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Review article

Spores and clinical impact of *Clostridium botulinum*: an integrative review

Esporos e impacto clínico de Clostridium botulinum: uma revisão integrativa

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ABSTRACT

Clostridium botulinum is a pathogen of relevance to public health due to its ability to produce potent neurotoxins and form highly resistant spores. This study consists of an integrative review of strategies for spore inactivation, identification, and control of *C. botulinum* in food, based on the analysis of 11 articles published between 2022 and 2024. Methods such as UV-C irradiation, supercritical carbon dioxide, qPCR, and the development of non-toxic surrogate strains were addressed. UV-C irradiation proved effective in spore inactivation, although with limitations in liquid foods. Molecular techniques, such as qPCR targeting the 16S rRNA gene, demonstrated high sensitivity in detecting *C. botulinum* in different food matrices. Additionally, non-toxic surrogate strains facilitated tests without health risks. The clinical impact of botulism was evidenced in outbreaks related to contaminated food, reinforcing the need for effective control methods. It is concluded that the integration of physical, molecular, and genetic techniques, combined with good hygiene practices, is essential to ensure food safety and prevent botulism outbreaks.

RESUMO

Clostridium botulinum é um patógeno de relevância para a saúde pública devido à sua capacidade de produzir neurotoxinas potentes e formar esporos altamente resistentes. Este estudo consiste em uma revisão integrativa das estratégias para a inativação de esporos, identificação e controle de C. botulinum em alimentos, com base na análise de 11 artigos publicados entre 2022 e 2024. Foram abordados métodos como irradiação UV-C, dióxido de carbono supercrítico, qPCR e o desenvolvimento de cepas substitutas não tóxicas. A radiação UV-C mostrou-se eficaz na inativação de esporos, porém com limitações em alimentos líquidos. Técnicas moleculares, como a qPCR direcionada ao gene 16S rRNA, demonstraram alta sensibilidade na detecção de C. botulinum em diferentes matrizes alimentares. Além disso, cepas substitutas não tóxicas facilitaram a realização de testes sem riscos à saúde. O impacto clínico do botulismo foi evidenciado em surtos relacionados a alimentos contaminados, reforçando a necessidade de métodos eficazes de controle. Conclui-se que a integração de técnicas físicas, moleculares e genéticas, aliada a boas práticas de higiene, é fundamental para garantir a segurança alimentar e prevenir surtos de botulismo.

INTRODUCTION

The bacterial species *Clostridium botulinum* is of significant importance to public health and food safety due to its ability to produce highly potent neurotoxins responsible for botulism, a severe and potentially fatal neuroparalytic disease[1]. The formation of highly resistant spores by *C. botulinum* represents a major challenge for the food industry, as these spores can survive preservation processes and germinate under favorable conditions, allowing the bacterium to return to its vegetative phase with active metabolism and toxin production[2]. Botulism is frequently associated with the consumption of contaminated foods, particularly those that are improperly preserved, such as canned foods, cured meats, and refrigerated products[3],[4].

Clinically, botulism is characterized by a symmetrical descending flaccid paralysis that may progress to respiratory failure and is therefore considered a medical emergency[5]. The main clinical forms include foodborne botulism, infant botulism, and wound botulism, varying according to the route of exposure to the toxin[5]. Treatment is based on early administration of botulinum antitoxin and ventilatory support, and prognosis depends on rapid diagnosis and antitoxin availability. However, management remains challenging, particularly due to the nonspecific nature of early symptoms and limited access to antitoxins in some regions[5].

Botulism occurs worldwide, with regional variation in the prevalence of clinical forms. In the United States, for example, infant botulism is the most prevalent form, accounting for approximately 70% of reported cases between 2001 and 2017, a period during which 326 foodborne cases were also recorded[6]. Several outbreaks have been reported in different countries, including a 2023 outbreak in France associated with canned sardines that resulted in 15 cases and one death[7]; a 2015 outbreak in the United States in which 29 individuals became ill and one died after consuming canned potatoes at a community event[8]; and a 2024 outbreak in Saudi Arabia, in which eight individuals were affected after consuming contaminated mayonnaise[9]. More recently, an unusual outbreak occurred in 2023 in Germany, Switzerland, Austria, France, and Turkey, related to the use of botulinum toxin in aesthetic procedures, resulting in 34 confirmed cases[10].

The morbidity and mortality associated with botulism are significant, particularly when there is a delay in diagnosis and treatment. The main sources of contamination include contaminated soils, preserved and canned foods that create anaerobic environments, and honey, which represents a well-known risk for infants[11]. Prevention involves safe food preservation and handling practices, inspection of packaging for signs of spoilage such as bulging and unpleasant odor, and strict wound care measures[12]. In Brazil, surveillance and control of botulism outbreaks are conducted by the National Health Surveillance Agency (ANVISA) and the Ministry of Health, in partnership with state and municipal health departments, which monitor notifications through the Notifiable Diseases Information System (SINAN).

Despite advances in preservation techniques and pathogen detection, the biological complexity of *C. botulinum*, including its ability to adapt to different environmental conditions and the diversity of its neurotoxins, requires integrated and multidisciplinary approaches for effective control. Thus, the prevention of botulism outbreaks remains a global challenge, particularly in regions with inadequate hygiene and food preservation practices[13]. In this context, reviewing updated strategies for the control of *C. botulinum* is essential, both to ensure food safety and to strengthen public health protection measures.

In light of this scenario, this integrative re-

view aims to synthesize the approaches that have been used for spore inactivation, as well as for the identification and control of *C. botulinum* along the food production chain. This review is justified by the need to consolidate recent information on pathogen control methods and to provide support for health professionals, the food industry, and surveillance authorities, in order to prevent botulism outbreaks and ensure food safety. To this end, recent studies addressing aspects ranging from basic microbial biology to methods that assist in detection and control will be analyzed, offering an overview of the topic and contributing to future approaches that may prevent botulism outbreaks and ensure food safety based on previously published data.

MATERIALS AND METHODS

This study is an integrative review of the scientific literature, using Google Scholar as the primary database. This platform was chosen due to its broad coverage and multidisciplinary nature, encompassing articles indexed in traditional databases such as PubMed, Scopus, and Web of Science. In addition, Google Scholar allows access to a wide range of national and international journals, enabling an inclusive and comprehensive search on *Clostridium botulinum* and its sporulation capacity.

The search strategy included the descriptors "*Clostridium botulinum*," "sporulation," "botulism," and "toxin," combined using the Boolean operator "AND" to refine the results. As inclusion criteria, only original articles published between 2022 and 2024 were considered, with the final search date set as May 11, 2024, and aligned with the objectives of this study. Documents such as monographs, theses, dissertations, books or book chapters, case reports, editorials, and review articles were excluded.

The article selection process occurred in two stages. In the first stage, original articles were separated from other types of publications. In the second stage, a manual analysis of abstracts was conducted, and only studies addressing the topic of this work were selected.

RESULTS AND DISCUSSION

In the initial stage of article selection, 305 documents were identified through the search. These results were categorized as shown in **Table 1**, with a predominance of review articles, followed by books, theses or dissertations, original articles, case reports, and editorials.

Table 1. Distribution of documents retrieved in the search on the relationship between *Clostridium botulinum* and sporulation and toxin production, conducted in Google Scholar between 2022 and 2024.

DOCUMENT TYPE	NUMBER
Review articles	191
Books	41
Theses or dissertations	40
Original articles	27
Case reports	4
Editorials	2
Total	**305**

Of the 27 original articles identified, full-text reading was performed, resulting in a final selection of 11 studies that met the criteria of the thematic approach of this study. These are summarized in **Table 2**, containing information such as title, year of publication, objectives, and main results. The temporal distribution of the selected articles showed greater representation in 2022 (n = 8). The 16 excluded articles did not align directly with the objectives of the review.

Table 2. Information on the 11 original articles selected in the search on *Clostridium botulinum*, sporulation, and toxin production, conducted in Google Scholar between 2022 and 2024.

ORIGINAL ARTICLE TITLE	CITATION	COUNTRY WHERE THE STUDY WAS CONDUCTED	ARTICLE OBJECTIVES	MAIN FINDINGS OF THE ARTICLE
Bacteriological isolation in the restaurant of Hawler city Kurdistan Region, Iraq	Ahmed[23] (2024)	Iraq	To isolate and identify bacteria present in three different Iraqi restaurants in order to assess the potential effects of food contamination on human health. Samples were collected from menus, tables, and containers.	Three restaurants were analyzed. In the first restaurant, the highest bacterial count was found on a table and the lowest on the menu. In the second restaurant, the container showed the highest bacterial count, while the table had the lowest. In the third restaurant, the table had the lowest number of bacteria, whereas the menu had the highest. The findings demonstrated that certain pathogens, such as *Shigella dysenteriae*, *C. botulinum*, and *Bacillus* cereus, are present and can be identified in restaurants.
Inactivation of Group I And Group II *Clostridium botulinum* spores by ultraviolet irradiation in water	Assal *et al.*[14] (2023)	Canadá	To evaluate the feasibility of using UV light to inactivate *C. botulinum* spores based on their sensitivity to irradiation.	*C. botulinum* spores showed moderate resistance to UV-C disinfection, requiring <55 mJ/cm² to achieve a 5-log inactivation.
Cellular and population strategies underpinning neurotoxin production and sporulation in *Clostridium botulinum* type E cultures	Mertaoja *et al.*[24] (2023)	Finland and Portugal	To analyze the relationship between botulinum neurotoxin (BoNT) production and sporulation in *C. botulinum* type E, investigating regulation by the transcription factor *Spo0A* and population heterogeneity under different environmental conditions.	The possible combinations of toxin production and sporulation tested in wild-type *C. botulinum* type E cultures indicated neither strict co-regulation nor complete independence between the two metabolic processes. In addition, Spo0A-independent BoNT production was observed in a small subpopulation of cells from the *spo0A-null* strain.
Prevalent toxin types of *Clostridium botulinum* in South Korean cattle farms	Park *et al.*[16] (2022)	South Korea	To investigate cattle farms in areas with recurrent outbreaks in South Korea in order to determine prevalent toxin types.	*C. botulinum* toxin types B and D were highly prevalent in feed and cattle feces on South Korean farms in 2012 and 2013 during botulism outbreaks.

Continuation **Table 2.**

Type C botulism outbreak in free-ranging waterfowl in Goiás	Martins *et al.*[17] (2022)	Brazil	To describe a type C botulism outbreak in a population of free-ranging waterfowl, including ducks (*Cairina moschata*), mallards (*Anas platyrhynchos*), and geese (*Anser cygnoides*), in Quirinópolis, Goiás, Brazil.	Botulinum toxin was identified in one of four water samples collected from the lake under study and in the intestinal contents of one necropsied mallard. Waterborne type C botulism was indicated as the cause of mortality in free-ranging waterfowl at Parque da Liberdade in Quirinópolis, Goiás, Brazil.
Genomic diversity, competition, and toxin production by group I and II *Clostridium botulinum* strains used in food challenge studies	Bowe *et al.*[20] (2022)	United States of America	To investigate a cocktail of ten *C. botulinum* strains (seven Group I and three Group II) used for food contamination challenge tests.	Whole-genome single-nucleotide polymorphism alignments revealed that the strain cocktail spans the major clades of Group I and II *C. botulinum*. Although growth competition appeared to occur among several strains, the cocktail as a whole resulted in high levels of BoNT production.
Selection and development of nontoxic nonproteolytic *Clostridium botulinum* surrogate strains for food challenge testing	Poortmans *et al.*[21] (2022)	Belgium and Germany	To develop strains capable of replacing nonproteolytic (Group II) *C. botulinum* strains, ensuring that the surrogate strains are non-toxic.	Phenotypic and genomic analyses of 31 non-toxic, nonproteolytic *C. botulinum* strains revealed three different lineages: type E and BEF (toxic nonproteolytic), and a distinct, poorly characterized cluster. Additional analyses showed that growth and spore heat resistance of these strains fall within the same range described for toxic nonproteolytic strains.
Extensive growth and growth boundary model for non-proteolytic *Clostridium botulinum* – evaluation and validation with MAP and smoked foods	Koukou, Dahl Devitt e Dalgaard[22] (2022)	Denmark	To evaluate and validate a recently developed mathematical model to predict growth of non-proteolytic *C. botulinum* and time to toxin formation (TTT) in smoked foods and foods stored under air, vacuum, or modified atmosphere packaging (MAP).	An existing growth and growth boundary model for non-proteolytic *C. botulinum* was successfully validated for seafood and poultry products, regardless of air, vacuum, or MAP packaging.
Inactivation of *Clostridium* spores in honey with supercritical CO_2 and in combination with essential oils	Dacal-Gutiérrez *et al.*[15] (2022)	Colombia and Spain	To explore the effectiveness of supercritical CO_2 ($scCO_2$), alone and combined with lemon, clove, and cinnamon essential oils, in inactivating *Clostridium sporo-*	$scCO_2$ applied to honey was unable to inactivate *Clostridium* spores at temperatures below 70 °C, likely due to the protective effect of honey. When combined with cinnamon essential oil

Continuation **Table 2.**

			genes (CECT 553) as a surrogate for *C. botulinum.*	(<0.4% w/w), improved spore inactivation was observed, achieving a 1.3 log_{10} CFU g^{-1} reduction at 60 °C.
Sporulation strategies and potential role of the exosporium in survival and persistence of *Clostridium botulinum*	Portinha *et al.*[18] (2022)	Finland	To analyze sporulation diversity in ten Group I, II, or III *C. botulinum* strains, focusing on (i) population structure dynamics in sporulating cultures, (ii) ultrastructure, and (iii) functional properties of individual spores.	Results showed two distinct population dynamics patterns during sporulation, possibly related to proteolytic properties of strains from different groups. Group I strains exhibited higher proportions of sporulating cells but slower spore release, resulting in higher spore counts than Groups II and III. Strains with lower heat resistance lacked an exosporium or had a thinner one, suggesting a role of the exosporium in heat resistance.
Membrane vesicles derived from *Clostridium botulinum* and related *clostridial* species induce innate immune responses via Myd88/Trif signaling *in vitro*	Kobayashi *et al.*[19] (2022)	Japan	To investigate the role of bacterial membrane vesicles produced by *C. botulinum* and phylogenetically related species (*C. sporogenes and C. scindens*) in host immunity and pathology during clostridial infections.	Membrane vesicles derived from all strains induced inflammatory cytokine expression in epithelial and intestinal macrophage cell lines via MyD88/TRIF signaling, revealing the influence of membrane vesicles on induction of the host innate immune response.

Inactivation of Clostridium botulinum spores

Of the 11 selected articles, three addressed methods for the inactivation of *C. botulinum* spores. Assal *et al.*[14] (2023) investigated the use of ultraviolet radiation (UV-C), demonstrating that Group II spores (types B, E, and F) were more resistant than those of Group I (types A, B, and F). To achieve a 5-log reduction in the spore population, UV-C doses below 55 mJ/cm² were required. However, spore aggregation reduced the effectiveness of the method, suggesting the need for complementary techniques to disperse spores in specific situations. The study highlighted that UV-C may be applied to foods, but that further experiments in liquid food matrices are necessary.

The study by Mertaoja *et al.*[24] (2023) investigated the behavior of *C. botulinum* type E at two different temperatures (10 °C and 30 °C), using fluorescence microscopy to monitor growth phases, toxin production, and sporulation, as well as ELISA to quantify toxin production. The results revealed that temperature significantly influences cellular dynamics and spore formation. At 30 °C, toxin production and sporulation occurred in an accelerated manner; however, cells underwent early lysis, limiting long-term microbial viability. In contrast, at 10 °C, a more stable balance between toxin production and sporulation was observed, with both processes occurring gradually over several weeks.

This behavior suggests that *C. botulinum* type E is capable of adapting to refrigerated

environments, which represents a significant challenge for food safety, particularly in products stored at low temperatures, such as seafood. In addition, the study identified four cellular subpopulations with distinct behaviors: (i) cells that produced toxins and sporulated; (ii) cells that produced toxins but did not sporulate; (iii) cells that sporulated but did not produce toxins; and (iv) cells that performed neither process. The proportion of these subpopulations was influenced by temperature and growth phase, highlighting the heterogeneity of cellular responses to different environmental conditions.

Another relevant result of this study was related to the role of the *spo0A* gene, as the authors discuss that this gene may control both sporulation and toxin production, suggesting that genetic regulation represents a promising target for spore inactivation strategies. These findings reinforce the complexity of *C. botulinum* spore inactivation and the need for multifactorial approaches that consider not only environmental conditions but also the genetic and physiological mechanisms involved in microbial survival and virulence.

Dacal-Gutiérrez *et al.*[15] (2022) tested supercritical carbon dioxide ($scCO_2$) to inactivate *C. sporogenes* spores (used as a surrogate for *C. botulinum* in this study due to morphological and genetic similarities) in water and honey. In water, inactivation was effective, achieving 99.7% spore inactivation at 80 °C; however, in honey, efficacy was lower due to its physicochemical properties. The addition of essential oils contributed to 94% spore inactivation at 60 °C. $scCO_2$ inactivation assays were performed at a pressure of 10 MPa, and increasing the pressure to 30 MPa was also tested but did not yield significant improvements; thus, the authors concluded that using the lower pressure is safer and more economically viable. The study concluded that $scCO_2$ is a viable method, but cost–benefit considerations and impacts on food quality must be taken into account.

Restaurant contamination by Clostridium botulinum

Only one article addressed contamination in restaurants. The study by Ahmed[23] (2024) investigated the presence of microorganisms in three restaurants located in the city of Erbil, Kurdistan Region, Iraq. Samples were collected from tables, menus, and containers (such as salt shakers), which were inoculated onto nutrient agar and incubated at 37 °C. After colony growth, Gram staining was performed to differentiate Gram-positive from Gram-negative bacteria. Subsequently, Gram-positive bacteria were cultured on blood agar, while Gram-negative bacteria were cultured on MacConkey agar.

Bacterial identification was based on colony characteristics, including color, shape, elevation, and diameter. The results revealed variable colony-forming unit (CFU) counts across the three restaurants. In the first restaurant, the highest contamination was found on a table (298 CFU), while the lowest was observed on the menu (220 CFU). In the second restaurant, the container showed the highest contamination (280 CFU), and the table showed the lowest (220 CFU). In the third restaurant, the menu presented the highest contamination (80 CFU), whereas the table showed the lowest (52 CFU). Among the identified bacteria, *Clostridium botulinum* and *Bacillus cereus* were notable, both of which are microorganisms associated with preserved foods. The study demonstrated that menus and containers were the most contaminated sites, suggesting failures in frequent and adequate hygiene practices.

Neurotoxins of Clostridium botulinum in animals

Only two articles addressed the neurotoxins produced by *C. botulinum* and their effects on animals. The study by Park *et al.*[16] (2022) investigated botulism outbreaks on cattle farms in South Korea, with the collection of 184 samples of feces, hay, silage, soil, drinking water, and bovine stomach contents. Using multiplex

PCR, the authors identified 33 samples positive for *C. botulinum*, with a prevalence of 33.3% (n = 11) for type B, 12.1% (n = 4) for type C/D, and 54.5% (n = 18) for type D. Most positive samples were found in feces (17/72) and stomach contents (11/15), while no soil samples tested positive for neurotoxins. The study highlighted that type B and D neurotoxins were the most prevalent during the 2012 and 2013 outbreaks in South Korea.

In contrast, the study by Martins *et al.*[17] (2022) described a type C botulism outbreak in waterfowl at Parque da Liberdade, in Quirinópolis, Goiás, Brazil. Samples of water, feed, liver, stomach, and intestinal contents from dead birds were collected. Type *C botulinum* toxin was detected in one water sample and in the intestinal contents of a duck, with confirmation by mouse bioassay and PCR. Mice inoculated with non-heat-treated samples exhibited signs of botulism and died within 12 to 24 hours, whereas those inoculated with heat-treated samples showed no symptoms. The feed was not contaminated, suggesting that lake water was the primary source of contamination.

Key structures involved in sporulation and neurotoxin synthesis by Clostridium botulinum

Three articles addressed sporulation, neurotoxin production, and the pathogenicity of *C. botulinum*. The study by Portinha *et al.*[18](2022) investigated sporulation diversity in ten *C. botulinum* strains from Groups I, II, and III. The results showed that Group I (proteolytic) strains exhibited a higher proportion of sporulating cells and higher spore counts, although they required a longer time to release spores; thus, they presented higher spore counts than strains from Groups II and III (non-proteolytic). All strains displayed similar morphological stages of sporulation. In addition, four spore morphotypes were identified, with differences among groups but without significant impact on spore hydrophobicity or autoaggregation. The study also highlighted that the presence of an exosporium increased spore heat resistance.

The study by Mertaoja *et al.*[24] (2023), previously cited, analyzed the role of the *spo0A* gene in sporulation and botulinum neurotoxin (BoNT) production in *C. botulinum* type E. The results showed that BoNT production occurred in cellular subpopulations and was released either by *spo0A*-mediated autolysis or after the release of mature spores. However, BoNT production was also observed in cells lacking the *spo0A* gene, indicating that other genes may be involved in this process. Culture heterogeneity was influenced by environmental conditions, such as temperature, suggesting that external factors also play a role in regulating toxin production.

The study by Kobayashi *et al.*[24] (2022) investigated the role of membrane vesicles (MVs) secreted by *C. botulinum* in inducing innate immune responses. The results showed that MVs derived from *C. botulinum* induced the expression of inflammatory cytokines in epithelial cells and intestinal macrophages. In addition, MVs from *C. botulinum* type E activated the expression of antimicrobial peptides of the Reg3 family, such as Reg3g and Reg3b, through MyD88/TRIF signaling. These findings suggest that membrane vesicles play an important role in activating the immune system against *C. botulinum* infections.

Methods for identifying food contamination by Clostridium botulinum in the food industry

Three articles addressed methods for evaluating food contamination by *C. botulinum* in the food industry. The study by Bowe *et al.*[20] (2022) investigated a cocktail of ten *C. botulinum* strains (seven from Group I and three from Group II), which has been used for more than 20 years in food challenge studies. The strains were cultured in specific media and had their genetic material sequenced, followed by bioinformatic analyses aimed at identifying genes

related to toxin production. The results showed that, despite competition among strains, the cocktail produced botulinum neurotoxins (BoNT) types A, B, and E, representing the major *C. botulinum* clades involved in foodborne intoxications.

Poortmans *et al.*[21] (2022) developed non-toxic surrogate strains of Group II *C. botulinum* that exhibit phenotypic and genotypic characteristics similar to those of toxic strains. Thirty-one non-toxic strains were analyzed, identifying three genomic clusters, including a novel, as yet uncharacterized group. The authors reported that, aside from the presence of a BoNT toxin gene cluster, non-toxic strains and toxic non-proteolytic Group II *C. botulinum* strains are genetically and phenotypically indistinguishable, indicating that this substitution can be effective for use in studies involving food contamination. Accordingly, five selected strains were tagged with an erythromycin resistance marker and tested in a selective medium that suppresses background microbiota, allowing quantitative recovery of the surrogate strains. This method facilitates food challenge testing without the risks associated with the use of toxic strains.

Koukou, Dahl Devitt, and Dalgaard[22] (2022) validated a qPCR method for the detection of *C. botulinum* targeting the 16S rRNA gene, which is not associated with toxin production. The method was validated for use in seafood and poultry products, regardless of packaging type (air, vacuum, or modified atmosphere packaging – MAP). The technique proved effective in detecting both proteolytic and non-proteolytic strains, even in the presence of other microorganisms, establishing itself as a promising tool for the food industry.

In this study, recent research addressing the challenges and advances in the control, detection, and inactivation of *Clostridium botulinum* was reviewed. This pathogen is of major relevance to food safety due to its ability to produce potent neurotoxins and to form spores that are highly resistant to adverse environmental conditions. The three studies reviewed that focused on the inactivation of *C. botulinum* spores highlighted the high resistance of these spores and the challenges associated with their inactivation in foods.

UV-C radiation proved to be a promising approach; however, its effectiveness is limited by spore aggregation, suggesting the need for complementary methods to optimize its application. The influence of temperature on sporulation and toxin production, as observed by Mertaoja *et al.*[24] (2023), reinforces the importance of considering variable environmental conditions when developing inactivation methods. In addition, although the use of supercritical CO_2 ($scCO_2$) was effective in water, it showed limitations in complex matrices such as honey, indicating that method selection must take into account the physicochemical properties of the food. These findings demonstrate the need for further research to adapt and optimize spore inactivation techniques, ensuring food safety without compromising product quality.

The study by Ahmed[23] (2024) highlights the presence of *C. botulinum* in restaurant environments, reinforcing the importance of strict hygiene practices to prevent food contamination. The predominance of contamination observed on menus and containers, such as salt shakers, suggests that these items are often neglected during cleaning procedures, becoming potential sources of cross-contamination. Furthermore, the identification of *Bacillus cereus*, another microorganism associated with foodborne diseases, underscores the need for continuous monitoring and adequate sanitation in commercial establishments. These results indicate that contamination in restaurants is not limited to food preparation surfaces but also affects items frequently handled by customers, such as menus and containers. Therefore, it is essential to implement more comprehensive cleaning protocols and to train

staff on the importance of hygiene in all areas of the establishment. In addition, future studies could investigate the effectiveness of different disinfection methods in these environments, aiming to reduce microbial load and ensure food safety.

The studies by Park *et al.*[16] (2022) and Martins *et al.*[17] (2022) emphasize the importance of *C. botulinum* neurotoxins as causative agents of botulism in animals. The former revealed that toxin types B and D are the most prevalent in outbreaks of bovine botulism in South Korea, with contamination associated with feces, hay, silage, and stomach contents. These results suggest that contamination of feed and water is a critical factor in disease dissemination, reinforcing the need for strict management and hygiene practices on farms. The absence of neurotoxin detection in soil samples may indicate that soil is not a significant reservoir for *C. botulinum* in these outbreaks; however, further studies are required to confirm this hypothesis.

The second study demonstrated that contaminated water was the primary source of type C botulism in waterfowl in Brazil, highlighting the role of aquatic environments as reservoirs of the toxin. Detection of type C toxin in water samples and intestinal contents, combined with the absence of contamination in feed, suggests that exposure of organic soil during lake drainage may have facilitated the proliferation of *C. botulinum*. This outbreak underscores the importance of monitoring aquatic environments, particularly in areas where birds and other animals have direct access to water. Both studies employed PCR techniques to identify neurotoxin types, demonstrating the effectiveness of this methodology for the detection and characterization of *C. botulinum*. These findings reinforce the need for prevention and control strategies that consider not only food contamination but also water quality and environmental conditions that favor microbial proliferation. In addition, the results highlight the importance of investigating botulism outbreaks in animals, as such events may provide relevant insights for the prevention of human cases and for the protection of public health.

The three studies reviewed on key structures involved in sporulation and neurotoxin synthesis by *C. botulinum* emphasize the complexity of the molecular mechanisms underlying sporulation, toxin production, and pathogenicity in this bacterium. The work by Portinha *et al.*[18] (2022) revealed that sporulation varies significantly among *C. botulinum* groups, with proteolytic strains (Group I) showing greater efficiency in spore formation. The finding that the exosporium contributes to spore heat resistance suggests that this structure may represent a potential target for spore inactivation strategies in foods and contaminated environments.

The study by Mertaoja *et al.*[24] (2023) demonstrated that the *spo0A* gene plays an important, though not exclusive, role in toxin production and sporulation. The observation that cells lacking *spo0A* are still capable of producing BoNT opens new perspectives for the identification of additional genes and regulatory pathways involved in this process. These results underscore the need for further research to understand the genetic network controlling *C. botulinum* virulence, which may contribute to the development of new therapeutic and control strategies.

The study by Kobayashi *et al.*[19] (2022) highlighted the role of membrane vesicles (MVs) in the activation of innate immune responses. The ability of MVs to induce the expression of inflammatory cytokines and antimicrobial peptides suggests that these structures may constitute promising targets for the development of vaccines or therapies aimed at modulating immune responses against *C. botulinum* infections. Moreover, understanding MyD88/TRIF signaling mechanisms may provide new avenues for the treatment of diseases associated

with clostridial toxins. Taken together, these studies advance the understanding of molecular targets involved in *C. botulinum* biology, providing a foundation for future research and practical applications in the prevention and control of botulism.

The reviewed studies on the implications of *C. botulinum* detection methods in the food industry highlight significant advances in the development and validation of methods for detecting this bacterium in foods, with relevant implications for food safety. The work by Bowe *et al.*[20] (2022) reinforces the utility of *C. botulinum* strain cocktails in food challenge studies, allowing the assessment of toxin production under controlled conditions. The strain competition observed in the study did not compromise toxin production, indicating that the cocktail constitutes a reliable tool for simulating real contamination scenarios in foods.

The study by Poortmans *et al.*[21] (2022) introduced an innovative approach by developing non-toxigenic surrogate strains of *Clostridium botulinum* Group II that retain phenotypic and genotypic characteristics similar to those of pathogenic strains. The insertion of an erythromycin resistance marker, combined with the development of a selective medium, facilitates the quantification of these strains in food challenge tests, reducing the risks associated with handling toxigenic strains. This method may make food safety testing more accessible and safer for the industry.

Finally, the study by Koukou *et al.*[22] (2022) validated a qPCR method based on the 16S rRNA molecular marker, which allows detection of *C. botulinum* regardless of toxin production. This technique proved to be robust across different packaging types and food matrices, such as seafood and poultry, and effective even in the presence of other microorganisms. This method represents a rapid and reliable alternative for monitoring *C. botulinum* contamination throughout the food production chain.

Taken together, these studies provide valuable tools for the food industry, contributing to the prevention of botulism outbreaks and to ensuring food safety. The combination of strain cocktails, non-toxic surrogate strains, and molecular methods such as qPCR can optimize detection and control processes for *C. botulinum*, reducing public health risks and promoting innovation in the food sector.

In summary, the reviewed studies highlight the complexity of *Clostridium botulinum* biology and the challenges associated with its detection, inactivation, and control in foods. Spore resistance, neurotoxin diversity, and the ability to adapt to different environmental conditions underscore the need for multifactorial approaches to ensure food safety. Methods such as UV-C radiation, the use of supercritical carbon dioxide, and the identification of molecular targets such as the *spo0A* gene offer promising perspectives for spore inactivation and reduction of toxin production. In addition, the development of non-toxic surrogate strains and advanced molecular techniques, such as qPCR, represents a significant advance in the detection and monitoring of *C. botulinum* in the food industry.

These tools, combined with rigorous hygiene and handling practices, are essential for preventing botulism outbreaks and protecting public health. Future research should emphasize the optimization of these methods, the exploration of new molecular targets, and the adaptation of techniques to different food matrices, aiming to ensure the effectiveness and feasibility of large-scale application. Thus, the integration of basic science, technological innovation, and good manufacturing practices can contribute to more effective control of *C. botulinum* and to food safety on a global scale.

AUTHOR CONTRIBUTIONS

DTM, BCD and AGCO were responsible for the conception and design of the study, data analysis and manuscript writing. DTM and AGCO data collection, statistical analysis,

and critical revision of the manuscript. All authors read and approved the final manuscript version and agree to take responsibility for its content.

CONFLICT OF INTEREST

We wish to confirm that there are no known conflicts of interest associated with this publication and that no significant financial support has influenced its results.

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DECLARATION REGARDING THE USE OF GENERATIVE AI

The authors declare that they used generative artificial intelligence tools (ChatGPT) to assist with linguistic revision. However, all analyses, interpretations, and conclusions presented are the sole responsibility of the authors. Artificial intelligence was not used for the generation of scientific data, the writing of critical sections of the manuscript, or methodological decision-making. The editorial board made the decision to utilize ChatGPT, an AI language model developed by OpenAI, for the translation of this manuscript from the original language

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