated). • Case reports and grey literature such as outbreak summaries.
Population	Studies reporting on <i>Bacillus</i> -related outbreaks in any population.
Outcome	• Studies reporting the occurrence of <i>Bacillus</i>-associated outbreaks (e.g., confirmed or suspected outbreaks). • Analytical and microbiological characterization methods: Detection and differentiation techniques, including molecular, biochemical, or toxin-detection methods.
Language	English
Databases	1. PubMed 2. Web of Science Core Collection 3. CABI: CAB Abstracts 4. Food Science and Technology Abstracts (FSTA) Search string found only abstract and title.
Publication Type	• Peer-reviewed journal articles (both primary and secondary research). • Excludes editorials, opinion pieces, and abstracts without full texts.
Search Terms	A combination of keywords such as " <i>Bacillus</i> " and "outbreak".
Geographic Scope	No restrictions on geographic location.
Time Period	No limitations on the time frame of the included studies.

Exclusion Criteria	• <i>B. anthracis</i> outbreak • Clinical Studies: Studies exclusively focusing on clinical or laboratory findings unrelated to outbreak scenarios. • Multi-pathogen Outbreaks: Reports of outbreaks involving multiple pathogens where <i>Bacillus</i> is not the primary focus. • Language Restrictions: Studies published in languages other than English. • Non-relevant Settings: Laboratory-only studies without real-world outbreak data.
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2.1.2. Study selection

The systematic review tool "DistillerSR®" (Evidence Partners, Ottawa, ON, Canada) was utilized to facilitate the study selection process, while the "PRISMA" framework was applied to ensure structured and transparent reporting. "PRISMA" (Preferred Reporting Items of Systematic Reviews and Meta-Analyses) is specifically designed to enhance clarity and reproducibility of systematic reviews (63, 64). A central element of this framework is the "PRISMA" flow diagram, a four-stage chart that depicts the progression of studies throughout the review process. It provides a visual representation of how studies are identified, screened, and subsequently included or excluded. The diagram enables readers to trace how the initial pool of articles identified through database searches is systematically reduced to the final set of studies deemed eligible for inclusion. The "PRISMA" flow diagram related to this study is presented in Fig. 2.

As part of the pre-screening process, 2,133 publications were gathered from the aforementioned databases. In the next step, the titles and abstracts of the obtained papers were evaluated in more detail to identify potentially suitable studies. 216 publications were eligible for inclusion. A full-text review was the final step in the study selection process. Finally, 191 papers were considered suitable for the data extraction step. These 191 papers consist of original papers, reviews, and surveillance reports. This diploma thesis focuses on reviews (n=39) and surveillance reports (n=40). Two reviews were subsequently excluded due to insufficient reference to *B. cereus*, resulting in a final total of 37 reviews included in the evaluation of this study. The list of the 37 reviews and 40 surveillance reports is provided in the supplementary tables at the end of this diploma thesis (Table S2 and S3).

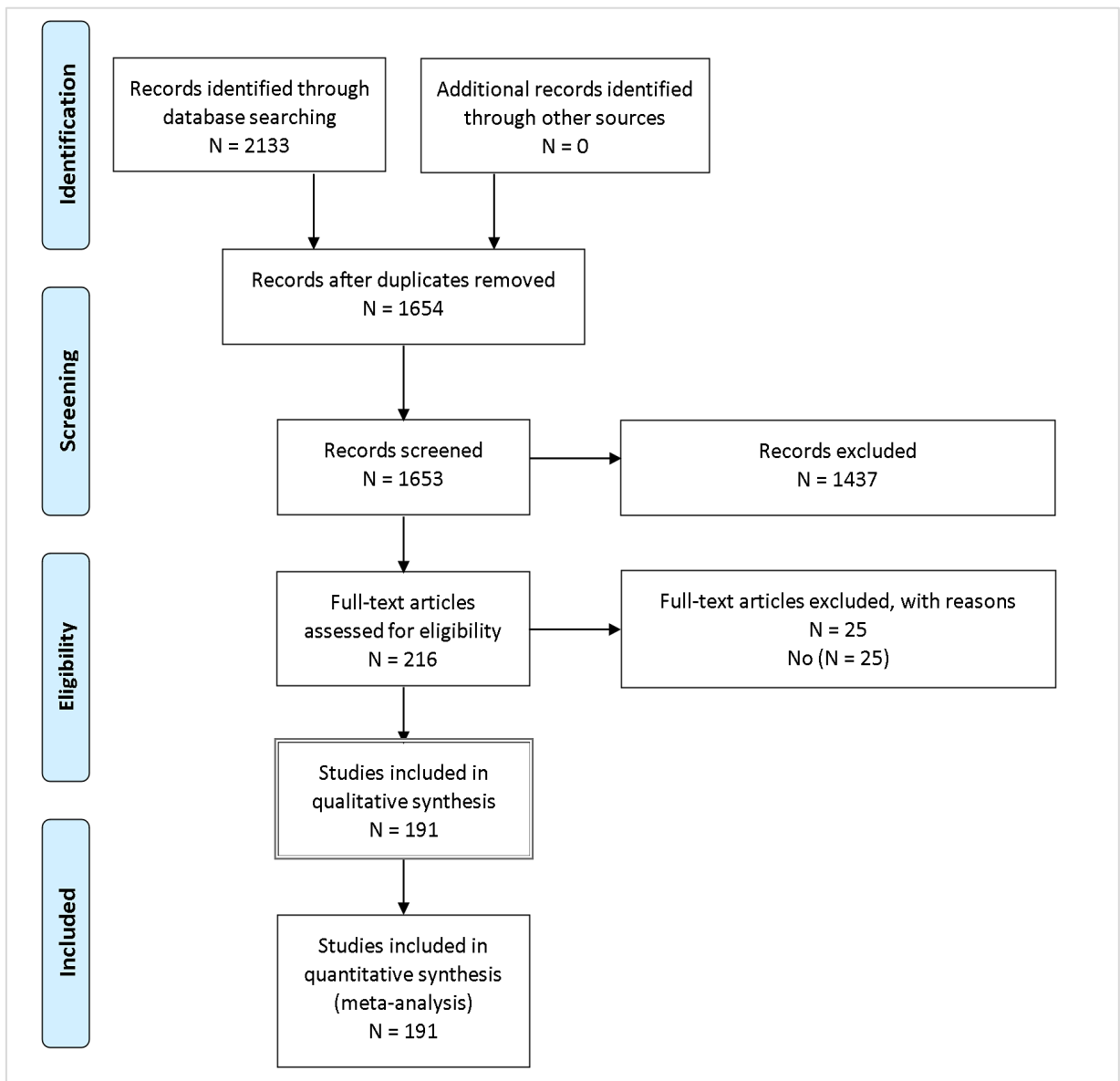


Fig. 2: “PRISMA” flow diagram illustrating the study selection process. Adapted from the presentation: “*From Search to Synthesis: The Process of Conducting a Systematic Review on Bacillus Outbreaks*” by M. Alinaghi, 2024. Data were extracted using the “DistillerSR” software. This chart reflects the most recent update available at the time of writing this diploma thesis, and the numbers may change at later stages.

2.2. Data extraction

The data extraction for this thesis is only performed for the reviews and the surveillance reports. The process was carried out in two distinct sections as follows.

2.2.1. Reviews

Following the study identification process, the 37 eligible reviews were entered into structured tables using Microsoft 365 Excel (Version 2506; Microsoft Corporation, Redmond, WA, USA). The Excel tables contained study characteristics, including title, authors, year of publication, journal, focus of the review, databases searched, country, and year of the outbreak, as well as more in-depth outbreak information in each review. This included common outbreak sources, epidemiological patterns, clinical symptoms, severity, and mortality. Furthermore, the tables encompassed data on microbiological or analytical characterization, presence of meta-analyses, study limitations and conclusions or key findings. Since such information is not available in all review papers, only a fraction of the studies provide complete data for each category. Consequently, some table entries remained blank.

In a subsequent step, the manually extracted data were compared with data extracted by artificial intelligence (AI) to identify potential inconsistencies. Review titles, authors, and publication years were entered into ChatGPT (GPT-4-Turbo, accessed in April 2025). Once the AI correctly identified the respective review, structured data extraction questions were posed, following the same categories as the manual Microsoft Excel tables (Version 2506; Microsoft Corporation, Redmond, WA, USA). The AI-generated responses were transcribed into the Excel tables. A protocol listing the exact questions posed to ChatGPT is provided in the supplementary material (Table S4).

2.2.2. Surveillance reports

In the second phase of data extraction, 40 surveillance reports were transferred into tables. The tables of each report included identification features such as author, title, journal, and year of publication. To summarize the outbreaks, the tables also captured the responsible agency or organization, the geographic scope, the time period covered, the total number of *B. cereus* outbreaks, the number of reported cases, the number of hospitalizations, and the associated mortality. Additionally, the tables contained outbreak characteristics such as common food sources, common reasons and/or causes, and observed seasonality. The

compilation was rounded off with information on microbiological or analytical methods employed, as well as conclusions and/or trends.

2.3. Information synthesis

Similar to the data extraction step, the information synthesis process was divided into two sections for reviews and surveillance reports. This process involved organizing extracted data, identifying relevant parameters, and generating graphical representations.

2.3.1. Reviews

Following data extraction, the collected information for narrative synthesis was compiled and organized in Microsoft 365 Word (Version 2506; Microsoft Corporation, Redmond, WA, USA). Extracted parameters included epidemiological characteristics, outbreak sources, and identification methods reported in the eligible literature. Additionally, the temporal distribution of studies, the focus on historical versus recent outbreaks, food sources, and geographic locations were recorded.

The extracted data were processed and summarized for subsequent visualization. Graphical representations were generated using Microsoft 365 (Microsoft Corporation, Redmond, WA, USA). Bar charts and stacked bar charts were created in Microsoft Excel (Version 2506) to depict temporal distributions. A Venn diagram was generated in Microsoft PowerPoint (Version 2507) to illustrate thematic focus areas. World maps were produced using Microsoft Excel (Version 2506) map charts to represent the country of the first author, the country of the corresponding author, and the frequency of country mentions. Selected extracted data are provided in the supplementary table (Table S2).

2.3.2. Surveillance reports

Data from surveillance reports were compiled and organized in Microsoft 365 Word (Version 2506; Microsoft Corporation, Redmond, WA, USA) using the same procedure applied for the reviews. Extracted parameters included outbreak trends over time, foods or settings consistently implicated, and the geographic coverage of the surveillance reports.

Graphical representations were generated using Microsoft 365 Excel (Version 2506; Microsoft Corporation, Redmond, WA, USA). Map charts were used to visualize author affiliations and outbreak-related country mentions, while bar charts and stacked bar charts summarized temporal trends. Selected extracted data are provided in the supplementary table (Table S3).

3. Results

The study was structured to identify key patterns, temporal developments, and outbreak trends within the reviewed literature. In addition, inconsistencies, knowledge gaps, and contradictions across the literature were examined. Key findings from outbreak reports were also synthesized to highlight recurring trends and notable discrepancies.

3.1. Reviews

3.1.1. Key epidemiological insights and focus areas of the reviews

The first sub-item in the information synthesis step of the reviews aimed to identify recurring patterns, inconsistencies and gaps in the eligible literature. The thematic foci of the reviews were systematically analyzed and the results were graphically represented using a Venn diagram (Fig. 3).

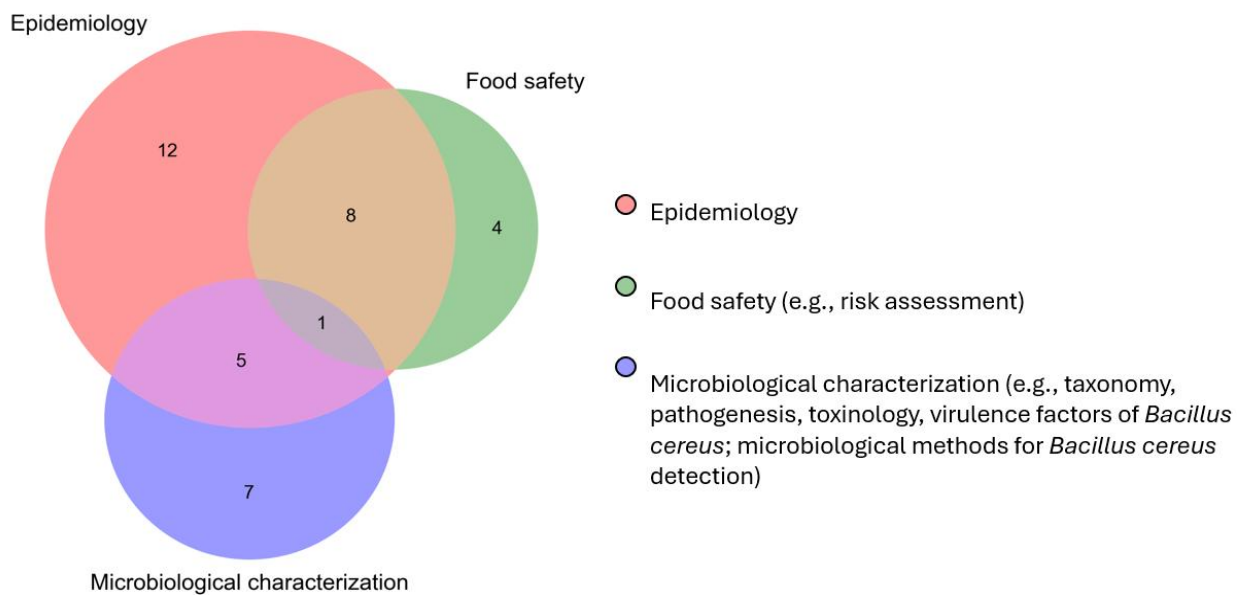


Fig. 3: Thematic foci of the eligible reviews. The Venn diagram illustrates the overlap and distribution among the three thematic categories: epidemiology, food safety, and microbiological characterization. The numbers within the circles indicate the number of reviews corresponding to the respective thematic focus. A total of 37 reviews were included.

The majority of the reviews concentrated on epidemiological aspects, including outbreak investigations, case numbers, symptoms exhibited by affected individuals, incubation periods, onset times, and mortality. Of the 37 reviews, four primarily addressed food safety concerns, particularly risk assessment related to the subject. Another 21% of the review articles focused on both food safety concerns and the epidemiological aspects of *B. cereus*. Seven of the eligible 37 reviews centered on the microbiological characterization of *B. cereus*, encompassing taxonomy, pathogenesis, toxicology, virulence factors, as well as the microbiological methodologies employed for its detection. Approximately 13% of the reviews addressed both microbiological characterization and epidemiology. Only one of the 37 eligible papers devoted attention to all three thematic categories, namely epidemiology, food safety, and microbiological characterization. The focus of each review is summarized in the supplementary table S2, which also provides additional information including publication year, countries of the outbreak and reference.

Furthermore, around 38% of the reviews emphasized the significance of hygiene measures in the prevention of illness (Table 5).

Table 5: Importance of hygiene measures. 14 out of 37 reviews emphasized the significance of hygiene measures. 1/0 indicates whether the importance of hygiene measures was mentioned (1) or not mentioned (0).

Title	Importance of hygiene measures mentioned
Paper I	0
Paper II	0
Paper III	1
Paper IV	0
Paper V	0
Paper VI	1
Paper VII	1
Paper VIII	1
Paper IX	1
Paper X	0
Paper XI	0
Paper XII	1
Paper XIII	1
Paper XIV	1
Paper XV	0
Paper XVI	1
Paper XVII	0
Paper XVIII	0
Paper XIX	0
Paper XX	0
Paper XXI	0
Paper XXII	0
Paper XXIII	0
Paper XXIV	0
Paper XXV	0
Paper XXVI	1
Paper XXVII	0
Paper XXVIII	0
Paper XXIX	1
Paper XXX	0
Paper XXXI	1
Paper XXXII	0
Paper XXXIII	0
Paper XXXIV	0
Paper XXXV	0
Paper XXXVI	1
Paper XXXVII	1

Regarding commonly used identification techniques, more than a quarter of the reviews discussed methods such as culture-based methods, biochemical assays (e.g., immunoassays), and molecular methods, particularly PCR-based assays. In this context, advancements in detection technology were defined as emerging or novel methods

recognized as useful for detecting *B. cereus* within the last decade. The distribution of detection methods reported across the 37 eligible reviews is summarized in Table 6.

Table 6: Presence (1) or absence (0) of detection methods mentioned in the 37 eligible reviews. 1/0 indicates whether the particular detection method (i.e., culture-based methods, biochemical tests, molecular methods, and advancements in detection technology) was mentioned (1) or not mentioned (0).

Title	Culture-based methods	Biochemical tests	Molecular methods	Advancements in detection technology
Paper I	0	0	1	0
Paper II	1	0	1	1
Paper III	0	0	0	0
Paper IV	0	0	0	0
Paper V	0	0	0	0
Paper VI	0	0	0	0
Paper VII	0	0	0	0
Paper VIII	1	0	0	0
Paper IX	0	0	0	0
Paper X	1	0	1	1
Paper XI	0	0	0	0
Paper XII	1	0	1	1
Paper XIII	0	0	0	0
Paper XIV	0	1	0	0
Paper XV	0	0	0	0
Paper XVI	1	1	1	0
Paper XVII	0	0	0	0
Paper XVIII	0	0	0	0
Paper XIX	1	1	0	0
Paper XX	1	0	1	0
Paper XXI	1	1	1	1
Paper XXII	1	1	1	1
Paper XXIII	0	0	1	1
Paper XXIV	1	1	1	0
Paper XXV	0	1	1	0
Paper XXVI	1	1	1	0
Paper XXVII	1	1	1	0
Paper XXVIII	1	0	0	0
Paper XXIX	1	0	0	0
Paper XXX	1	1	1	1
Paper XXXI	0	0	1	0
Paper XXXII	1	1	1	0
Paper XXXIII	1	1	1	0
Paper XXXIV	0	0	0	0
Paper XXXV	1	1	1	1
Paper XXXVI	1	1	1	1
Paper XXXVII	0	0	0	0

3.1.2. Temporal trends

In addition, time-based gaps were examined in greater detail. Over 55% of the reviews referenced outbreak periods spanning the 1950s–1970s, 1980s–2000, and 2006–2010. In contrast, fewer than 11% of the reviews addressed outbreaks occurring between 2021–2024. A bar chart displays the number of published reviews on *B. cereus* per year between 1972 to 2024 (Fig. 4). Between 1972 and 2004, only 11 reviews were published. From 2009 onwards, however, the number of publications increased substantially. In the past 15 years alone, 24 reviews have been published, which is approximately twice as many as in the preceding three decades. Additionally, a stacked bar chart illustrates the temporal distribution of mentioned outbreaks across each review. The figure is provided in the supplementary materials (Fig. S5). The timeline analysis indicates a notable increase in citation activity after the mid-twentieth century, with the majority of papers being cited predominantly from the 1970s onwards. In the last two decades (2000–2020), citation spans appear more densely clustered.

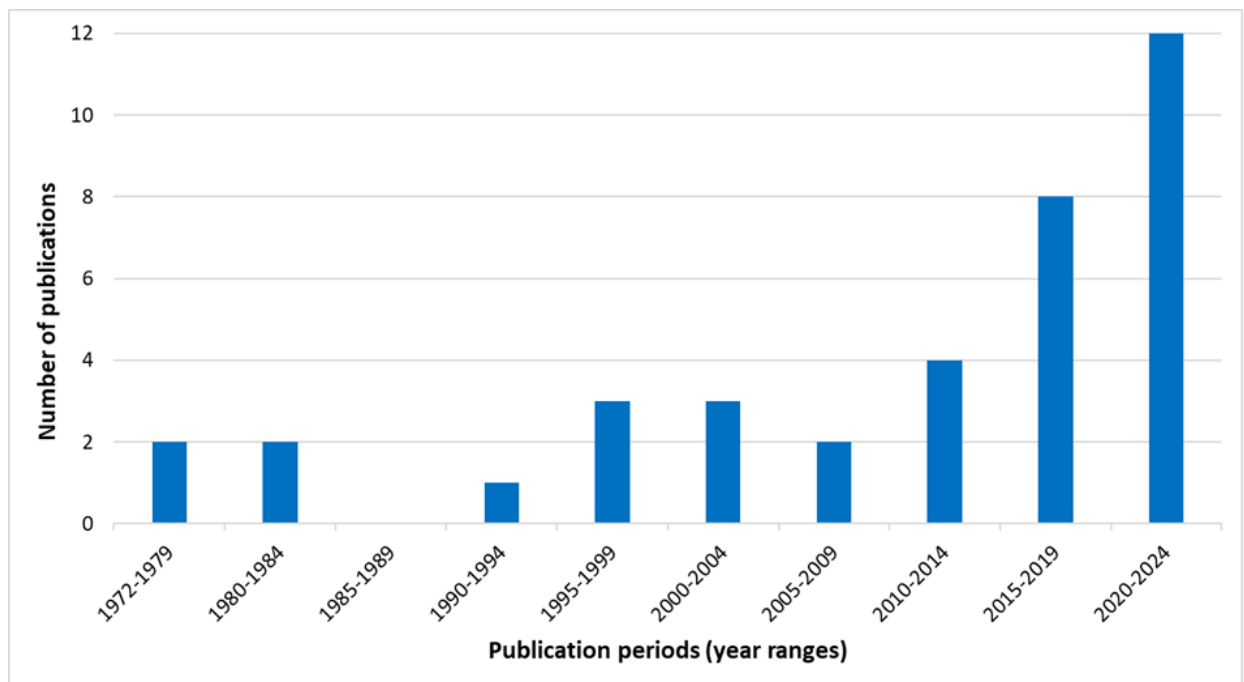


Fig. 4: Number of review publications including information on *Bacillus cereus* outbreaks. Data are shown for five-year periods (1972–2024), except for the initial interval (1972–1979), which spans eight years. The highest number of review publications was recorded during 2020–2024.

While some emerging detection methods were introduced earlier, the majority, including endolysin cell wall-binding domain-based techniques, artificial neural network (ANN)-assisted FTIR spectroscopy, and ultra-performance liquid chromatography coupled with electrospray ionization tandem mass spectrometry (UPLC-ESI-MS/MS), have appeared more frequently in reviews published from 2020 onward (Table 7). Nonetheless, more recent reviews continue to reference conventional methods, including culture-based methods and biochemical assays (Table 7). Table 7 provides a chronological overview of conventional and emerging detection methods mentioned in reviews from 1972 to 2024, highlighting the first appearance of emerging methods and showing that conventional methods are still widely cited.

Table 7: Overview of conventional and emerging detection methods mentioned in reviews from 1972 to 2024. Conventional methods include culture-based, biochemical, molecular assays, and advanced techniques such as HPLC-MS analysis. Emerging methods, appearing from 2020 onward, include novel molecular and spectroscopic approaches. The symbol “/” indicates that no detection methods were reported in the review.

Title	Publication year	Conventional methods	Emerging methods
Paper XIX	1972	culture-based, biochemical	/
Paper XXIX	1978	culture-based	/
Paper XXVIII	1981	culture-based	/
Paper VIII	1984	culture-based	/
Paper XVIII	1989	/	/
Paper IX	1990	/	/
Paper XIII	1995	/	/
Paper XXV	1997	biochemical, molecular	/
Paper XXVII	2000	culture-based, biochemical, molecular	/
Paper XXXIII	2000	culture-based, biochemical, molecular	/

Paper XXVI	2004	culture-based, biochemical, molecular	/
Paper XXIV	2005	culture-based, biochemical, molecular	/
Paper XXXV	2008	culture-based, biochemical, molecular	HPLC-MS analysis
Paper VI	2010	/	/
Paper XXII	2011	culture-based, biochemical, molecular	HPLC-MS analysis
Paper IV	2013	/	/
Paper XX	2013	culture-based, molecular	/
Paper VII	2015	/	/
Paper XXXI	2015	molecular	/
Paper XXXII	2017	culture-based, biochemical, molecular	/
Paper III	2018	/	/
Paper XI	2018	/	/
Paper XV	2018	/	/
Paper V	2019	/	/
Paper XVI	2019	culture-based, biochemical, molecular	/
Paper II	2020	culture-based, molecular	endolysin cell wall-binding domain
Paper XIV	2020	biochemical	/
Paper XXX	2020	culture-based, biochemical, molecular	LCMS², UPLC-ESI-MS/MS, droplet digital PCR
Paper I	2021	molecular	/
Paper X	2021	culture-based, molecular	MALDI-TOF MS, metabolomics, ANN-assisted FTIR spectroscopy
Paper XVII	2021	/	/

Paper XXI	2021	culture-based, biochemical, molecular	PMA-qPCR, UPLC-MS/MS
Paper XXIII	2022	molecular	BType3 software, FTIR spectroscopy
Paper XXXVI	2022	culture-based, biochemical, molecular	WGS (core genome MLST), MALDI-TOF updates, Biotyper/Vitek databases
Paper XII	2023	culture-based, molecular	single-copy protein-coding genes, locally collinear blocks (LCBs) conserved protein-coding genes, whole-genome SNPs, dDDH
Paper XXXIV	2024	/	/
Paper XXXVII	2024	/	/

3.1.3. Outbreak trends

Another focus of the information synthesis process was the identification of outbreak trends. Approximately 59% of the reviews cited rice-based products as the food source most frequently associated with *B. cereus* outbreaks (Table 8). In addition, dairy products and vegetables were also identified as significant vehicles for *B. cereus* infections. Proteinaceous foods such as meat, vegetables, and dairy products (e.g., milk) were most commonly associated with the diarrheal form of *B. cereus* foodborne illness, whereas farinaceous foods, including rice-based dishes, pasta (e.g., noodles), and pastries were predominantly linked to the emetic syndrome caused by *B. cereus* (Fig. 6). An overview of the food sources and associated outbreak settings reported across the 37 eligible reviews is provided in Table 8.

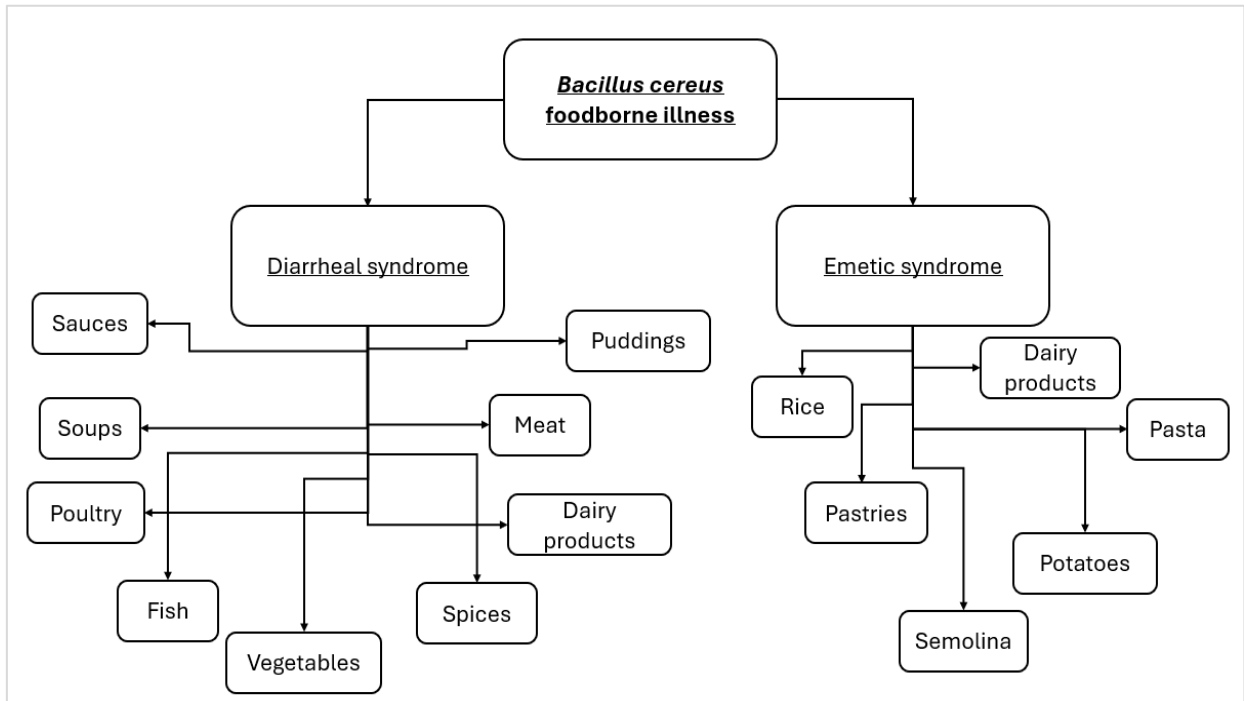


Fig. 6: Overview of foodborne illnesses caused by *Bacillus cereus*. The figure illustrates the two principal syndromes associated with *Bacillus cereus* intoxication: the diarrheal and the emetic syndrome. The diarrheal type is commonly linked to protein-rich foods, including meat, poultry, fish, sauces, and soups. In contrast, the emetic type is primarily associated with starchy foods such as rice, pasta, potatoes, pastries, and semolina.

Table 8: Overview of the food sources and associated outbreak settings reported across the eligible reviews. The table differentiates between single food products (e.g., milk, eggs) and composite dishes (e.g., mashed potatoes, pasta salad), highlighting those most commonly linked to *Bacillus cereus* outbreaks, such as rice-, dairy-, and vegetable-based items. *Bacillus cereus* food poisoning presents in two distinct gastrointestinal syndromes, the emetic and the diarrheal forms. The emetic syndrome is characterized by the acute onset of nausea and vomiting, whereas the diarrheal syndrome is associated with nausea, abdominal cramps, and diarrhea. The symbol “/” indicates the absence of reported information regarding food sources, gastrointestinal symptoms, or outbreak settings.

Title	Food sources	Gastrointestinal symptoms	Outbreak settings
Paper I	potato-based products (potatoes, potato products), cereal-based products	diarrheal syndrome	educational institutions (school

	(semolina, dried food products), insect-based products (edible fried insects), vegetable- and fish-based dishes (mashed celery, fish)		canteens), health/social care institutions
Paper II	pasta-based dishes (pasta salad, spaghetti with tomato sauce), bean-based products (beans, refried beans), water-based products (underground sources)	emetic and diarrheal syndrome	educational institutions (nurseries), food service establishments (fast-food restaurants)
Paper III	starchy-based dishes (rice), vegetable-based dishes, fish- and seafood-based products, dairy-based products (cheese), dessert-based dishes (cake)	emetic and diarrheal syndrome	catering services (banquets, weddings, pubs, clubs, prisons)
Paper IV	rice-based products , meat-based products, poultry-based products, Asian-style dishes	emetic and diarrheal syndrome	food service establishments (restaurants, delis), catering services
Paper V	/	/	/
Paper VI	starchy-based dishes (rice , pasta), dairy-based products (milk), vegetable-based products, meat-based products, spice- and herb-based products	emetic and diarrheal syndrome	/
Paper VII	cereal-based products (cereal, grains), spice- and herb-based products, tea-based products	/	/
Paper VIII	starchy-based dishes (macaroni and cheese, rice), vegetable-based dishes (salads), dessert-based dishes (vanilla slices), meat-based dishes, dairy-based products, casserole-based dishes, dried	emetic and diarrheal syndrome	/

	products, bakery/confectionery products, spice- and herb-based products		
Paper IX	protein-based products, vegetable-based products, sauce-based dishes, dessert-based dishes (puddings), starchy-based dishes (cooked rice)	emetic and diarrheal syndrome	food service establishments (Chinese restaurants, take-away outlets)
Paper X	vegetable-based products (fresh produce (e.g., raw vegetables like tomatoes)), dairy-based products (milk), pasta-based products, cooked chilled dishes, spice- and herb-based products	emetic and diarrheal syndrome	/
Paper XI	fruit-based products (fruit pulp), vegetable-based products (salad, cooked carrot, lettuce, cucumber, watermelon, beet)	emetic and diarrheal syndrome	workplace dining facilities (job refectories), food service establishments (restaurants, bakeries), health/social care institutions (hospital, residences)
Paper XII	starchy-based dishes (cooked rice , pasta)	emetic and diarrheal syndrome	food service establishments (Chinese restaurants, cafeterias)
Paper XIII	sprout-based products (soy, mustard, cress sprouts grown in seed-sprouting kits)	/	/

Paper XIV	starchy-based dishes (cooked rice , pasta), vegetable-based products (potatoes, mushrooms), dairy-based products (milk), meat-based products, poultry-based products, sauce-based dishes, soup-based dishes, dessert-based dishes (puddings, pastries), spice- and herb-based products, sprout-based products	emetic and diarrheal syndrome	/
Paper XV	/	/	/
Paper XVI	spice- and herb-based products (coriander, oregano, basil, sesame seed, black pepper, cumin, curry powder)	/	/
Paper XVII	vegetable-based products, fruit-based products, meat-based products, mixed food products	/	health/social care institutions (hospitals, nursing homes, rehabilitation centers)
Paper XVIII	meat-based products, poultry-based products, dairy-based products (milk), egg-based products	/	/
Paper XIX	meat-based products, dessert-based dishes (puddings), sauce-based dishes (vanilla sauce), poultry-based dishes (chicken soup), potato-based dishes (mashed potatoes), vegetable-based products, rice-based dishes , egg-based dishes	emetic and diarrheal syndrome	/

Paper XX	spice-and herb-based products (black pepper, red pepper, white pepper)	/	workplace dining facilities (workplace restaurant), educational institutions (school/kindergarten canteens)
Paper XXI	mixed food products (canned foods, drinks), cereal-based products, rice-based products , poultry-based products, fish- and seafood-based products (fish, crustaceans, shellfish, mollusks), pasta-based products, egg-based product, dairy-based products, vegetable-based products (potatoes), spice- and herb-based products, bakery/confectionery products (sweets, chocolates), seed- and pulse-based products	emetic and diarrheal syndrome	/
Paper XXII	meat-based products, vegetable-based dishes, pasta-based products/dishes, dessert-based products (cakes), sauce-based dishes, dairy-based products ((pasteurized) milk, milk pudding, instant formulas), rice-based dishes	emetic and diarrheal syndrome	/
Paper XXIII	vegetable-based products ((e.g., raw vegetables like tomatoes)), starchy-based dishes (rice , semolina, pasta, mashed potatoes), meat-based products, sauce-based dishes, dairy-based products	emetic and diarrheal syndrome	/
Paper XXIV	protein-based products, meat-based products, poultry-based products, fish-	emetic and diarrheal syndrome	/

	and seafood-based products, vegetable-based products, soup-based dishes, sauce-based dishes (vanilla sauce), dairy-based products (pasteurized cream, milk pudding, infant formulas), rice-based products , pasta-based products		
Paper XXV	/	emetic and diarrheal syndrome	/
Paper XXVI	meat-based products, soup-based dishes, vegetable-based products, sauce-based dishes, dairy-based products (milk), starchy-based dishes (rice , noodles, pasta), dessert-based dishes (pastries)	emetic and diarrheal syndrome	/
Paper XXVII	rice-based products , meat-based products, sauce-based products, dairy-based products	emetic and diarrheal syndrome	/
Paper XXVIII	rice-based products , mixed food products	emetic and diarrheal syndrome	/
Paper XXIX	rice-based products (fried rice) , meat-based products, vegetable-based products	emetic and diarrheal syndrome	/
Paper XXX	starchy-based dishes (fried rice , pasta, noodles), protein-based products	emetic and diarrheal syndrome	/
Paper XXXI	rice-based products , pasta-based products, dairy-based products (milk), vegetable-based products, meat-based products	emetic and diarrheal syndrome	food service establishments (Chinese restaurants, take-away shops), event-based products (cultural/religious

			events), educational institutions (school canteens)
Paper XXXII	dairy-based products (cheese), pasta-based dishes (noodles), vegetable-based dishes (salad)	/	/
Paper XXXIII	rice-based products , pasta-based products, dairy-based products (milk), vegetable-based products, meat-based products	emetic and diarrheal syndrome	/
Paper XXXIV	rice-based products (fried rice, rice balls) , pasta-based products, meat-based products, dairy-based products	emetic and diarrheal syndrome	/
Paper XXXV	starchy-based dishes (fried/cooked rice , pasta, noodles), dessert-based dishes (pastries, puddings), protein-based products (including meat), soup-based dishes, sauce-based dishes, dairy-based products (milk), vegetable-based products	emetic and diarrheal syndrome	/
Paper XXXVI	vegetable-based products, starchy-based dishes, dairy-based products (breast milk)	/	health/social care institutions (neonatal intensive care units (NICUs))
Paper XXXVII	plant-based products, rice-based products , pasta-based products, cereal-based products	emetic and diarrheal syndrome	food service establishments (restaurants, in-house kitchens), educational institutions (preschools)

The examined literature did not indicate any significant changes in food contamination patterns over time. In general, improper holding temperatures and inadequate kitchen or food hygiene were identified as key contributing factors to *B. cereus* infection. The emetic form of *B. cereus* infection was reported as predominant in Japan (8, 27, 65, 21, 67, 68) and the United Kingdom (27, 21, 67, 68). In contrast, the diarrheal form was more frequently observed in Norway (27, 21, 68), Hungary (27, 21, 68), Finland (27, 21, 68), and North America (8, 65–67). Seasonal trends were noted in four reviews, all of which reported a higher incidence of *B. cereus* infections between March and October. The majority of documented cases originated from China, Malaysia, Japan, and the United States. A world map illustrates the frequency with which individual countries were referenced in relation to outbreaks across the included reviews (Fig. 7). Canada, the United States, and the United Kingdom were mentioned most frequently. Mentions ranged from 1 to 23 per country, with the majority of countries referenced fewer than five times. Most African, South American, and Middle Eastern countries were absent. Additionally, a separate world map depicts the countries of the first and corresponding authors of the included studies (Fig. 8). The United States had the highest number of authors, whereas several countries were represented by only a few. Author affiliations per country ranged from 1 to 12, with the majority of countries appearing fewer than five times. Notably, many countries in Africa, South America, and the Middle East were underrepresented or absent.

No significant differences in susceptibility were identified across age groups, including neonates, infants, children, adults, and the elderly. School and workplace canteens, healthcare settings, and Chinese restaurants were most frequently stated as common outbreak settings. Table 8 presents a detailed overview linking these outbreak settings to the specific food sources involved.

For instance, Li et al. reported that the mortality rate associated with *B. cereus* infections was highest during the period from 1974 to 1984, followed by a general decline over subsequent years (69). Lotte et al. indicated that the fatality rate may reach up to 30% among preterm neonates and immunocompromised individuals (70).

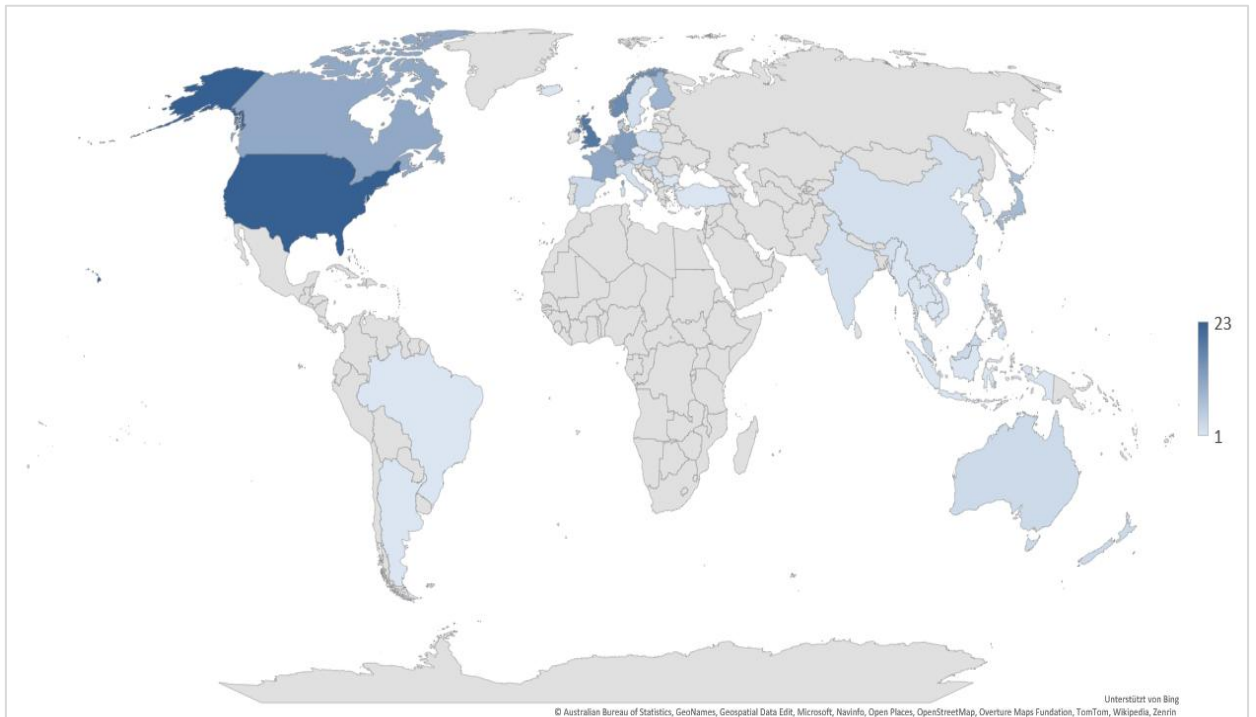


Fig. 7: A global overview of the frequency with which countries are mentioned in eligible reviews. Canada, the United States, and the United Kingdom were mentioned most frequently.

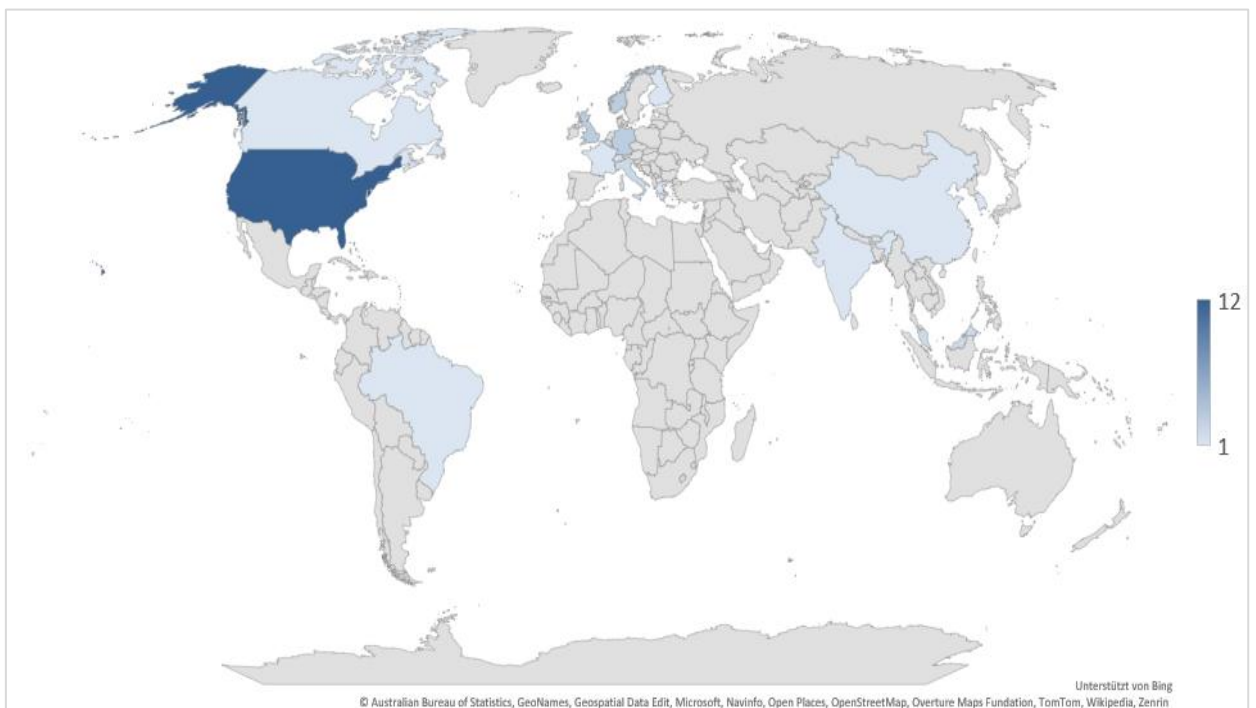


Fig. 8: Countries of first and corresponding authors of the included reviews. Since the countries of the first and corresponding authors are identical, only a single world map is presented.

3.1.4. Inconsistencies or contradictions in the literature

The final component of the information synthesis process aimed to identify inconsistencies or contradictions in the literature. Approximately 25% of the reviews identified limitations in various reporting systems, and the prevalence of underreporting, as contributing factors to gaps within the existing literature. No substantial differences in reported outbreak sources, or conflicting results among the reviews, were detected. Likewise, no significant disparities were observed in the epidemiological data presented across the studies.

3.1.5. Comparison with Artificial Intelligence (AI)

In a subsequent step, the manually extracted data were compared with the data extracted by the AI to identify potential inconsistencies. AI-generated responses were manually transcribed into the Microsoft Excel tables (Version 2506; Microsoft Corporation, Redmond, WA, USA). All data extraction by the AI was performed using GPT-4-Turbo (accessed in April 2025). A protocol listing the exact questions presented to ChatGPT is provided in the supplementary materials (Table S4).

Approximately 38% of the thematic foci identified by the AI partially correspond with manual extraction and analysis, while around 8% show no alignment. Roughly 35% of the databases extracted by the AI from the reviews did not correspond with those I identified. When tasked with identifying the country of the outbreak, the AI misclassified approximately 27% of the reviews. More than half of the reviews, more precisely around 68%, were not accurately processed by the AI with regard to extracting the year of the outbreak. In 12 of the 37 analyzed reviews, the AI's analysis of outbreak characteristics was incomplete or inaccurate. AI processing exhibited deficiencies in accurately representing the microbiological and analytical characterization in approximately 49% of the reviews. In roughly 97% of the cases, the AI correctly determined the presence or absence of meta-analyses within the reviews. The AI accurately identified the author-reported limitations in approximately 62% of the reviews. The conclusions were accurately interpreted by the AI in 32 of the 37 reviews. The percentage of AI responses evaluated as correct, partially correct, or incorrect across the extraction questions is shown in Fig. 9.

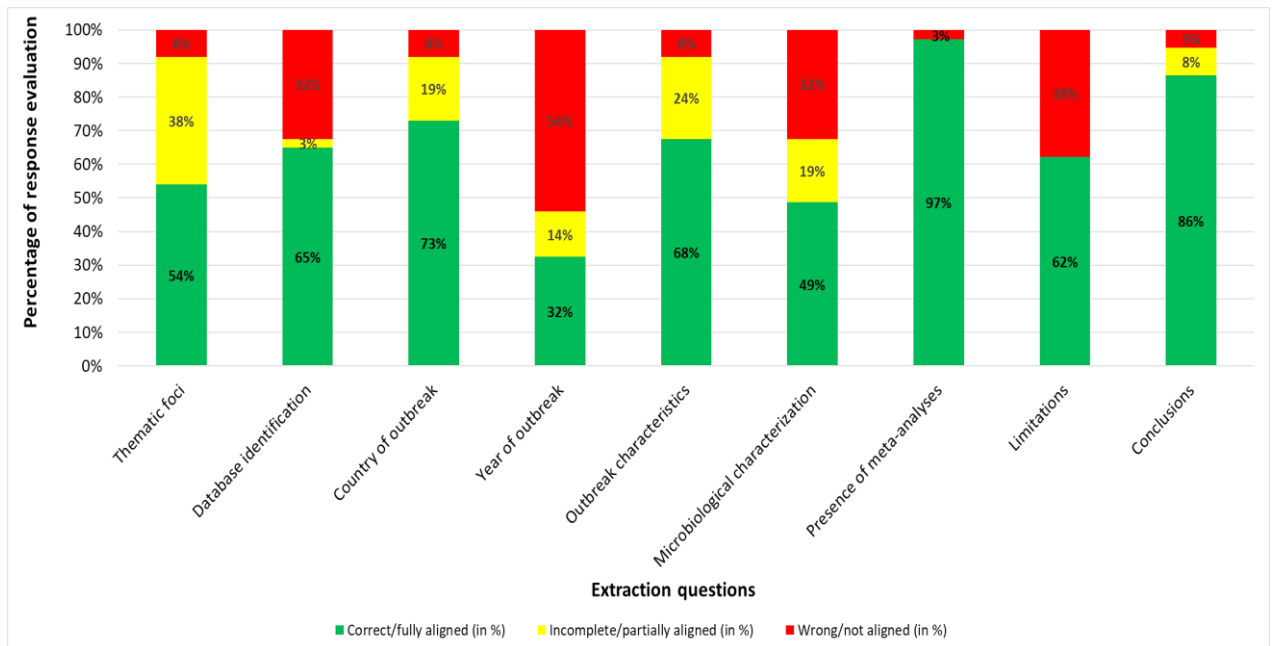


Fig. 9: Percentage of AI response evaluations across different extraction questions. Stacked bar charts use the following color scheme: correct/fully aligned (green), incomplete/partially aligned (yellow), and wrong/not aligned (red). The prompt "Presence of meta-analyses" shows the highest alignment, with approximately 97% of AI responses fully correct, whereas "Year of the outbreak" has the lowest alignment, with only about 32% fully correct.

3.2. Surveillance reports

3.2.1. Outbreak trends and patterns

No substantial evidence of temporal variation in the number of reported outbreaks was observed. Separately, a bar chart depicts the annual distribution of published surveillance reports from 1977 to 2024 (Fig. 10). Of the total 40 surveillance reports, ten were published between 1985 and 1989, with six originating from Canada. Between 2010 and 2024, six surveillance reports were published in each five-year interval. A timeline, shown as a stacked bar chart, displays the temporal distribution of outbreaks mentioned across the analyzed reports, and is provided as a supplementary figure (Fig. S11). Outbreaks occurring between 2000 and 2015 are prominently represented in the included surveillance reports. Additionally, another bar plot illustrates the relative frequency of *B. cereus* cases as a percentage of total foodborne outbreak cases (Fig. 12). Across the studies, the reported frequencies of *B. cereus* outbreaks ranged from 1% to 21% of total foodborne outbreaks. Three studies,

namely Paper XXXI, Paper XXXII, and Paper XXXIX, reported frequencies exceeding 15%. Papers XXXI and XXXII covered Taiwan, with one study spanning the 1980s to 1990s and the other reporting data from 2014 to 2018. Only surveillance reports that included both the number of *B. cereus* cases and the total number of foodborne outbreak cases were considered for the graphical representation shown in Fig. 12. Detailed information on the eligible surveillance reports is provided in supplementary table S3, including publication year, countries of the outbreak, the number of *B. cereus* cases per outbreak, the relative frequency of *B. cereus* cases as a percentage of total foodborne outbreak cases, and reference.

The majority of cases identified (fried) rice as the primary vehicle for *B. cereus* foodborne illness. Food service establishments, particularly restaurants, were most frequently cited as common outbreak settings. Common contributing factors included improper holding temperature (71–77), storage of food at warm temperatures (78, 71, 16, 73, 74, 76, 77), and inadequate cooking (6, 73, 77). Only 4 out of the 40 surveillance reports specifically addressed the seasonality of *B. cereus* foodborne disease. The respective reports indicate a *B. cereus* poisoning peak during the warmer months of the year, particularly between April and October. Over one-quarter of the surveillance reports identified rice as the primary food vehicle for *B. cereus* infections. Additionally, meat products (e.g., pork and poultry), dairy products, and certain "Chinese foods" were frequently cited as significant vehicles of transmission. Food service establishments, notably Chinese restaurants, as well as private homes, were identified as the primary settings for outbreaks.

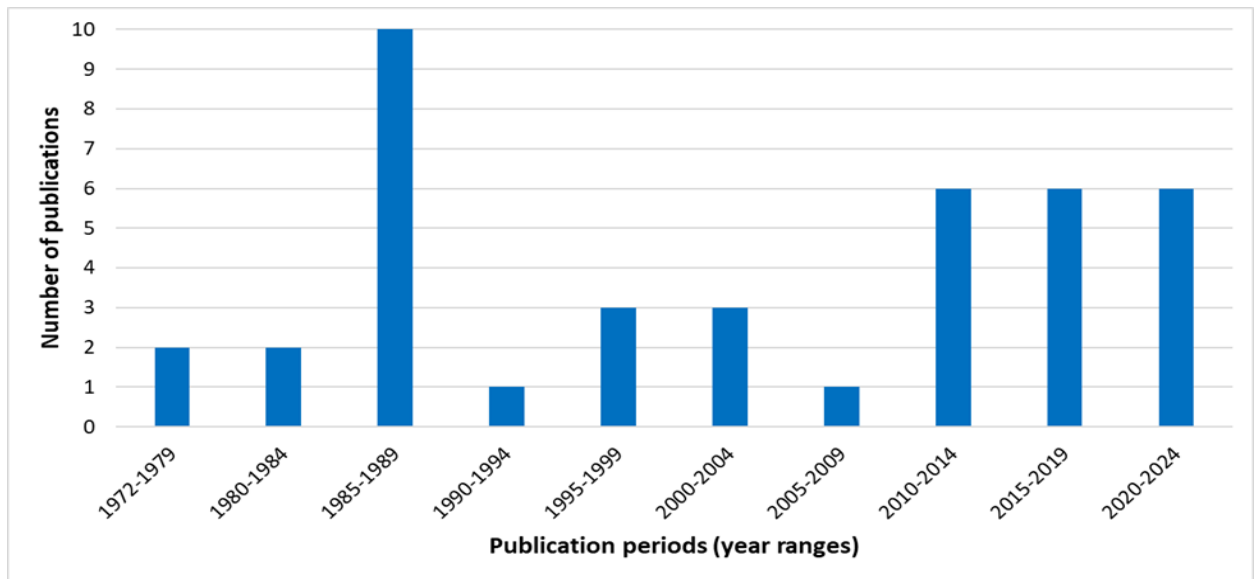


Fig. 10: Number of surveillance report publications including information on *Bacillus cereus* outbreaks. Data are shown for five-year periods (1972-2024), except for the initial interval (1972-1979), which spans eight years. The highest number of surveillance report publications was recorded during 1985-1989, with ten surveillance reports in total, six of which were from Canada.

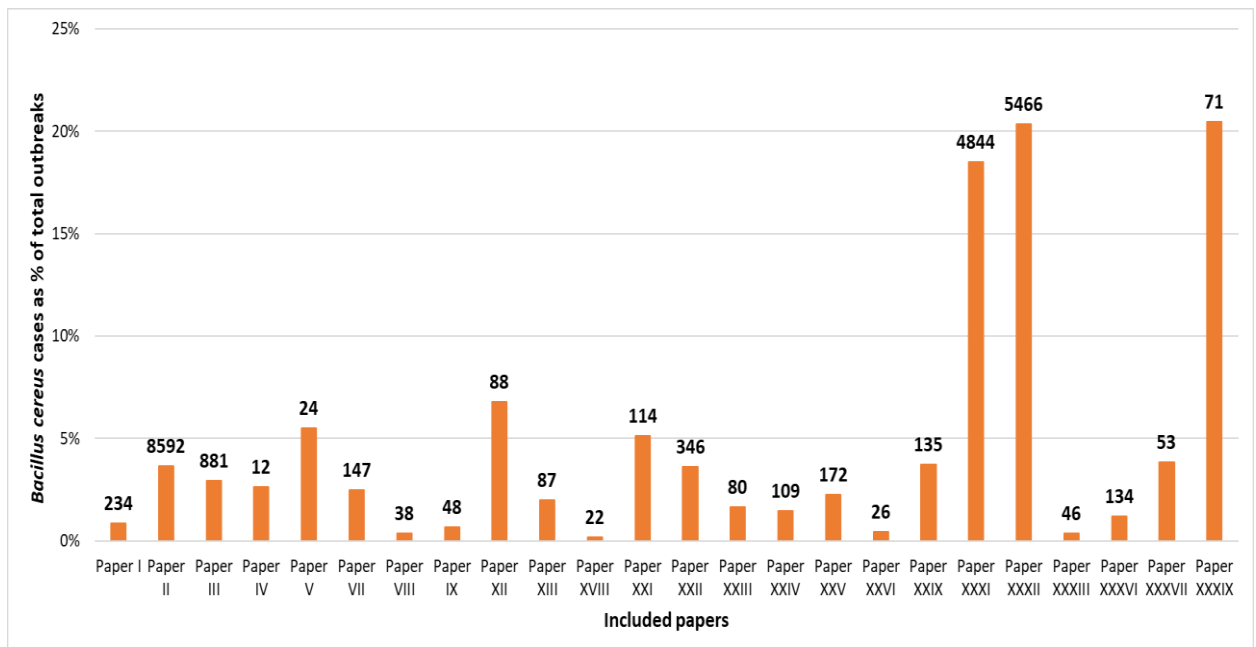


Fig. 12: Relative frequency of *Bacillus cereus* cases of the total number of foodborne outbreak cases (in %). The numbers above the bar of the bar plot indicate the number of *Bacillus cereus* cases in each surveillance report.

3.2.2. Inconsistencies and gaps in surveillance materials

A world map illustrating the geographic distribution of outbreak mentions and author affiliations of the included surveillance reports was compiled (Fig. 13). As the countries of the first and corresponding authors, as well as the frequency with which individual countries were referenced in relation to outbreaks, are identical, a single world map is presented. Canada, the United States, Europe, and Australia and Oceania were mentioned most frequently. What becomes immediately evident is the complete absence of any surveillance reports concerning two entire continents, namely Africa and Antarctica. Furthermore, Brazil was the sole South American country represented in the eligible dataset. Similarly, only China and Taiwan were mentioned in relation to Asia. Europe accounted for over 35% of the surveillance reports, making it the most frequently represented continent. However, only a limited number of countries, namely Sweden, the Netherlands, the United Kingdom, Germany, and Italy, had published individual surveillance reports. Organizations, including the EFSA and CDC, as well as national ministries, function as key reporting systems, although their structures and roles vary across countries. Both the EFSA and the World Health Organization (WHO) contributed surveillance materials covering Europe as a whole. North America followed as the second most frequently reported region, with the United States and Canada represented. Notably, no surveillance reports originated from Middle America. Surveillance reports concerning *B. cereus* outbreaks in the United States lacked detailed information regarding their distribution across states and/or territories. In contrast, Canadian surveillance reports provided substantial detail regarding the geographic distribution of outbreaks across the country. With regard to the continent of Australia and Oceania, Melanesia, Micronesia, and Polynesia were not mentioned in any of the examined surveillance materials. Although the Commonwealth of Australia appeared in only 10% of the surveillance reports, those reports provided detailed information concerning the distribution of cases across the country.

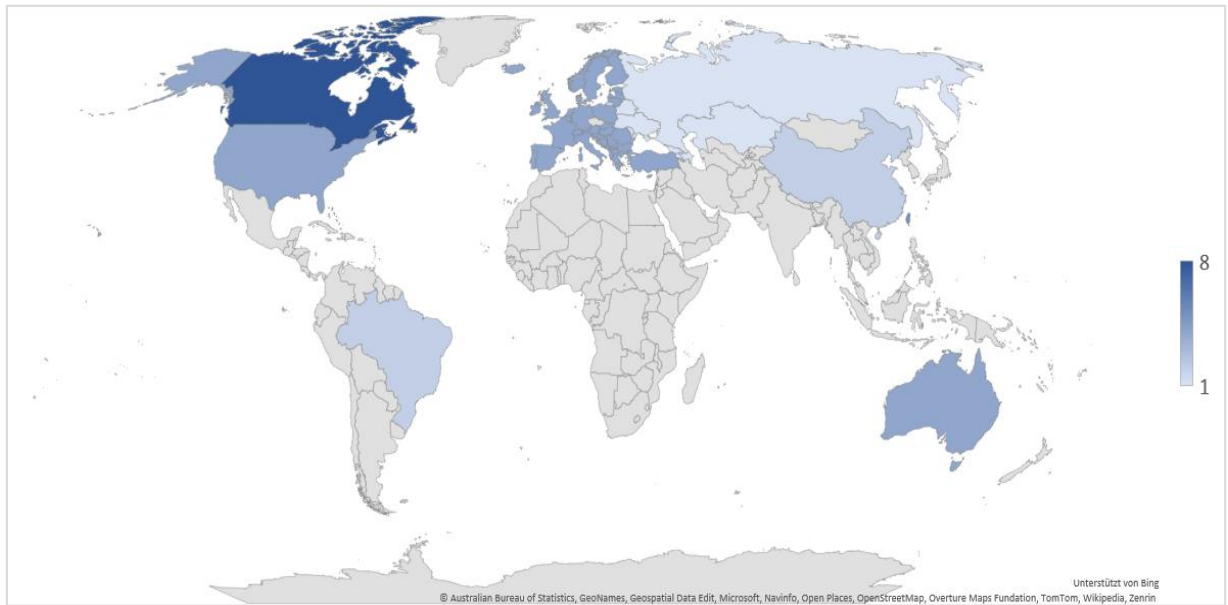


Fig. 13: Geographic distribution of outbreak mentions and author affiliations of the included surveillance reports. As the countries of the first and corresponding authors, as well as the frequency with which individual countries were referenced in relation to outbreaks in the included surveillance reports, are identical, a single world map is presented. Canada, the United States, Europe, and Australia and Oceania were mentioned most frequently.

4. Discussion

4.1. Trends in *Bacillus cereus* outbreak reporting

This diploma thesis demonstrates that although surveillance data show a persistent high incidence of *B. cereus* outbreaks in specific regions, the number of review articles has steadily increased since the 1990s (Fig. S5), likely reflecting growing scientific interest in *B. cereus* (79, 80, 39). This trend may be driven not only by elevated food safety concerns, but also by improvements in diagnostics, including molecular-based methods, MALDI-TOF (81), and FTIR spectroscopy (82), as well as by increased consumption of processed and ready-to-eat (RTE) foods (83, 84). Furthermore, the expanding role of systematic reviews in synthesizing outbreak data facilitates the integration of findings from diverse studies, the identification of knowledge gaps, and the formulation of evidence-based recommendations for public health interventions (39, 55, 85).

However, the diagnosis of *B. cereus* in outbreak situations is still a challenge due to the ubiquitous presence of *B. cereus* group strains including the non-pathogenic, probiotic, and growth-promoting strains (13, 86, 87). Entrance of *B. cereus* group strains into the food chain cannot be entirely prevented, which makes the identification of causative *B. cereus* strains in outbreak situations particularly challenging (85).

4.2. Discrepancies between surveillance data and published literature

Notable discrepancies emerge between surveillance reports and published literature. For example, surveillance systems in China documented hundreds of outbreaks over multi-year periods, far exceeding the numbers captured in academic publications. This divergence suggests a publication bias, as most *B. cereus* outbreaks are mild and often underreported. In alignment with this, Li et al. reported that China had the highest number of *B. cereus* cases worldwide in their literature search prior to 2024 (69). In contrast, our own search (Fig. 13) did not identify China among the countries with the highest numbers of reported outbreaks, and review articles rarely mentioned outbreaks in China (Fig. 7). A similar discrepancy is visible for Canada (Fig. 13), where surveillance data primarily document outbreaks during the 1970s and 1980s (88–95), while review articles mention only a limited number of outbreaks, mainly from the 1960s and 1990s (Fig. 7).

The United States is mentioned in 23 of the 37 review articles and appears as the country of the first or corresponding author in twelve of these reviews, reflecting its prominent representation in the literature. By contrast, it is represented by only four surveillance reports,

highlighting a substantial imbalance between literature coverage and actual outbreak data. This discrepancy may reflect the United States' high research output, the predominance of English-language publications, and strong academic infrastructure, which together contribute to more published outbreak analyses than are captured by national surveillance reports.

Due to discrepancies between reporting systems worldwide, it remains challenging to accurately determine the number of *B. cereus* intoxications. High-quality reviews from developing countries are scarce (69). In several Asian and other developing regions, food safety often receives limited prioritization from both the government and the general public, partly due to the economic circumstances. This lack of prioritization likely contributes to the underrepresentation of *B. cereus* outbreaks in the scientific literature, even when surveillance data indicate a substantial burden (9). Consequently, reported case numbers tend to be higher in developed countries, while they remain lower or absent in less developed ones (69).

Norway, Germany, and France also demonstrate notable discrepancies. Norway appears frequently in reviews (16 instances) but has only four surveillance reports. Similarly, Germany and France are well covered in reviews yet have limited surveillance data, much of which is included in European compilations by EFSA, ECDC, and WHO rather than being country-specific surveillance reports (5, 96). This pattern likely reflects the common practice in European countries of contributing data to European regional reports (e.g., EU-wide compilations) rather than publishing national outbreak analyses, as well as potential underreporting of mild outbreaks in routine surveillance. The United Kingdom shows a similar pattern, being mentioned in 19 review articles but represented by only seven surveillance reports, four of which are part of European compilations (96, 16, 97, 77).

Overall, these discrepancies underscore the limited generalizability of outbreak patterns derived solely from published literature and emphasize the need for the systematic inclusion of surveillance data in outbreak investigations.

4.3. Regional disparities in outbreak data

Geographic mapping of both reviews and surveillance reports demonstrates robust data from high-income countries, including China, the Netherlands, the United States, Australia, and parts of the European Union (EU) (98–100). In contrast, large regions such as much of Africa and South America lack systematic surveillance data and sufficient publications (101, 102). Standardizing reporting requirements and strengthening laboratory capacities in low- and

middle-income regions are essential steps to reduce this imbalance. Such measures would improve the detection, documentation, and comparability of outbreaks, thereby enabling more accurate estimates of the global burden and facilitating timely public health responses. Furthermore, harmonizing reporting standards across countries would reduce regional disparities in outbreak documentation, ultimately contributing to a better understanding of *B. cereus* epidemiology worldwide.

4.4. Patterns and characteristics of *Bacillus cereus* outbreaks

No major discrepancies were identified between review articles and surveillance reports with regard to food sources, predominant outbreak types, typical outbreak settings, or reported mortality rates. Across both data sources, temperature misuse, cross-contamination, and inadequate hygiene consistently emerge as the primary risk factors for *B. cereus* infection.

This finding corroborates the claims of food authorities and scientists that temperature control and food hygiene are the utmost important parameters to be controlled (103, 104, 12, 105). Furthermore, rice-based products were most frequently identified as the primary food vehicle, with food service establishments, particularly Asian-style restaurants, representing the predominant outbreak settings. In agreement across data sources, the mortality rate associated with *B. cereus* outbreaks remains consistently low.

4.5. Methodological considerations

Heterogeneity in reporting practices and variable completeness of surveillance records present major obstacles for synthesizing global outbreak data. Many surveillance reports omit information on seasonality or mortality, reducing their epidemiological utility. In addition, the high genetic similarity within the *B. cereus* group complicates precise species-level identification, which may lead to misclassification in outbreak statistics (70). Moreover, only a minority of outbreak reports provide complete socio-demographic (e.g., age, sex, pre-existing health conditions) and/or traceable clinical data (e.g., disease onset, incubation period, clinical manifestation, outcome) (6).

Standardized extraction templates, such as those employed in systematic reviews, could improve data consistency and comparability. Of the 37 eligible reviews, 26 addressed detection methods. Within this subset, conventional approaches were referenced universally. In contrast, fewer than one-quarter reported the application of advanced techniques, such as PMA-qPCR, CBD-based assays, dDDH, ANN-assisted FTIR spectroscopy, and UPLC-ESI-MS/MS (12, 106, 22, 17, 11, 9). Expanding the application of these innovative methods could

improve outbreak investigation accuracy and ultimately contribute to more effective food safety monitoring and risk assessment strategies.

4.6. Limitations of this study

This synthesis is constrained by the heterogeneity of both published literature and surveillance records. Underreporting, regional disparities, and incomplete methodological details limit the applicability of the conclusions. A particularly substantial limitation arises from the surveillance search itself, where shortcomings in search strategy and study selection may have led to the omission of relevant data, thereby compromising the comprehensiveness of the findings. Despite these limitations, the analysis provides valuable insights into global patterns, risk factors, and methodological gaps.

4.7. Future directions

Considering the regional disparities and methodological inconsistencies observed, future research should prioritize the standardization of reporting practices and the reduction of geographic imbalances in outbreak documentation. Strengthening laboratory capacity, particularly in low- and middle-income regions, and adopting innovative detection methods are critical to improving global *B. cereus* surveillance. Moreover, international collaboration and investment in harmonized reporting protocols would facilitate a more accurate understanding of *B. cereus* epidemiology and better inform public health interventions.

The adoption of systematic and scoping review methodologies, paired with AI-assisted data extraction, offers promising avenues for improving outbreak monitoring and review efficiency. Given the time-consuming nature of manual data extraction, leveraging a human-in-the-loop AI approach can already enhance the accuracy of the data extraction. Future work should validate and refine these tools to facilitate comprehensive, high-quality data extraction and synthesis in epidemiological and microbiological research.

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6. Appendix

6.1. List of abbreviations

B. cereus s.l.: *Bacillus cereus sensu latu*, the *Bacillus cereus* group

B. cereus (s.s.): *Bacillus cereus (sensu stricto)*

CDC: Centers for Disease Control and Prevention (USA)

China CDC: Chinese Center for Disease Control and Prevention

CytK: cytotoxin K

ECDC: European Centre for Disease Prevention and Control

EFSA: European Food Safety Authority

Hbl: hemolysin BL

Nhe: non-hemolytic enterotoxin

PCR: polymerase chain reaction

WHO: World Health Organization

6.2. Supplementary materials

6.2.1. Supplementary tables

Table S2: Overview of key data extracted from the 37 eligible reviews.

Title	Name*	Publication year	Countries	Focus of the review	Reference
<i>Bacillus cytotoxicus</i> — A potentially virulent food-associated microbe	Paper I	2021	France, Germany, Switzerland	Epidemiology	Cairo et al. (19)
Outbreaks, Germination, and Inactivation of <i>Bacillus cereus</i> in Food Products: A Review	Paper II	2020	Worldwide: Belgium, the United Kingdom, the United States, South Korea	Epidemiology, food safety	Choi et al. (106)
<i>Bacillus cereus</i> foodborne outbreaks in mass catering	Paper III	2018	European Union (EU): Austria, Belgium, France, Germany, Italy, Spain, the United Kingdom	Epidemiology	Osimani et al. (107)
Foodborne Disease Outbreaks Caused by <i>Bacillus cereus</i> , <i>Clostridium perfringens</i> , and <i>Staphylococcus aureus</i> —United States, 1998–2008	Paper IV	2013	The United States	Epidemiology	Bennett et al. (6)

Risk presented to minimally processed chilled foods by psychrotrophic <i>Bacillus cereus</i>	Paper V	2019	/	Food safety, epidemiology	Webb et al. (108)
<i>Bacillus cereus</i> : an important foodborne pathogen	Paper VI	2010	European Union, Italy, Norway, the Netherlands, Spain, Switzerland, the United Kingdom, Canada, the United States, Asia (e.g., Japan), Australia and New Zealand	Epidemiology, food safety	Pexara et al. (8)
Application of a Rapid Knowledge Synthesis and Transfer Approach to Assess the Microbial Safety of Low-Moisture Foods	Paper VII	2015	Worldwide	Food safety	Young et al. (109)
<i>Bacillus cereus</i> Foodborne Illness-An Update	Paper VIII	1984	Worldwide: the Netherlands, Scandinavia, the United Kingdom, Canada, the United States, Japan	Microbiological characterization	Johnson, K. M. (110)
Foodborne disease due to <i>Bacillus</i> and <i>Clostridium</i> species	Paper IX	1990	Worldwide: Finland, Hungary, the Netherlands, the United Kingdom, Canada	Epidemiology, food safety	Lund, B. M. (111)

Evaluation of <i>B. thuringiensis</i> -based biopesticides in the primary production of fresh produce as a food safety hazard and risk	Paper X	2021	Worldwide: France, Canada	Food safety	Bock et al. (17)
Foodborne outbreaks in Brazil associated with fruits and vegetables: 2008 through 2014	Paper XI	2018	Focus on Brazil	Epidemiology, (food safety)	Oliveira Elias et al. (112)
<i>Bacillus cereus</i> : A review of “fried rice syndrome” causative agents	Paper XII	2023	Focus on Asia (e.g., India, Korea, Malaysia, Philippines)	Epidemiology	Leong et al. (22)
Pathogenic Microorganisms Associated with Fresh Produce	Paper XIII	1995	Focus on the United States	Epidemiology, (food safety)	Beuchat, L. R. (113)
The <i>Bacillus cereus</i> Food Infection as Multifactorial Process	Paper XIV	2020	Worldwide: Austria, Finland, Germany, Poland, Canada, the United States	Microbiological characterization	Jessberger et al. (10)
A Systematic Review of Bacterial Foodborne Outbreaks Related to Red Meat and Meat Products	Paper XV	2018	Worldwide: Denmark, Germany, Italy, Norway, Sweden, the United Kingdom, the United States	Epidemiology	Omer et al. (114)

Microbiological Safety of Dried Spices	Paper XVI	2019	Focus on the United States	Epidemiology, food safety	Gurtler et al. (115)
Healthcare-associated foodborne outbreaks in high income countries: a literature review and surveillance study, 16 OEDC countries, 2001 to 2019	Paper XVII	2021	57 Healthcare-associated foodborne (HA-FBO) outbreaks from 16 Organisation for Economic Co-operation and Development (OECD) countries (primarily Germany, the United Kingdom, the United States) and further 28 HA-FBO were retrieved from the German surveillance system	Epidemiology, food safety	Boone et al. (116)
Agents, vehicles and, causal inference in bacterial foodborne disease outbreaks: 82 reports (1986-1995)	Paper XVIII	1998	The United States	Epidemiology	Petersen et al. (117)
<i>BACILLUS CEREUS</i> : FOOD POISONING ORGANISM. A. REVIEW	Paper XIX	1972	Worldwide: Hungary, the Netherlands, Norway, the United States	Microbiological characterization	Goepfert et al. (118)
Foodborne illness outbreaks from microbial contaminants in	Paper XX	2013	Focus on the United States	Epidemiology	Van Doren et al. (119)

spices, 1973-2010					
<i>Bacillus cereus</i> food intoxication and toxicoinfection	Paper XXI	2021	Worldwide: Belgium, European Union (EU), France, Norway, the United States, Japan, Australia	Microbiological characterization, epidemiology	Jovanovic et al. (12)
<i>Bacillus</i> and relatives in foodborne illness	Paper XXII	2011	Norway, the United Kingdom	Microbiological characterization	Logan, N. A. (27)
Recent paradigm shifts in the perception of the role of <i>Bacillus thuringiensis</i> in foodborne disease	Paper XXIII	2022	Austria, France, Germany	Food safety	Biggel et al. (11)
<i>Bacillus cereus</i> Food Poisoning and Its Toxins	Paper XXIV	2005	Worldwide: Finland, the Netherlands, the United Kingdom, Canada, The United States, Japan, Taiwan	Microbiological characterization, (epidemiology)	Schoeni et al. (14)
<i>Bacillus cereus</i> and its food poisoning toxins	Paper XXV	1997	Worldwide: Denmark, Finland, Iceland, the Netherlands, Norway, the United Kingdom, Canada, the United States, Japan	Microbiological characterization	Granum et al. (65)
<i>Bacillus cereus</i> , the causative agent of an emetic type of food-borne illness	Paper XXVI	2004	Worldwide: Finland, Germany, Hungary, Norway, the United Kingdom, the United States, Japan	Microbiological characterization	Ehling-Schulz et al. (21)

Prevalence and expression of enterotoxins in <i>Bacillus cereus</i> and other <i>Bacillus</i> spp., a literature review	Paper XXVII	2000	/	Microbiological characterization	McKillip, J. L. (66)
<i>Bacillus cereus</i> Food Poisoning	Paper XXVIII	1981	Norway, the United States	Epidemiology	Morris, J. G. (120)
<i>Bacillus cereus</i> Food Poisoning	Paper XXIX	1978	Norway, the United States	Epidemiology	Terranova et al. (121)
Pathogenic <i>Bacillus cereus</i> , an Overlooked Food Contaminants in Southeast Asia	Paper XXX	2020	Southeast Asian countries: Brunei, Cambodia, Indonesia, Laos, Malaysia, Myanmar, Philippines, Singapore, Thailand, Vietnam	Epidemiology, microbiological characterization, (food safety)	Rusnan et al. (9)
<i>Bacillus cereus</i> food poisoning: international and Indian perspective	Paper XXXI	2015	Worldwide: Finland, France, Hungary, the Netherlands, Norway, the United Kingdom, Canada, the United States, India, Japan, Taiwan	Epidemiology	Tewari et al. (67)
In defence of <i>Bacillus thuringiensis</i> , the safest and most successful microbial insecticide available to humanity—a response to EFSA	Paper XXXII	2017	Germany	Food safety	Raymond et al. (122)

Epidemiology and pathogenesis of <i>Bacillus cereus</i> infections	Paper XXXIII	2000	Worldwide: Bulgaria, Finland, Hungary, the Netherlands, Norway, the United Kingdom, Canada, the United States, Japan, Taiwan	Epidemiology, microbiological characterization	Kotiranta et al. (68)
Global Epidemiology and health risks of <i>Bacillus cereus</i> Infections: Special focus on infant foods	Paper XXXIV	2024	32 countries worldwide covered: focus on China	Epidemiology	Li et al. (69)
From soil to gut: <i>Bacillus cereus</i> and its food poisoning toxins	Paper XXXV	2008	Worldwide: France, the Netherlands, Norway, the United Kingdom	Microbiological characterization	Stenfors Arnesen et al. (57)
<i>Bacillus cereus</i> Invasive Infections in Preterm Neonates: an Up-to-Date Review of the Literature	Paper XXXVI	2022	Worldwide: France, Germany, Israel	Epidemiology, microbiological characterization	Lotte et al. (70)
Characteristics of Gastroenteritis Outbreaks Investigated in Singapore: 2018–2021	Paper XXXVII	2024	Focus on Singapore	Epidemiology	Fua'di et al. (123)

*The **Name** column corresponds to the labels presented in Fig. S5.

Table S3: Overview of key data extracted from the 40 eligible surveillance reports.

Title	Name*	Publication year	Countries	Number of <i>Bacillus cereus</i> cases per outbreak (relative frequency of <i>Bacillus cereus</i> cases as a percentage of total foodborne outbreak cases)	Reference
Surveillance and characteristics of food-borne outbreaks in the Netherlands, 2006 to 2019	Paper I	2022	The Netherlands	234 cases (0.8%)	Friesema et al. (124)
Surveillance of foodborne disease outbreaks in China, 2003–2017	Paper II	2020	China	8592 cases (3.6%)	Li et al. (125)
Foodborne Disease Outbreaks Caused by <i>Bacillus cereus</i> , <i>Clostridium perfringens</i> , and <i>Staphylococcus aureus</i> —United States, 1998–2008	Paper III	2013	The United States	881 cases (3.0%)	Bennett et al. (6)
OZFOODNET QUARTERLY REPORT, 1 OCTOBER TO 31 DECEMBER 2011	Paper IV	2012	Australia	12 cases (2.6%)	OzFoodNet Working Group (126)

OZFOODNET QUARTERLY REPORT, 1 OCTOBER TO 31 DECEMBER 2010	Paper V	2011	Australia	24 cases (5.5%)	OzFoodNet Working Group (127)
Investigation of food and water microbiological conditions and foodborne disease outbreaks in the Federal District, Brazil	Paper VI	2013	Brazil	/	Nunes et al. (128)
Foodborne and Waterborne Disease in Canada -1983 Annual Summary	Paper VII	1989	Canada	147 cases (2.5%)	Todd, E. C. D. (93)
Foodborne and Waterborne Disease in Canada -1984 Annual Summary	Paper VIII	1989	Canada	38 cases (0.4%)	Todd, E. C. D. (94)
Foodborne and Waterborne Disease in Canada — 1980 Annual Summary	Paper IX	1987	Canada	48 cases (0.7%)	Todd, E. C. D. (90)
Food Poisoning and <i>Salmonella</i> Infections in England and Wales, 1973-75	Paper X	1977	England, Wales	/	Vernon, E. (78)
Risks Associated with Vehicles of Foodborne Pathogens and Toxins	Paper XI	1988	The United States	/	Bryan, F. L. (71)

Incidence of Foodborne Disease in the Netherlands: Annual Summary - 1980	Paper XII	1985	The Netherlands	88 cases (6.8%)	Beckers, H. J. (129)
Food borne Disease in Canada -1974 Annual Summary	Paper XIII	1977	Canada	87 cases (2.0%)	Todd, E. C. D. (88)
Incubation periods of enteric illnesses in foodborne outbreaks, United States, 1998–2013	Paper XIV	2019	The United States	/	Chai et al. (130)
Risks for public health related to the presence of <i>Bacillus cereus</i> and other <i>Bacillus</i> spp. including <i>Bacillus thuringiensis</i> in foodstuff	Paper XV	2016	European Union (EU)	/	EFSA BIOHAZ Panel (EFSA Panel on Biological Hazards) (16)
Investigation on the trend of food-borne disease outbreaks in Taiwan (1991-2010)	Paper XVI	2013	Taiwan	/	Cheng et al. (131)
Food poisoning and <i>salmonella</i> surveillance in England and Wales: 1982	Paper XVII	1984	England, Wales	/	Public Health Laboratory Service (Communicable Disease Surveillance Centre) (132)
Food poisoning and salmonellosis surveillance in	Paper XVIII	1980	England, Wales	22 cases (0.2%)	Public Health Laboratory Service (Communicabl

England and Wales: 1979					e Disease Surveillance Centre) (133)
Epidemiological Evaluation of <i>Bacillus cereus</i> - Induced Foodborne Outbreaks — China, 2010–2020	Paper XIX	2023	China	/	Duan et al. (7)
The use of clinical profiles in the investigation of foodborne outbreaks in restaurants: United States, 1982– 1997	Paper XX	2008	The United States	/	Hedberg et al. (72)
EPIDEMIOLOGY OF BACTERIAL TOXIN- MEDIATED FOODBORNE GASTROENTERITIS OUTBREAKS IN AUSTRALIA, 2001 TO 2013	Paper XXI	2016	Australia	114 cases (5.1%)	May et al. (73)
<i>Bacillus cereus</i> as the main casual agent of foodborne outbreaks in Southern Brazil: data from 11 years	Paper XXII	2018	Southern Brazil	346 cases (3.6%)	Lentz et al. (74)
Foodborne and Waterborne Disease in Canada -1981 Annual Summary	Paper XXIII	1987	Canada	80 cases (1.7%)	Todd, E. C. D. (91)
Foodborne and Waterborne Disease	Paper XXIV	1988	Canada	109 cases (1.5%)	Todd, E. C. D. (92)

in Canada -1982 Annual Summary					
Investigations of Foodborne Diseases by Food Inspection Services in The Netherlands, 1991 to 1994	Paper XXV	1997	The Netherlands	172 cases (2.3%)	Simone et al. (134)
Foodborne and Waterborne Disease in Canada - 1978 Annual Summary	Paper XXVI	1985	Canada	26 cases (0.4%)	Todd, E. C. D. (89)
Impact of the Revision of European Food Hygiene Legislation and the Introduction of Convenience- based Food on Food Safety in the German Military	Paper XXVII	2023	Germany (German military)	/	Nau et al. (135)
FOOD-BORNE DISEASE OUTBREAKS IN TAIWAN, 1994	Paper XXVIII	1996	Taiwan	/	Pan et al. (136)
Incidence of Foodborne Disease in the Netherlands: Annual Summary - 1981	Paper XXIX	1986	The Netherlands	135 cases (3.7%)	Beckers, H. J. (137)
Bacterial Foodborne Outbreaks in Central Taiwan, 1991-2000	Paper XXX	2003	Taiwan	/	Chang et al. (138)
Food-Borne Disease Outbreaks Due to	Paper XXXI	1997	Taiwan	4844 cases (18.5%)	Pan et al. (139)

Bacteria in Taiwan, 1986 to 1995					
Surveillance of foodborne diseases in Taiwan: A retrospective study	Paper XXXII	2021	Taiwan	5466 cases (20.4%)	Yu et al. (75)
Describing the Epidemiology of Foodborne Outbreaks in New South Wales from 2000 to 2017	Paper XXXIII	2020	New South Wales (NSW)	46 cases (0.4%)	Franklin et al. (76)
The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2017	Paper XXXIV	2018	European Union (EU): 37 European countries	/	European Food Safety Authority (EFSA) and European Centre for Disease Prevention and Control (ECDC) (77)
The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2016	Paper XXXV	2017	European Union (EU): 37 European countries	/	European Food Safety Authority (EFSA) and European Centre for Disease Prevention and Control (ECDC) (97)
A Summary of Reported Foodborne Disease Incidents in	Paper XXXVI	2000	Sweden	134 cases (1.2%)	Lindqvist et al. (140)

Sweden, 1992 to 1997					
Incidence of Foodborne Diseases in The Netherlands: Annual Summary 1982 and an Overview from 1979 to 1982	Paper XXXVII	1988	The Netherlands	53 cases (3.9%)	Beckers, H. J. (141)
WHO Surveillance Programme for Control of Foodborne Infections and Intoxications: Preliminary Results and Trends Across Greater Europe	Paper XXXVIII	2001	Europe: 49 participating countries	/	Tirado et al. (96)
Foodborne outbreaks surveillance in the Piedmont Region, Italy (2002-2009)	Paper XXXIX	2011	Piedmont Region (Italy)	71 cases (20.5%)	Zicari et al. (142)
Foodborne Disease in Canada a 10-year Summary from 1975 to 1984	Paper XXXX	1992	Canada	/	Todd, E. C. D. (95)

*The **Name** column corresponds to the labels presented in Fig. S11 and Fig. 12. Only reports that provided both the number of *B. cereus* cases and the total number of foodborne outbreaks were included in Fig. 12.

6.2.2. ChatGPT protocol for data extraction

The manually selected review publications (based on inclusion criteria in Table 1, Search strategy 2.1.1.) were checked with ChatGPT in chat mode. For this purpose, structured prompts, as listed in Table S4, were asked from ChatGPT (GPT-4-Turbo, accessed in April 2025).

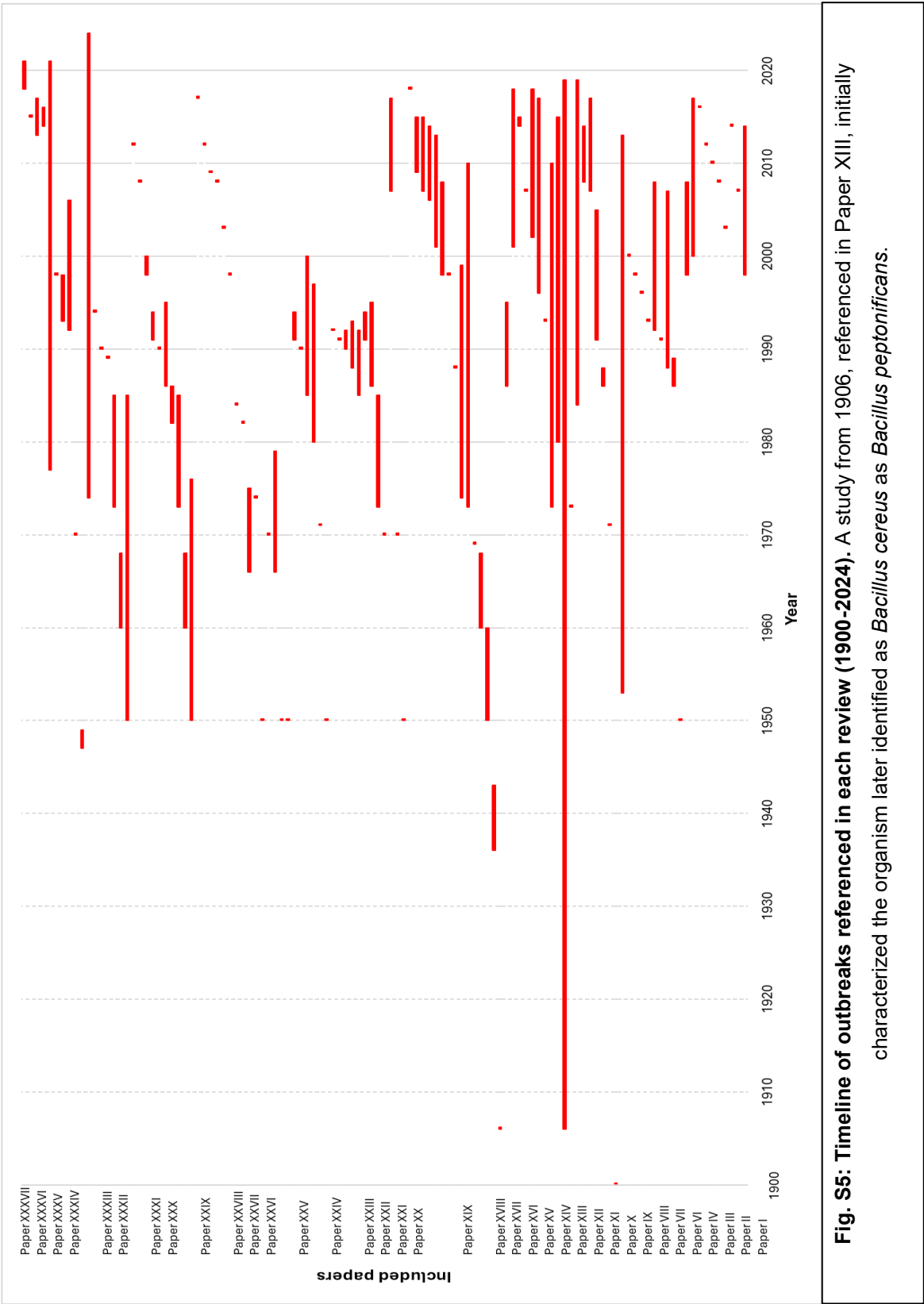
Table S4: ChatGPT prompts for data extraction from the 37 eligible review articles. The protocol begins with an introductory statement and comprises ten specific questions addressing study characteristics, review focus, databases searched, geographic and temporal scope, outbreak characteristics, microbiological and analytical characterization, inclusion of meta-analyses, reported study limitations, and key findings.

Introductory statement: "I would like to ask questions regarding the article entitled 'Title' by 'Author1 et al.', published in 'Publication year.'"
Prompt 1: "In which journal was this article published?" (I would verify all study characteristics and confirm that the link provided by ChatGPT corresponds to the correct publication.)
Prompt 2: "What is the focus of the review (e.g., epidemiology, microbiological methods, analytical methods, clinical studies, food safety, or more)?"
Prompt 3: "Which databases were searched as part of the review methodology?"
Prompt 4: "In which country or region did the outbreak occur (geographic scope)?"
Prompt 5: "In which year did the outbreak occur (time period)?"
Prompt 6: "Which outbreak characteristics are reported (e.g., common outbreak sources, epidemiological patterns, clinical symptoms, severity, and mortality)?"
Prompt 7: "Which microbiological and analytical characterization is described (e.g., culture-based methods, biochemical tests, molecular methods, advancements in detection technology, validation, and standardization issues)?"
Prompt 8: "Does the review include any meta-analyses?"
Prompt 9: "What are the limitations of the studies acknowledged by the authors?"
Prompt 10: "What conclusions and key findings are presented in the review?"

The AI-generated outputs were manually reviewed against the already extracted data from the reviews. For each category of information, including thematic foci, searched databases,

outbreak country and year, outbreak characteristics, microbiological and analytical characterization, presence of meta-analyses, author-reported limitations, and conclusions, any discrepancies, such as missing, misclassified, or differently recorded information, were marked. The proportion of errors per category was calculated by dividing the number of columns with discrepancies by the total number of columns across all reviews and multiplying by 100%.

6.2.3. Supplementary figures



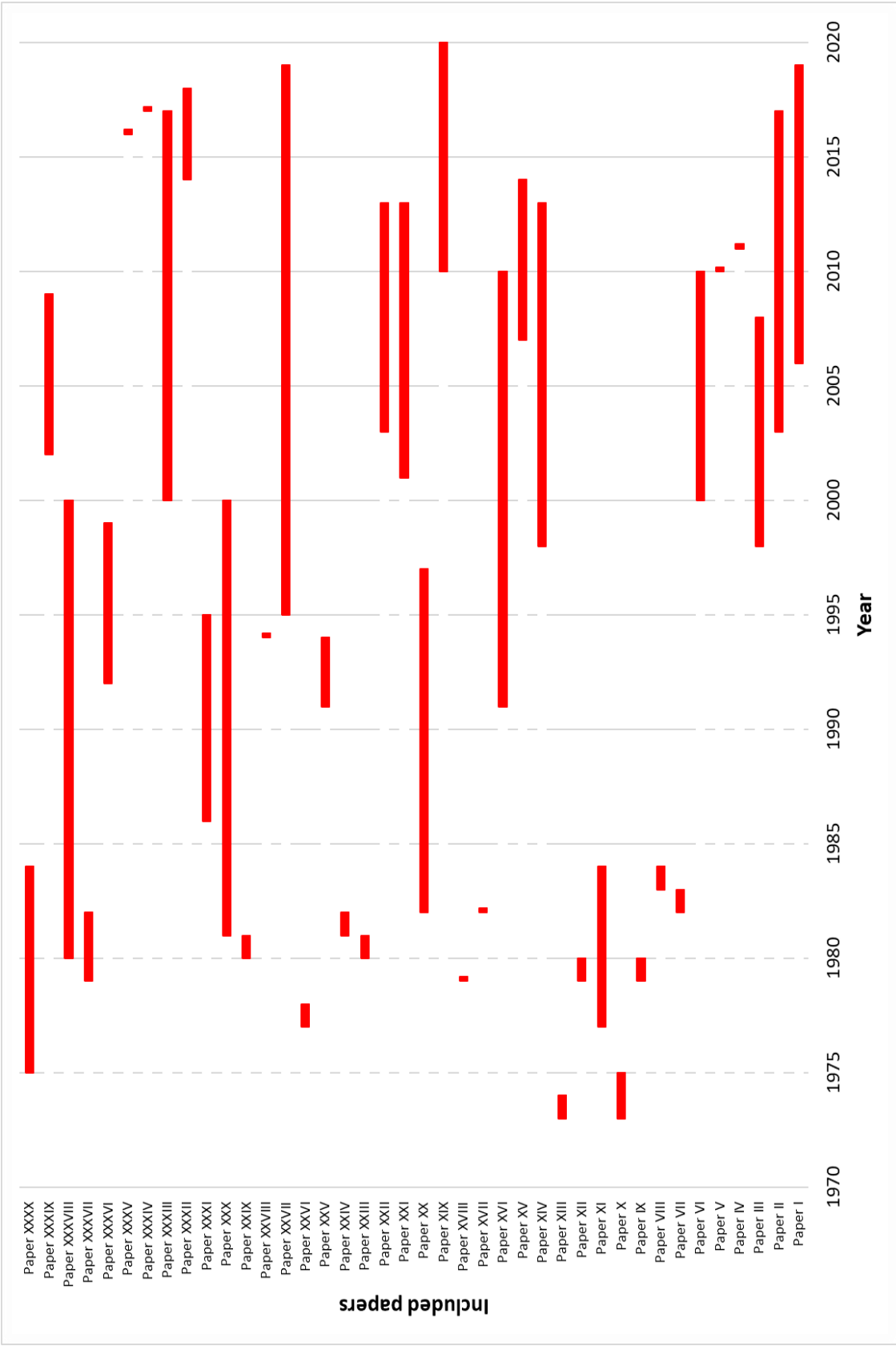


Fig. S11: Timeline of outbreaks referenced in each surveillance report (1973-2020).

7. List of figures and tables

Fig. 1: Search terms used during the literature search.....	22
Fig. 2: “PRISMA” flow diagram illustrating the study selection process.....	25
Fig. 3: Thematic foci of the eligible reviews.....	28
Fig. 4: Number of review publications including information on <i>Bacillus cereus</i> outbreaks (1972-2024).....	32
Fig. S5: Timeline of outbreaks referenced in each review (1900-2024).....	83
Fig. 6: Overview of foodborne illnesses caused by <i>Bacillus cereus</i>	36
Fig. 7: Global overview of the frequency of country mentions.....	44
Fig. 8: Countries of first and corresponding authors of the included reviews.....	44
Fig. 9: Percentage of AI response evaluations across different extraction questions.	46
Fig. 10: Number of surveillance report publications including information on <i>Bacillus cereus</i> outbreaks (1972-2024).....	48
Fig. S11: Timeline of outbreaks referenced in each surveillance report (1973-2020).....	84
Fig. 12: Relative frequency of <i>Bacillus cereus</i> cases of the total number of foodborne outbreak cases.....	48
Fig. 13: Geographic distribution of outbreak mentions and author affiliations of the included surveillance reports.....	50
Table 1: Eligibility criteria for the study.....	23
Table S2: Overview of key data extracted from the 37 eligible reviews.....	67
Table S3: Overview of key data extracted from the 40 eligible surveillance reports.....	74
Table S4: Protocol of questions posed to ChatGPT for data extraction from the 37 eligible review articles.....	81
Table 5: Importance of hygiene measures.....	30
Table 6: Presence or absence of detection methods mentioned in the eligible reviews.....	31

Table 7: Overview of conventional and emerging detection methods mentioned in reviews (1972-2024).....	33
Table 8: Overview of food sources and the associated outbreak settings reported across the eligible reviews.....	36

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