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CLOSTRIDIUM BOTULINUM: PATHOGENIC BACTERIA-KNOWN FOR BOTULINUM NEUROTOXIN (BONT)

Ravindra B. Malabadi [1, 3, *], Satvir Kaur [2] and Raju K. Chalannavar [1]

[1] Department of Applied Botany, Mangalore University, Mangalagangotri-574199, Mangalore (DK), Karnataka State, India.
[2] Department of Allied Health Sciences, Sant Baba Bhag Singh University, Village Khiala, P. O Padhiana, Jalandhar-144030, Punjab State, India.
[3] Miller Blvd, NW, Edmonton, Alberta, Canada.
* Corresponding Author

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ABSTRACT

Clostridium botulinum is a Gram-positive, spore-forming bacterium with ubiquitous distribution in soils and aquatic sediments. It produces botulinum neurotoxin (BoNT), which is responsible for botulism (mainly characterized by flaccid paralysis) in humans and animals. This neurotoxin is among the most toxic substances known; even microscopic amounts can cause illness or death. The majority of cases in humans are food borne botulism. Therefore, it is a crucial food hazard. Decomposition of plants, algae, and animals creates anaerobic environments that facilitate growth of *C. botulinum*, which may then enter into food webs leading to intoxication of animals. This toxin is responsible for causing botulism, which is considered a severe neurological disease. All *C. botulinum* strains produce botulinum toxin, which paralyzes animals by inhibiting acetylcholine release from synaptic vesicles at neuromuscular junctions. Botulism is a severe muscle paralysis disease mediated by the botulinum toxin. Early symptoms include fatigue, blurred or double vision, dry mouth, ptosis, and difficulty speaking or swallowing. This toxin is classified into eight serotypes designated A– H of which A, B, E, and F are shown toxic to humans. Botulinum toxin-producing bacteria are divided into six groups: *C. botulinum* Groups I–IV as well as some strains of *C. baratii* and *C. butyricum*. Group I includes the proteolytic *C. botulinum* strains that produce botulinum toxin serotypes A, B, and F. Group II comprises non-proteolytic strains that produce toxin serotypes B, E, and F. The strains in Group III produce serotypes C and D, or mosaic C/D toxins. Group VI strains, referred to as *C. argentinense*, produce toxin serotype G. Among the other species, *C. butyricum* produces botulinum toxin serotype E and *C. baratii* produces serotype F. This literature review paper intends to provide a brief overview of the current state of knowledge regarding *C. botulinum*, and the common physical treatments to eliminate this biological hazard in the food industry.

Keywords: *Clostridium botulinum*; Botulism; Botulinum Neurotoxin (BoNT); Health sciences, Sporulation; Germination; Food Contamination; Therapeutics

1 INTRODUCTION

Clostridium botulinum, a polyphyletic Gram-positive taxon of bacteria, is classified purely by their ability to produce botulinum neurotoxin (BoNT) [1-63-95-100]. Botulinum toxins or neurotoxins (BoNTs) are the most potent neurotoxins known, and are currently extensively studied, not only for their potential lethality, but also for their possible therapeutic and cosmetic uses. *Clostridium botulinum* (*C. botulinum*) is known for producing one of the strongest neurotoxins, namely botulinum neurotoxin (BoNT) [1-62-100]. *Clostridium botulinum* are rod-shaped bacteria (also called *C. botulinum*). They are anaerobic, meaning they live and grow in low oxygen conditions. The bacteria form protective spores when conditions for survival are poor. The spore has a hard protective coating that encases the key parts of the bacterium and has layers of protective membranes. Within these membranes and the hard coating, the dormant bacterium is able to survive for years. *C. botulinum* is responsible for a disease called botulism. This toxin is responsible for causing botulism, which is considered a severe neurological disease [1-62]. A notable, historically significant outbreak of food-borne botulism in India occurred in 1996, linked to *Clostridium butyricum* (rather than *C. botulinum*) in a residential school in Gujarat, which prompted investigation into rare botulinum toxin producers [58-62].. BoNT (botulinum neurotoxin) is the primary virulence factor and the causative agent of botulism [1-60]. A potentially fatal disease, botulism is classically characterized by a symmetrical descending flaccid paralysis, which is left untreated can lead to respiratory failure and death. Botulism cases are classified into three main forms dependent on the nature of intoxication; food-borne, wound and infant [1-60-100]. Botulism is a severe muscle paralysis disease mediated by the botulinum toxin. *Clostridium botulinum* (*C. botulinum*) is an anaerobic bacterium mostly producing a potent botulinum toxin (BoNT), which causes severe disease with a high mortality rate [1–4-50]. The botulinum toxin comprises a heavy chain (100 kDa) and a light chain (50 kDa) [1-60]. As a zinc metalloprotease, its light chain can access the cytoplasm of nerve cells, where it specifically cleaves the soluble N-ethylmaleimide-sensitive factor attachment protein receptors (SNARE) associated with the release of the neurotransmitter acetylcholine, thereby effectively inhibiting its release [5–7-57-100]. This ultimately leads to fatal flaccid muscle paralysis [1-60]. BoNTs (botulinum neurotoxin) can be classified into eight types (A–G-H types) [1-60-95]. Types A, B, E, and F can cause poisoning in humans, while types C and D mainly cause disease in animals [1-10–12-60]. *Clostridium botulinum* is a Gram-positive, spore-forming bacterium with ubiquitous distribution in the environment [1-60-95]. It produces botulinum neurotoxin (BoNT), which is responsible for botulism (mainly characterized by flaccid paralysis) in humans and animals [1-45-60-95]. The majority of cases in humans are food borne botulism [2], therefore, it is a crucial food hazard [1-3-60-95].

The BoNT (botulinum neurotoxin) regarded as the most potent biological substance known, is a zinc metalloprotease that specifically cleaves SNARE proteins at neuromuscular junctions, preventing exocytosis of neurotransmitters, leading to muscle paralysis [1-3-62-100]. The BoNT (botulinum neurotoxin) is now used to treat numerous medical conditions caused by overactive or spastic muscles and is extensively used in the cosmetic industry due to its high specificity and the exceedingly small doses needed to exert long-lasting pharmacological effects [1-3-63-100]. Additionally, the ability to form endospores is critical to the pathogenicity of the bacteria [1-3-62]. Food-borne botulism occurs when food contaminated with botulinum neurotoxins (BoNTs) is ingested [1-3-62]. This contamination happens during storage under conditions that allow bacterial growth together with BoNT production [1–3-62]. Early symptoms include fatigue, blurred or double vision, dry mouth, ptosis, and difficulty speaking or swallowing [1,4-62]. Gastrointestinal signs such as constipation may follow [1–3-63-100]. The extent of pathophysiological disorders varies significantly depending on the dose of toxin and route of exposure, with oral uptake being far less efficient than parenteral administration [1-4-57-63-95].

The BoNT family is produced by *Clostridium botulinum* and related toxigenic anaerobic spore-forming *Clostridium* species, including *C. butyricum, C. baratii, C. novyi sensulato*, *C. sporogenes* and *C. argentinense* [1-4-57-62-100]. Botulinum neurotoxins (BoNTs) are classified into eight major serotypes (A–G-H), which share 37–70% pairwise amino acid identity, and collectively encompass at least 40 subtypes that have significant differences in their primary amino acid sequences [1-13,14-63-95].

Food borne botulism is caused by the ingestion of food contaminated with preformed BoNT, with as little as a few milligrams of contaminated food enough to cause symptoms and perhaps death if left untreated [1-4-10-34-45-57-62-100]. Symptoms typically present 12–72-h post-consumption of the contaminated food, dependent on the quantity ingested [1-4-10-34-45-57-62-100]. Food borne botulism was historically the most prevalent form of human botulism and is usually associated with uncooked foods or the inadequate processing of food stuffs both commercially and when home prepared [1-4-10-34-45-57-62-100]. Preservation techniques such as fermentation or pickling, followed by canning or bottling of foods without prior heat treatments pose the greatest threat as they create the anaerobic conditions required for spore germination and bacterial growth [1-4-10-34-45-57-63]. The introduction of certain food control measures; the “botulinum cook” (121 °C heat treatment for 3 min), freezing, and refrigeration (below 4 °C), when applied correctly, have greatly decreased food borne outbreaks [1-4-10-34-45-57-62-100].

2 *CLOSTRIDIUM BOTULINUM*: DISTRIBUTION

C. botulinum is prevalent in soil and marine sediments worldwide, most commonly as spores. These spores are found everywhere [1-34-45-57-63-95-100]. While the spores are generally harmless, the danger can occur once the spores begin to grow out into active bacteria and produce neurotoxins [1-34-45-57-63-95]. A neurotoxin is a poisonous chemical that affects the central nervous system. It can destroy, paralyze, or adversely affect nerves or nerve tissue [1-34-45-57-63-95-100]. *C. botulinum* produces seven different types of neurotoxins designated by the letters A through G; only types A, B, E, and F cause illness in humans. *Clostridium botulinum* is ubiquitously present in the environment in soils, dust, the marine and freshwater sediments of wetlands, rivers, and lakes [1-34-45-57-63-95]. Spores in soil may be mobilized by surface waters in heavy rain, or dust carried away by wind [1-34-45-57-63-100]. Botulism has been characterized as a particularly substantial risk to humans in northern climatic regions, due to intoxication from poorly preserved food. Serotype E is dominant in sediments of the arctic and subarctic regions, whereas serotype B is most prevalent in soil [1-34-45-57-63-95]. The temperate climate zone of Europe shows the same distribution pattern, in which serotype B is most prevalent in soil and serotype E is found in sediments although serotypes C and D are also commonly found [1-34-45-57-63-95-100]. In the temperate zone of Northern America, serotype A is most common west of the Mississippi river, and serotype B east of the Mississippi river, whereas serotype E is most common in the areas of the Great Lakes and the Pacific Northwest [1-34-45-57-63]. In China, serotypes A–F have all been detected in the soil [1-34-45-57-63]. In Japan, the presence of botulinum toxin serotypes B, C, and E has been documented In general, environmental botulism outbreaks have been connected to serotypes C, mosaic C/D, and E [1-34-45-57-63-95].

Clostridium botulinum spores released into the environment are robust, potentially persisting in soils and sediments for decades [1-34-45-57-63-100]. The bacterium has been found in the intestinal tract of healthy fish, birds, and mammals. *C. botulinum* serotype E does not multiply in the fish gut, and fish fed 500,000 spores per day (in pellets) did not acquire botulism [1-34-45-57-63-95]. Thus, the initial proliferation of bacterial germination and vegetative growth must occur somewhere in the environment. Once established, a botulism outbreak is self-perpetuating [1-34-45-57-63]. During an avian botulism outbreak, the disease spreads through necrophagous flies depositing eggs on dead and toxic animal carcasses [1-34-45-57-63-100]. The resulting maggots feed on the carcasses and concentrate the botulinum toxin. When other animals ingest the toxic maggots, they become the next victims (the carcass–maggot cycle) [1-34-45-57-63]. During outbreaks in fish, decomposing invertebrates and decaying fish sink to the lake bottom and are consumed by scavenging fish in an amplifying cycle. A study of channel catfish showed that their lethal dose of botulinum toxin E was less than the median lethal dose for mice. Toxin levels may persist and remain lethal over the winter in larvae [1-34-45-57-63-95]. A wide variety of organisms—such as algae, plants, and invertebrates—have been shown to contain botulinum toxin or *C. botulinum* DNA [1-34-45-57-63]. These organisms represent a biotic reservoir for *C. botulinum*, and may themselves become toxic upon anaerobic decomposition [1-34-45-57-63-95].

3 *C. BOTULINUM* CONTAMINATION IN FOOD

C. botulinum spores are often found on the surfaces of fruits and vegetables and in seafood [1-34-45-57-63-95]. The organism grows best under low-oxygen conditions and produces spores and toxins. The toxin is most commonly formed when food is improperly processed (canned) at home [1-34-45-57-63-95-100]. *C. botulinum* cannot grow below a pH of 4.6, so acidic foods, such as most fruits, tomatoes, and pickles, can be safely processed in a water bath canner [1-34-45-57-63-95-100]. However, foods with a higher pH (most vegetables and meats) must be processed under pressure. Therefore, a pressure cooker should be used. The pressure cooker will reach high enough temperatures to destroy the *C. botulinum* spores [1-34-45-57-63-95].

For example, if a low-acid food, such as green beans, is canned improperly (not canned under pressure or improperly canned using a pressure canner), *C. botulinum* bacteria and other bacteria present will be destroyed by the boiling of water and food, but the *C. botulinum* spores will not be destroyed [1-34-45-57-63-95]. The canning process will remove the oxygen from the jar, creating a low-oxygen environment that will allow the spores to grow into active bacteria [1-34-45-57-63-95]. When the jars are stored at room temperature, the spores can germinate and produce the toxin. However, the toxin is sensitive to heat and can be destroyed if the food in question is boiled for 10 minutes (longer at high altitudes) [1-34-45-57-63-95-100]. One of the most common causes of food borne botulism is improperly home-canned food, especially low-acid foods such as vegetables and meats. Only a pressure cooker/canner allows water to reach 240 to 250 degrees F, a temperature that can kill the spores [1-34-45-57-63-95]. The control of food borne botulism is based almost entirely on thermal destruction (heating) of the spores or inhibiting spore germination into bacteria and allowing cells to grow and produce toxins in foods [1-34-45-57-63-95-100].

4 BOTULISM REPORTED IN 1820

Across Southwest Germany, an increasing number of fatal food poisoning cases were reported after consumption of a traditional uncooked blood sausage meal [1-4-10-34-45-57-62-100]. This led a young local medical officer and poet,

Justinus Kerner, now known as the godfather of botulinum research, to connect the blood sausage to the paralytic illness. He published the first complete descriptions of botulism in 1820 [1-4-10-34-45-57-62-100]. He termed the ailment "sausage poisoning" making it a reportable disease, and was the first to suggest the cause as a biological poison, performing experiments with the poison upon himself. Kerner even postulated the use of the "poison" to treat a variety of diseases in 1822, which was realized over 150 years after it was first envisaged [1-4-10-34-45-57-62-100].

Botulism is a serious and potentially fatal neuroparalytic disease of humans and animals, in which the sole causative agent is the BoNT [1-4-10-34-45-57-62-100]. Human botulism is characterized as a bilateral descending muscle weakness, symptoms generally begin in the cranial nerves presenting as blurred or double vision, dry mouth, and difficulty speaking [1-4-10-34-45-57-63-100]. The classical early physical presentation of botulism can be remembered using the "four D's" mnemonic: dysarthria, diplopia and dysphonia, dysphagia [1-3-12-44-49-58-62-95]. As the disease progresses to the extremities of the body with the dissemination of the neurotoxin, weakness to the muscles of the torso and upper limbs occurs. If left untreated, the respiratory muscle groups are affected, and death is caused by respiratory failure [1-4-10-34-45-57-62-100].

Clostridium botulinum represents a remarkable paradox in biology, an organism capable of producing one of the deadliest toxins known, yet simultaneously a cornerstone in modern therapeutic innovation [1-4-10-34-45-57-62-100]. In fact, this toxin's dual nature is based on its selective targeting of BoNTs. In this regard, the systemic entry and distribution of this poison in the body - or, in other words, intoxication with it - poses a life-threatening aspect [1-4-10-34-45-57-62-100]. On the other hand, the use of this bacterium's toxin for therapeutic and medicinal purposes is progressing due to its topical and controlled administration and entry into the body make it a targeted and effective therapeutic tool in the field of medicine [1-4-10-34-45-57-62-100]. A better and more precise understanding of the mechanisms of action and, if necessary, future structural and engineered modifications in the toxin could lead to the emergence of a new generation of BoNTs that hold promise for the development of esthetic and therapeutic applications in medicine [1-4-10-34-45-57-63-95-100].

5 THE BACTERIUM *CLOSTRIDIUM BOTULINUM*: 3 GROUPS

The Bacterium *Clostridium botulinum* is an anaerobic, straight or slightly curved, motile rod (3–22 μm by 0.2–0.6 μm in size), Gram-positive, catalase-negative, and mainly sub-terminal to rarely central spore-forming bacterium [1-3,20,21-62-95]. The genus *Clostridium* is a member of the family *Clostridiaceae*, in the order *Clostridiales*, class *Clostridia,* and phylum *Bacillota* (synonym *Firmicutes*) [1-22-62-100]. This genus consists of approximately 200 species, about 15 of which synthesize toxins that cause disease in humans or animals [1-22-62-100]. Based on biochemical and proteolytical properties, *C. botulinum* is divided into three basic groups (I–III). Strains of Group I are proteolytic, and strains of groups II and III are non-proteolytic [1-22-62-95]. For the sake of thoroughness, it is important to note that other species of the genus *Clostridium* than *C. botulinum*, such as *C. baratii*, also produce botulinum toxins and constitute groups IV, V, and VI of botulinum toxin-producing *Clostridium*. Group IV is proteolytic and groups V and VI are non-proteolytic [1-22-62-100]. Neurotoxigenic *Clostridium* strains are also classified according to the type of toxin they produce. Currently, nine types of BoNTs have been identified: A, B, C, D, E, F, G, H, and X, but only the first seven (A to G) are considered for this classification [1-22-63-100]. These species are the bacteria that have a similar biochemical and proteolytic profile to *C. botulinum* but do not produce botulinum toxin [1-22-62-100]. Botulism is a severe paralytic disease caused by BoNT. This illness has different forms based on the mode of contamination and exposure to the BoNTs [1-22-63-100]. Food borne botulism occurs after ingesting BoNT-containing foods (canned vegetables— A, preserved meat—B, and fish—E) [1-22-62-100]. Inhalation botulism has a similar clinical footprint to food botulism, and it occurs by inhaling an aerosol that is accidentally or intentionally [1-22-63-100].

6 TYPES OF BOTULISM

Smith [77] reviewed that botulism is a neuroparalytic intoxication caused by a collection of large proteins, known as botulinum neurotoxins (BoNTs), that are related in amino acid sequence and structure [1-77-95-100]. The extreme potency of BoNTs can be traced to their ability to access and enter cholinergic nerve terminals, their enzymatic nature, and their persistence within these cells [1-77]. The extreme diversity seen among the BoNTs (7 serotypes and 44 subtypes) and the bacteria that produce them (7 species) stands in stark contrast to its close relative, tetanus toxin, which exists as a single protein entity produced by a single bacterial strain [77]. Botulism may take many forms [1-77]. It can be due to direct ingestion with BoNT (food-borne), or it may be the consequence of germination and toxin production within the body (infant and adult toxicoinfections, wound botulism) [1-77]. As BoNT-producing organisms are soil inhabitants, the cycle that results in botulism begins when the spores of these bacteria are moved to a location that is favourable for its growth and toxin production, be that in foods, humans, or animals [1-77]. Multiple researchers in the United States did pioneering work concerning the etiology of botulism, including the identification of different types, the recognition of various host sensitivities, and the necessary conditions for germination and toxin production of these bacteria [1-77-100].

Botulism is a life-threatening disease caused by the ingestion of a potent neurotoxin produced during growth of the *C. botulinum* bacteria [1-77-95]. This neurotoxin is among the most toxic substances known; even microscopic amounts can cause illness or death [1-77-95-100]. In the past, botulism was linked primarily to home-canned foods. In recent decades, however, botulism illnesses have been linked to foods such as unrefrigerated homemade salsa, baked potatoes sealed in aluminum foil, honey (the primary cause of botulism in infants), garlic in oil, and traditionally prepared salted or fermented fish [1-77-95].

Botulism is a rare but life-threatening neuroparalytic syndrome caused by the botulinum neurotoxin, most often produced by the bacterium *Clostridium botulinum* [1-40-57-63-100]. The condition begins with symmetrical cranial nerve paralysis and progresses to descending, symmetrical weakness affecting the trunk and extremities, potentially leading to complete flaccid paralysis [1-4-10-34-45-57-62]. Severe respiratory compromise is common, requiring mechanical ventilation, and mortality can occur if treatment is delayed [1-6-17-37-46-57-62-100]. Diagnosis is confirmed by detecting botulinum toxin in blood, stool, or food samples or by identifying the bacteria in stool. Prompt administration of botulinum antitoxin and supportive care is essential, though recovery can take months to years [1-4-10-34-45-57-63].

Botulism is a rare but serious illness caused by a bacterium called *Clostridium botulinum*, which occurs in soil. It produces a neurotropic toxin. [1-4-10-34-45-57-62]. There are three main kinds of botulism viz., food-borne botulism, wound botulism and infant botulism [1-4-10-34-45-57-62-100]. According to one more classification, Five types of botulism are commonly identified in human cases, including food-borne botulism, infant botulism, wound botulism, iatrogenic botulism, and inhalational botulism [1-4,13-62]. Food borne botulism is a poisoning caused by the consumption of food containing the botulinum toxin, and as little as 30 ng of the neurotoxin is enough to cause illness or even death [1-14-62-100]. In Canada and France, the most common food products for food borne *C. botulinum* poisoning are home-made traditional processed fish products and canned fish [1-15,16-62-100]. Fermented homemade items such as stinky tofu and noodle sauce have primarily caused food-borne botulism in China [1-14-62]. The highest incidence of botulism in China is in the North Western region, with Xinjiang province having the highest incidence rate [1-17,18-62-100]. Studies have demonstrated that the predominant BoNT serotypes were type A in China. Food borne botulism cases mainly occur from June to August due to high temperature and high humidity, which is suitable for the reproduction of C. botulinum and the production of toxins [1-17-63-95].

Food-borne botulism comes from eating foods contaminated with the toxin. Wounds infected with toxin-producing bacteria resulted in wound botulism [1-4-10-34-45-57-62-100]. And infant botulism occurs when *C. botulinum* spores germinate and produce toxin in the gastrointestinal tract of infants by consuming the bacteria, usually from honey [1-4-10-34-45-57-62-100]. All these three forms of botulism can be deadly and are medical emergencies. *C. botulinum* is an anaerobic, Gram-positive, spore-forming rod shape bacteria that produce botulinum toxin. Botulinum toxin is one of the most powerful known toxins (about one microgram is lethal to humans) that causes the severe neuroparalytic illness [1-4-10-34-45-57-62-95]. There are seven serologically distinct types of botulinum neurotoxin – types A, B, C, D, E, F, and G1. Comparison of 16S rRNA sequences showed that *C. botulinum* strains form four distinct clusters that correspond to four physiological groups (I–IV), which supported the historical classification scheme based upon biochemical and biophysical parameters [1-4-10-34-45-57-62]. Group I (proteolytic *C. botulinum*) strains produce one or sometimes two toxins of types A, B or F; Group II (non-proteolytic C. botulinum) strains produce toxins of types B, E, or F; Group III strains produce toxins of types C or D; and Group IV strains produce toxin of type G3, Botulinum neurotoxin types A (BoNT/A) is the most widely studied and best characterized of the BoNT serotypes [1-4-10-34-45-57-63-95].

In addition to the neurotoxin, a key virulence factor in the pathogenesis of *C. botulinum* and prevalence of botulism, is the sporulation and germination survival strategy [1-4-10-34-45-57-62-100]. This capability enables the bacterium to protect itself from adverse environmental conditions as a dormant and metabolically inert endospore, returning to vegetative cell growth once conditions become favourable, through the process of germination [1-4-10-34-45-57-62-100]. It is precisely these processes that enable the ubiquitous environmental persistence of *C. botulinum* and its infiltration and neurotoxin production in the specific environmental niches associated with the different forms of botulism [1-4-10-34-45-57-63-96].

In general, the most commonly used classification is related to the type of toxin produced. This approach also considers the fact that different strains of *C. botulinum* are capable of producing two or more types of BoNTs simultaneously, usually with different rates of production of the different toxins [1-23,31-62-100]. In the literature, by convention, the type of toxin produced in the majority is indicated in capital letters and the type of toxin produced in the minority is indicated in lowercase, e.g., Ba, Bf, Ab, Af, Bx, and Bh [1-23,31-63-95].

7 BONT (BOTULINUM NEUROTOXIN): BIOTERRORISM

The use of biological agents for bioterrorism or bio-crime purposes continues to be of global concern envisaged [1-4-10-34-45-57-62-100]. BoNT (botulinum neurotoxin) is classified as among the highest risk threats, being listed as a Category A agent by the CDC in the US and a Schedule 5 biological agent in the United Kingdom envisaged [1-4-10-34-45-57-62-100]. Due to its potential to be utilized as a biological weapon, the storage and handling of BoNT neurotoxic strains requires enhanced security arrangements and regular audits. BoNT is one of the most potent biological agents having the lowest lethal dose of any natural substance known envisaged [1-4-10-34-45-57-62-100]. The human lethal dose (LD_{50}) is dependent on the route of entry into the body, but assuming an average body weight of 70 kg, 70 μg of ingested, 0.7–0.9 μg of inhaled and 0.09–0.15 μg of intravenously administered concentrated BoNT/A toxin would be lethal envisaged [1-4-10-34-45-57-62-100]. In humans, the disease is classified into three main clinical types dependent on the route of entry into the body; food borne, wound, and infant botulism. Symptoms across all forms of botulism are broadly uniform, with the exception that food borne cases may include additional gastrointestinal symptoms of diarrhoea, nausea, vomiting, and abdominal cramps prior to neurological symptoms [1-4-10-34-45-57-62-100]. The development of new botulism treatments and diagnostics has remained an active area and will hopefully provide greater safety to populations in the event of a bioterror attack, as well as allow improved ethical surveillance of imminent risks [1-4-10-34-45-57-62-100]. Food borne botulism is a neurological disease with clinical manifestations distinct from other food poisonings and less gastrointestinal symptoms. The clinical symptoms of botulism are primarily neurological, which usually start with blurred vision and flaccid paralysis of the respiratory muscles or myocardium, which may occur in some severe cases [1-41,42-62-100]. Existing studies indicated that the rate of misdiagnosis in domestic hospitals in our country reaches 27.50% [1-17-62-95].

8 BONT (BOTULINUM NEUROTOXIN): SYMPTOMS AND DETECTION

According to the literature survey, Food borne botulism were reported to the Centers for Disease Control and Prevention (CDC) [1-17-62-95-100]. The majority of these cases were caused by intoxication by BoNT/A (76 cases) or BoNT/E (46 cases), with only 10 cases directly linked to consumption of food contaminated with BoNT/B [1-17-62]. However, during the same period, BoNT/B was the causative agent of 387 of the 663 cases of infant botulism (58.4%) recorded by the CDC [1-17-62]. Botulinum toxin acts by blocking nerve function and leads to respiratory and musculoskeletal paralysis [1-17-62]. Specifically, the toxin acts by blocking the production or release of acetylcholine at synapses and neuromuscular junctions [1-17-62-100]. Death occurs due to respiratory failure. Symptoms include double vision, blurred vision, drooping eyelids, slurred speech, diffi culty in swallowing, dry mouth and muscular weakness [1-17-62-100]. In all cases illness is caused by the toxin produced by *C. botulinum*, not by the bacterium itself. Physicians may consider diagnosing botulism if the patient's history and physical examination suggest botulism [1-17-62-100]. However, these clues are often not enough to allow a diagnosis. Other diseases such as GuillainBarré syndrome, stroke, and myasthenia gravis can appear similar to botulism, and special tests may be needed to rule out these other conditions [1-17-62]. These tests may include brain scan, cerebrospinal fluid examination, nerve conduction test (electromyography, or EMG), and an edrophonium chloride (Tensilon) test for myasthenia gravis [1-17-62-100]. A definite diagnosis can be made if botulinum toxin is identified in the food, stomach or intestinal contents, vomit or faeces [1-17-54-62-100he toxin is occasionally found in the blood in acute cases [1-17-54-62-95].

Once in the body, the toxin binds to nerve endings that join muscles. This prevents the nerves from signaling the muscles to contract [1-17-54-62-95-100]. The first symptoms of botulism are nausea, vomiting, weakness, and vertigo (dizziness) [1-17-54-62-95-100]. These are followed by neurological symptoms: visual impairments (blurred or double vision), loss of normal throat and mouth functions (difficulty speaking and swallowing; dry mouth, throat, and tongue; and sore throat), general fatigue, lack of muscle coordination, and difficulty in breathing. Gastrointestinal symptoms may include abdominal pain, diarrhea, or constipation [1-17-54-62-95-100]. Death is usually caused by respiratory failure and airway obstructions. When the diaphragm and chest muscles become fully involved, breathing is affected and results in death from asphyxia [1-17-54-62-95]. If botulism is caught in the early stages, the injection of an antitoxin can lessen the severity of the disease by neutralizing any toxin that has not yet bound to nerve endings. However, due to the risk of serious side effects, the antitoxin cannot always be used. A human-derived antitoxin is used to treat cases of infant botulism and is available from the California Department of Public Health [1-17-54-62-95-100].

The toxin produced by the bacterium *Clostridium botulinum* (*C. botulinum*), known as botulinum neurotoxin (BoNT), is the cause of the neurological paralysis disease botulism [1-17-54-62-95]. It is also increasingly recognized for its therapeutic applications in treating various medical conditions characterized by overactive or spastic muscles [1-17-54-62-100]. The effectiveness of BoNT in reducing muscle contractions not only addresses medical disorders but also meets aesthetic needs, such as reducing facial wrinkles [1-17-54-62-95]. In recent years, despite significant advancements in understanding *C. botulinum* in the fields of microbiology and neurotoxins, scientific understanding of its duality as both a pathogen and a source of therapeutic potential represents a significant knowledge gap [1-17-54-62-95-100]. Most previous studies have generally focused on the descriptive aspects of pathogenesis and conventional

treatments and have paid less attention to examining the dual nature of the toxin produced by this organism [1-17-54-62-100].

Botulinum toxin can be detected by a variety of techniques, including enzyme-linked immunosorbent assays (ELISAs), electrochemiluminescent (ECL) tests and mouse inoculation or feeding trials with neutralization tests in mice [1-17-54-62-95-100]. Sensitive and rapid detection of botulinum neurotoxins is essential for the detection of poisoned food, as well as for response to potential bioterrorist threats. Currently, the most reliable method for detection of botulinum neurotoxin is mouse bioassay, while this assay is sensitive, it is slow, expensive, has limited high throughput and requires sacrificing the animals. In toxico-infectious botulism, the organism can be cultured from tissues [1-17-54-62-100]. On egg yolk medium, toxin-producing colonies usually display surface iridescence that extends beyond the colony [1-17-54-62-100]. These diagnostic tests are mentioned in literature, but are not easily available in India [1-17-54-62-95]. Lack of availability of commercial diagnostic kits such as ELISA, lack of anaerobic culture facility in most of the hospital and private set-ups as well as technical and ethical difficulties of doing neutralization test in mice make confirmation of diagnosis of botulism very challenging [1-17-54-62-100]. To investigate suspected cases of Botulism outbreaks in India, as no ELISA kits were available, researchers resorted to mouse neutralization test and molecular tests such as PCR [1-17-54-62-100]. However, botulinum antitoxin required to do neutralization tests in mice is not easily available and a large number of mice will be required to do this test [1-17-54-62]. As these tests are not routine diagnostic tests, cost involved and formalities required to indent mice delays the results [1-17-54-62-95].

As Botulinum toxin is an important bioterrorism agent, there is an urgent need to develop in-house diagnostic asssay [1-17-54-62-95-100]. An ELISA test was developed for the detection of botulinum neurotoxin and the minimum detection limit was also estimated. Recombinant BoNT/B specific antibody was used to capture the antigen [1-17-54-62]. Results showed that the recombinant BoNT/B could be detected up to a concentration of approximately 15 ng/ml. ELISA is a potential technique which can replace the bioassay [1-17-54-62]. The developed ELISA system was highly specifi c, rapid and could be applied to the testing of a large number of specimens [1-17-54-62-95-100].

9 *CLOSTRIDIUM BOTULINUM* HAZARD IN FOOD PRODUCTS

Various physical (e.g., heating and irradiation), chemical (e.g., nitrate, nitrite, organic acid, and natural antimicrobial compounds), and biological (e.g., bacteriophage and enzymes) treatments are used to control biological hazards in food [1-17-54-62-95-100]. For example, nitrite is a chemical additive used for curing meat products against vegetative *C. botulinum* [1-17-54-62-100]. However, this practice is being questioned, because nitrite has carcinogenic effects [1-17-54-62-95]. As the spores of *C. botulinum* are very resistant to environmental conditions, biological and chemical treatments are not very effective against them and can even elicit secondary food safety concerns [1-17-54-62]. Therefore, physical treatments are the main choice to control C. botulinum hazards including bacterium, spores, and toxins [1-17-54-62-100]. In fact, *C. botulinum* spores are one of the most heat-resistant spores among pathogenic microorganisms [1-17-54-62-95-100].

Therefore, their inactivation is considered a standard for thermally processed, commercially sterile food products, and the awaited 12-log reduction is named “Botulinum cook” [1-17-54-62-95-100]. Among physical food treatments, heating is the principal and traditional method of microbial inactivation. Furthermore, the spores of *C. botulinum* are the reference point for establishing thermal treatment efficiency scales for “low-acid canned foods” [1-17-54-62-95]. These heat treatment benefits come with some undesired effects on food, though, such as changes in the physicochemical properties and organoleptic characteristics [1-17-54-62-100]. Therefore, alternative technologies have always been in demand in order to conserve these product properties while inactivating the C. botulinum hazard [1-17-54-62-95]. Ionizing radiation was tested for food safety in the 1960s, and since then, there have been many recent advances in other nonthermal technologies, such as high-pressure processing (HPP), cold plasma (CP), pulsed electric field (PEF), intense light pulses (ILP), ultraviolet (UV), ultrasound waves, etc [1-17-54-62-100]. Understanding and analyzing the specific mode of action of different technologies aids in the design and implementation of strategies for exploiting their potential cumulative or synergistic effects in order to control the C. botulinum hazard in food products [1-17-54-62-95-100].

10 THERMAL TREATMENT FOR CONTROLLING *C. BOTULINUM*

Overall, *C. botulinum* is a rare but extremely severe pathogen. Its transmission through food is proven in all its forms: vegetative, spores, and toxins [1,22,28-62-95-100]. These characteristics make it imperative to be able to control all the different forms of this hazard during food production and until consumption [1-22,28-62-100]. As with all food borne biological hazards, the control strategy can be preventive and/or curative [1-49-62]. In terms of curative strategies, physical food processing is an excellent means of control, because it is reliable, effective, predictable, and measurable [1-22,28-62-100]. It is also important to mention that the classic studies on *C. botulinum* are very old, because this microorganism is now declared a potential bioterrorism threat and there are only a few laboratories authorized to work

on this bacterium [1-22,28-62-95]. This is the reason that thermal treatments are well-studied for this microorganism. Meanwhile, most of the ionizing radiation research regarding *C. botulinum* was conducted in the 1950–60s [1-22,28-62-100]. Thermal treatment remains the main method of controlling the *C. botulinum* hazards in food. To achieve commercial sterility through 12-log reduction in low-acid canned foods, thermal treatment or 'botulinum cook' is the main method of choice. The thermal treatments, even with a short duration of application, can change the organoleptic quality of the product. Therefore, adapting non-thermal technologies or combining them with thermal treatments can preserve the nutritional and sensory properties of the products and meet the food safety objectives [1-22,28-62-95].

Irradiation is successfully used in combination with heat treatment to inactivate *C. botulinum* in different food products. The availability of the technology and customer acceptance are the main limitations in adapting irradiation for food [1-22,28-62-95-100]. C*. botulinum* spores are generally resistant to HPP. However, the combination of thermal and HPP treatments has shown promising results in effectively inactivating spores in different food products. As a non-thermal alternative, the combination of HPP and irradiation can also be used to exert synergistic effects for inactivating *C. botulinum* [1-22,28-62]. Pulsed electric fields, intense light pulses, cold plasma, and other emerging technologies are still new to the food safety industry. There is limited research on the ability of these technologies to control C. botulinum in food [1-22,28-62-95-100].

11 CONCLUSION

Clostridium botulinum is a Gram-positive, anaerobic, endospore-forming bacillus responsible for botulism, a severe neuroparalytic disease that affects humans and vertebrate animals. *Clostridium botulinum* produces Botulinum neurotoxins (BoNTs), causing a rare but potentially deadly type of food poisoning called food borne botulism. The causative agent is botulinum neurotoxin (BoNT), renowned as the most potent biological substance known to humankind. Although cases of botulism have decreased over the last decade, *C. botulinum* remains a notable pathogen of importance to public health, not least due to the potential bioterrorism risk and the extensive pharmaceutical applications of the BoNT. Currently, seven types of antigenically distinct toxins are known and characterized, produced by a rod-shaped bacterium, Clostridium botulinum. Human poisoning by botulism (presenting with severe neuromuscular paralytic disease) is usually caused by toxins A, B, E, and F type. The BoNTs are divided into antigenically distinguishable types (i.e., A, B, C1, C2, D, E, F, and G). The BoNTs A, B, E, and F are produced by strains of groups I and II and they are the principal cause of botulism in humans. The BoNT types C, D, and E cause illness in other mammals, birds, and fish. The relevance of BoNT in the medical and cosmetic fields has stimulated research interest in pathogens. Due to dangers associated with handling the highly toxic neurotoxin and its listing as a select biological agent, research into this pathogen is restricted to only a few authorized laboratories across the world. This has been the main barrier to elucidating a greater understanding of the pathogen. An established set of genetic tools to produce mutants in the bacteria is now available which can allow the development of safe, non-toxigenic strains of *C. botulinum,* which may offer the possibility for further work outside of these select facilities in the future. Numerous regulators of neurotoxin expression in the bacterium have been identified, but a full picture of the regulatory network underpinning neurotoxin production would remain useful for public safety measures and pharmaceutical synthesis.

Poisoning from contaminated food preparations is the most common cause of noniatrogenic botulism. The spores are highly resistant to heat but are easily destroyed at 80 ◦C for thirty minutes. Type A and B toxins are resistant to digestion by the enzymes of the gastrointestinal system. After their entry, BoNTs irreversibly bind to cholinergic nerve endings and block the release of acetylcholine from the synapses. In contrast, in wound botulism, the neurotoxin is instead product by the growth of C. botulium in infected tissues. The contamination by BoNT inhalation does not occur by a natural route but it is certainly the most dangerous. It can be caused by the dispersion of the botulinum toxin in the atmosphere in the form of an aerosol and therefore can be deliberately used for bioterrorist purposes (e.g., during CBRN (chemical, biological, radiological, and nuclear) unconventional events). In addition, BoNTs are currently used to treat a variety of diseases or alleviate their symptoms, such as the botulinumtoxinA for migraine attacks and for cosmetic use.

STATEMENTS ON COMPLIANCE WITH ETHICAL STANDARDS

Disclosure of conflict of interest

The authors declare no conflicts of interest regarding this literature review paper.

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