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RELEVANCE OF PRINCIPLES FOR IMPROVING LABORATORY DIAGNOSIS AND PREVENTION OF BOTULISM

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Abstract. Botulinum neurotoxin is the cause of botulism, a potentially fatal paralytic illness. A variety of anaerobic spore-forming bacteria, such as Clostridium botulinum groups I and II, Clostridium butyricum and Clostridium baratii, produce human pathogenic neurotoxins of types A, B, E, and F. The identification of botulinum neurotoxin in the patient is the basis for the standard laboratory diagnosis of botulism. The diagnosis is confirmed if toxin-producing clostridia are found in the patient or the car. The mouse lethality test provides the basis for neurotoxic detection. Although quick and sensitive in vitro tests have been created, they have not yet undergone the necessary validation on food and clinical matrices. Effective isolation and identification technologies are inadequate, and C. botulinum culture techniques are underdeveloped. Although molecular methods that target the neurotoxic genes are perfect for identifying and detecting C. botulinum, they should not be utilized in isolation as they do not detect biologically active neurotoxin. In addition to providing a quick diagnosis, laboratory botulism diagnostics should focus on improving our knowledge of the disease's epidemiology and prevention. As a result, it is important to regularly separate the organisms that produce toxins from both the patient and the vehicle. It is necessary to identify the isolates' genetic characteristics and physiological group.

Keywords. Botulism, diagnosis, treatment, food, poisoning, intoxication, poison center rehabilitation.

Introduction. Since the first cases of food-borne botulism were documented in the late 18th century, botulism has drawn attention as a threat to food producers and consumers as well as a possible cause of infant crib death, a lethal trip for intravenous drug abusers, and an inspiration for bioterrorists. Botulinum toxins, which are extremely strong neurotoxins produced during the growth of the spore-forming bacterium Clostridium botulum, are the cause of botulism.C. botulinum strains are classified into toxinotypes A through G based on the serological

characteristics of the toxins they generate. The bacterium species C. botulinum is notable for its antiquated taxonomy; the ability to manufacture neurotoxins that cause flaccid paralysis is the only trait that all strains of the species have in common. The most potent natural toxins are botulinum neurotoxins (BoNTs), which are primarily associated with Clostridia species. These BoNTs have the potential to cause "botulism," a potentially fatal neuroparalytic disease. Traditionally, an acute, afebrile, symmetric descending flaccid paralysis is the hallmark of the clinical presentation of human botulism. From a clinical perspective, this severe intoxication may be an emergency that calls for a quick diagnosis and early source identification [1-5]. Additionally, every case of botulism may potentially constitute a public health emergency if commercial product consumption is suspected. The doctor should notify the Ministry of Health or the national reference agencies as soon as they detect a case of botulism. Different types of botulism, such as foodborne, intestinal, wound, iatrogenic, and inhalation botulism, may be characterized by different routes of exposure. Some authors also report botulism from an unidentified source. Because of the toxin-induced neuromuscular paralysis, all of these distinct types share a clinical syndrome. The most common type of botulism in the EU is foodborne, which is caused by consuming preformed BoNT-complexes in food [6-11]. The effects of a particular xenobiotic, the BoNTs, are linked to the harmful mechanism in all of its manifestations. Another possible source of botulism is what Winston Churchill referred to as "perverted science," even if the prevalence of foodborne botulism attests to both the human capacity for error and the inevitability of close contacts with the omnipresent microbial world. The biological weapons programs of several nations have weaponized botulinum toxin for use on the battlefield. Purified botulinum toxin, the most lethal agent known, could be used to intentionally poison a large number of people by foodborne or aerosolized methods. Thus, the threat of the toxin being used as a bioterror agent is added to the public health professional's constant concern about the potential for a significant botulism outbreak due to an unintentional mistake in food preparation or handling. C. botulinum neurotoxin (BoNT) and C. tetani neurotoxin (TeNT) are the two rare but potentially deadly pathogens that cause botulism and tetanus, respectively [12-17]. BoNT especially targets the peripheral nervous system, where it internalizes into motor neurons' presynaptic nerve terminals to prevent the release of the neurotransmitter acetylcholine. This stops nerve signals from reaching the muscle, which causes botulism's typical bilateral flaccid paralysis. TeNT is incorporated into peripheral nerve terminals and selectively targets the central nervous system. From there, it travels to nerve cells in the brain and spinal cord by retrograde axonal transport. Clinical observations and laboratory confirmation, such as the identification of toxin in bodily fluids (often serum or material from infected wounds) and/or the isolation of C. botulinum or C. tetani from infected wounds, are used to diagnose wound botulism and tetanus. Mouse bioassays have historically been used for toxin detection as well as the isolation and identification of toxic C. botulinum or C. tetani.In order to investigate instances of food-borne and baby botulism, we previously discussed the use of sensitive and specific real-time PCR tests for BoNTA, -B, and -E gene segments. Here, we detail the creation of a real-time PCR assay for the C. tetani neurotoxic gene and its application, along with the BoNT gene assays, to material from wound infection cases among illicit IDUs [18-23].

The main purpose of the presented manuscript is to provide a brief analysis of the relevance of the principles for improving the laboratory diagnosis of botulism and its prevention based on the results of authoritative scientific works.

The past. In 1735, the first botulism epidemics were reported. Following this, six people over the age of thirteen died in Wildbad, in the German state of Baden-Württemberg, in 1793. The common consumption of a popular blood sausage (black pudding) has been recognized as the cause

of intoxication. Following this initial well-reported outbreak, the number of cases of sausage intoxication quickly rose until German doctor Kerner reported 230 instances along with the distinctive clinical presentation. Simultaneously, some Russian doctors described "fish poisoning," a condition with similar clinical features. The German physician Muller coined the term "botulism" in 1870 from the Latin word botulus, which means "sausage." Following an exceptional outbreak in Ellezelles, Belgium, in December 1895, the microbiologist Emilie Pierre Van Ermengem carried out a thorough examination that resulted in the identification of the clostridial bacterium causing the botulism in the 19th century [5-12]. Specifically, 23 out of 34 participating musicians experienced neuromuscular paralysis (mydriasis, diplopia, dysphagia, dysarthria, and progressive muscle paralysis) over the course of the following two days following a funeral dinner that primarily consisted of raw-salted ham; three of them passed away. As a result, Van Ermengem characterized botulism as an intoxication rather than an infection and determined that the toxin was produced by "Bacillus botulinus," an obligatory anaerobic bacteria that forms spores. The pathogen name was eventually modified to Clostridium botulinum in order to properly convey both its spindle shape (kloster is Greek for spindle) and its anaerobic metabolism [20-25].

Human foodborne botulism's toxic mechanism. The well-known human form of foodborne botulism is typified by a neurological toxidrome that results from the toxin's blocking of voluntary motor and autonomic cholinergic connections. In this form, food is consumed together with the produced BoNT. The onset (time) of the first clinical manifestations and the severity of the toxidrome are influenced by the quantity and type of BoNT consumed, rather than the toxic mechanism, which is quite similar. Only when the meal has an anaerobic environment, a pH more than 4.6, a low salt and sugar content, and a temperature between 4 and 45 °C can C. botulinum and other BoNT-producing clostridia develop and manufacture toxin [13-17]. Traditional local cuisine or home-canned meals are the main causes of intoxication. In order to establish or validate the diagnosis of foodborne botulism, it is crucial to gather the patient's food history for the previous seven days, if at all possible. By attaching to receptors on the presynaptic membrane, the bont-producing clostridia produce a polypeptide toxin (150,000 Daltons) that selectively affects neuromuscular junctions and cholinergic sites in the autonomic nervous system (all ganglionic synapses and post-ganglionic parasympathetic synapses). The toxin then prevents the typical calcium-associated quantal release of acetylcholine from the presynaptic nerve terminals through endocytosis and a number of intricate processes, such as the translocation of light chain (Lc) into the cytosol and the cleavage of soluble N-ethylmaleimide-sensitive factor attachment protein receptors (SNAREs), by the metalloproteinase activity of the Lc. This process is irreversible. At the presynaptic level, acetylcholine molecules are typically found in vesicles called "quanta." Acetylcholine vesicles are first produced in the neuronal soma and then transported to the terminals, where they can be recycled multiple times. Quanta are found in three distinct stores at the terminal level: the primary store, which is immediately accessible and has about 1,000 quanta and is closest to the presynaptic membrane; the secondary store, also known as the mobilization store, has 10,000 quanta that are accessible in 1-3 seconds; and the tertiary store, also known as the reserve, has over 100,000 quanta and is situated on the axon or neuronal soma. Botulinum toxin entirely changes this physiological advantage [18-24].

Differential diagnosis and diagnosis. Clinical suspicion and the choice to provide a particular antidotal medication are the primary factors in the diagnosis of botulism. In order to identify the many BoNTs implicated and the source of intoxication, the laboratory plays a critical role in confirming the clinical diagnosis, especially when it comes to regulatory agencies. The initial symptoms of foodborne botulism are not pathognomonic and can be mistaken for more

common clinical conditions like stroke, myasthenia gravis, the autoimmune acute demyelinating polyneuropathy known as Guillain-Barré syndrome (Miller-Fisher variant), Eaton-Lambert syndrome, tick paralysis, and shellfish or tetrodotoxin poisoning. This is due to the rarity of foodborne botulism. It is more challenging to suspect botulism when the gastrointestinal condition is widespread, and some instances are definitely completely undetected, particularly while the neurological syndrome is still unclear [6-13]. When doctors are dealing with a significant outbreak, the diagnosis is simpler. However, identifying an outbreak is difficult, and initial cases are frequently misdiagnosed. For instance, once an outbreak was identified, a second cluster of cases was linked to the same food vehicle. Unfortunately, cases of botulism intoxication often happen in isolation, and it is not unusual for patients to be referred to various specialists (such as an oculist, otolaryngologist, or neurologist) before being sent to the emergency room due to worsening clinical symptoms. To get laboratory confirmation of botulism, several days—up to four days—are typically required. It is widely established that early administration of botulinum antitoxin increases its effect, therefore it is crucial to emphasize that treatment must be started before to laboratory confirmation. In this approach, patients who have been poisoned will benefit greatly from a quick, accurate, and sensitive assay for identifying BoNTs, particularly when special patients like children are involved. To sum up, endopep-mass spectrometry assays, electrochemiluminescence (ECL) immunoassays, immuno-PCR, and enhanced chemiluminescence-based ELISA all show high sensitivity, have detection limits similar to the in vivo mouse lethality bioassay, and can quickly identify active BoNTs in sera or other clinical matrices [15-23].

Summary. An acute, afebrile, symmetric descending flaccid paralysis is the hallmark of botulism. Sometimes it can lead to a medical emergency, in which case it's critical to identify the sources as soon as possible. Because of these factors, every botulism case is a public health emergency that needs to be reported right once to national agencies or the ministry of health. The most well-known type, foodborne botulism, is the most prevalent in Europe and is caused by ingesting preformed toxin, primarily from inadequately preserved home-canned meat, fish, or vegetables. Despite this, the initial clinical suspicion may be complicated by the unfamiliarity of the doctors. When assessing and treating patients with foodborne botulism, conventional precautions should be taken because the illness is not communicable and patients do not need to be isolated. Neurological symptoms follow nonspecific initial clinical symptoms, such as nausea, vomiting, and xerostomia [2-9]. Even while the laboratory's job is to confirm the clinical diagnosis, identify the many BoNTs implicated, and ultimately determine the source, the diagnosis of botulism is fundamentally clinical. New motor axon twigs that sprout and re-innervate paralyzed muscle lead to recovery; this process can take months to finish. Botulism is treated with (i) gastrointestinal decontamination (if necessary), (ii) antidote (antitoxin), and (iii) respiratory assistance. Only free circulating toxins in the circulation that are not yet attached to nerve endings (at the presynaptic level) are neutralized by antitoxins. As soon as a clinical suspicion is raised, treatment might begin. The botulism antitoxin heptavalent (HBAT), which is safe and efficacious, is currently available and utilized in the EU. Many attempts have been undertaken over the past 20 years to develop novel medications, particularly to inhibit BoNTs' catalytic activity. Research on certain inhibitors that effectively block BoNTs' neuroparalytic function is now undertaken and seems promising for the future [9-19].

Views for the future. Even though the development of molecular biological tools for the detection and characterization of C. botulinum and in vitro tests to potentially replace the mouse assay for the detection of botulinum neurotoxins has advanced significantly, much more work needs to be done. All seven toxin types should preferably be detected at the same time, and the in

vitro toxin tests require additional speed and sensitivity in a single test. In addition to testing with pure toxins, the tests should be verified using a variety of complex matrices, including foods, excrement, and environmental samples. The traditional culture methods should not be disregarded, even though the molecular biological approaches for the identification and detection of C. botulinum are promising. To extract all potential toxin-producing strains from sample materials including competing bacteria, selective medium appropriate for both group I and II C. botulinum and other toxin-producing clostridia are required [3-11]. The taxonomy of the species should be reevaluated to reflect the genuine genetic variation among the botulinum neurotoxin-producing strains, since the diverse physiologies of the various groups of C. botulinum appear to hamper the detection and isolation methods. A more comprehensive understanding of the epidemiology, risk factors, and prevention of botulism caused by either of the two groups of C. botulinum should be the aim of appropriate laboratory procedures, even though the primary goal of laboratory diagnostics of botulism is to achieve a rapid diagnosis and save the patient. Whenever feasible, C. botulinum should be isolated and genetically characterized from both the patient and the source. The sequencing of bacterial genomes has opened up new avenues for genetic typing and the study of the genetic processes underlying various metabolic characteristics, including the production and regulation of neurotoxins. Within a few years, DNA microarrays—which allow for genome-wide examination of microorganisms—are likely to surpass more traditional genetic typing instruments. More genomic data about closely related species and bacterial strains is required, even if the speed at which bacterial genomes are being read is accelerating [14-19]. A C. botulinum microarray has been made possible by the elucidation of the genome of a group I C. botulinum strain. However, the type A array may not be as useful for genomic analysis of group II organisms due to the differences between group I and II organisms. Thus, efforts to sequence the genome of a group II C. botulinum are required. Naturally, a comprehensive picture of the genetic diversity within the species cannot be obtained from genomic data on a single strain.In order to shed light on the genetic variety and the mechanisms driving various metabolic phenomena among botulinum neurotoxin-producing clostridia, more research funding allocated to C. botulinum genome sequencing programs is required [20-25].

Discussion. All age groups are susceptible to the potentially fatal neuroparalytic disease botulism, which is very difficult to diagnose because of its vague symptoms. The most prevalent type of botulism in the US is infant botulism, which is followed by wound and foodborne botulism. Both adult and pediatric doctors must be aware of its symptoms because the majority of patients need to be hospitalized. Severe cases of botulism usually appear soon after ingesting the toxin and worsen quickly. Botulism is still a public health concern even though it is uncommon in the US since even one case could indicate an outbreak. The normal clinical course of botulism is outlined in this paper, along with suggestions for diagnosis and treatment. In the UK in 2003 and 2004, there was an increase in wound infections caused by Clostridium botulinum and Clostridium tetani among users of illegal injectable medications (IDUs) [1,4,5,7]. In addition to previously published assays for C. botulinum neurotoxin types A, B, and E (BoNTA, -B, and -E), a real-time PCR assay was created to identify a portion of the neurotoxin gene of C. tetani (TeNT). Both bacteria thriving amid mixed microflora in enrichment cultures and in pure culture on solid medium, as well as infections among IDUs utilizing DNA taken directly from wound tissue, could be investigated using these tests, which were sensitive, specific, quick to conduct, and useful. Ten out of twenty-five suspected cases of botulism and two out of five suspected cases of tetanus among IDUs had their clinical diagnoses verified by a combination of bioassay and PCR test results. When taking into account results from various samples taken from the same patient, the PCR assays were nearly

entirely consistent with the traditional bioassays. Real-time PCR shows sensitivity and specificity comparable to traditional methods when used in place of bioassays for the isolation and identification of both C. botulinum and C. tetani. However, by replacing experimental animals and speeding up results, real-time PCR techniques significantly enhance the diagnostic procedure. A better approach to the laboratory examination of suspected tetanus and wound botulism among IDUs is suggested. The most powerful known natural toxins are botulinum neurotoxins (BoNTs), which are produced by Clostridium species [3-12]. The classic description of the toxic neurological condition is an acute symmetric descending flaccid paralysis that is (afebrile). The foodborne variant of botulism is the most well-known typical clinical condition. The same symptoms, which are brought on by toxin-induced neuromuscular paralysis, are present in all forms. Both the decision to use a particular antidotal medication and the diagnosis of botulism are primarily clinical. In order to identify the BoNTs involved and the source of intoxication, the laboratory must verify the clinical suspicion in reference to regulatory agencies. The identification of BoNTs in clinical specimens or food samples and the separation of BoNTs from feces provide the foundation for the laboratory diagnosis of foodborne botulism. Because the initial symptoms of foodborne botulism intoxication might be mistaken for more prevalent clinical diseases (such as stroke, myasthenia gravis, Guillain-Barré syndrome—Miller-Fisher variant, Eaton-Lambert syndrome, tick paralysis, and shellfish or tetrodotoxin poisoning), the condition is frequently underdiagnosed. Decontamination, antidote administration, and, if necessary, respiratory function support are all part of the treatment; there aren't many variations because of the various exposure methods [16-22].

Conclusions. A prompt diagnosis is crucial since botulism is a potentially fatal illness. Thus, it is necessary to conduct active research on assay development. All forms of botulinum toxin must be detected quickly and sensitively using in vitro assays, and these tests must be well validated using a variety of complicated matrices. To reduce the usage of the mouse bioassay in the interim, screening and monitoring should make use of molecular biological detection and identification technologies whenever feasible. To separate group I and II strains from clinical, food, and environmental sample materials, more advanced and effective instruments are required. The taxonomy of the organism should be reevaluated to reflect the actual genetic variety among the bacteria that produce botulinum neurotoxin in order to improve the development of culture techniques for group I and II (and III) C. botulinum.

A comprehensive epidemiological study of botulism outbreaks and the causative organism is required to improve our understanding of the various forms of the disease and their prevention, even if the quick laboratory diagnosis of botulism is dependent on the detection of toxin in the patient. Every attempt to separate C. botulinum from both the patient and the source should be part of the laboratory diagnosis for every case of human botulism. These isolates should be typed to determine the physiological group of C. botulinum in addition to identifying the type of toxin they generate. Regular diagnostics and epidemiological research should incorporate the isolates' genetic characteristics.

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