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Commentary

Botulism : A diagnostic challenge

Botulism is a rare but serious illness caused by a bacterium called *Clostridium botulinum*, which occurs in soil. It produces a neurotropic toxin. There are three kinds of botulism *viz*., foodborne botulism, wound botulism and infant botulism. Foodborne botulism comes from eating foods contaminated with the toxin. Wounds infected with toxin-producing bacteria result in wound botulism. 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. 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. There are seven serologically distinct types of botulinum neurotoxin – types A, B, C, D, E, F, and G[1]. 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[2]. 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 G[3,4].

Botulinum neurotoxin types A (BoNT/A) is the most widely studied and best characterized of the BoNT serotypes. A survey of the scientific literature indicates that there are approximately three times as many publications about BoNT/A than the next most frequent serotype, BoNT/B[5]. In the United States from 2001 to 2007, a total of 139 cases of foodborne botulism were reported to the Centers for Disease Control and Prevention (CDC). 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. 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[6].

Botulinum toxin acts by blocking nerve function and leads to respiratory and musculoskeletal paralysis. Specifically, the toxin acts by blocking the production or release of acetylcholine at synapses and neuromuscular junctions. Death occurs due to respiratory failure. Symptoms include double vision, blurred vision, drooping eyelids, slurred speech, difficulty in swallowing, dry mouth and muscular weakness. 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. However, these clues are often not enough to allow a diagnosis. Other diseases such as Guillain-Barré syndrome, stroke, and myasthenia gravis can appear similar to botulism, and special tests may be needed to rule out these other conditions. These tests may include brain scan, cerebrospinal fluid examination, nerve conduction test (electromyography, or EMG), and an edrophonium chloride (Tensilon) test for myasthenia gravis.

A definite diagnosis can be made if botulinum toxin is identified in the food, stomach or intestinal contents, vomit or faeces. The toxin is occasionally found in the blood in acute cases. 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. The toxins can be typed

with neutralization tests in mice. In toxico-infectious botulism, the organism can be cultured from tissues. On egg yolk medium, toxin-producing colonies usually display surface iridescence that extends beyond the colony. These diagnostic tests are mentioned in literature, but are not easily available in India. 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.

To investigate suspected cases of Botulism outbreaks in India, as no ELISA kits were available, we resorted to mouse neutralization test and molecular tests such as PCR[7]. 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. As these tests are not routine diagnostic tests, cost involved and formalities required to indent mice delays the results.

Molecular tests cannot detect toxin but can detect only the BONT genes if organism can be cultured. BONT genes can also be directly amplified from food and clinical samples if organisms are still present there.

As Botulinum toxin is an important bioterrorism agent, there is an urgent need to develop in-house diagnostic asssay. The article by Jain *et al*[8] published in this issue was aimed to develop an immunodetection system for botulinum neurotoxin serotype B using synthetic gene approach. 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. 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. The developed ELISA system was highly specific, rapid and could be applied to the testing of a large number of specimens.

Earlier Scotcher *et al*[5] reported sandwitch ELISA for detection of BoNT/A toxin using specific monoclonal antibodies with good sensitivity and specificity. Various ELISA formats have been developed utilizing fluorescent or chemiluminescent substrates to improve assay sensitivity 10-100 fold but require more specialized equipment[9]. Several rapid, sensitive, and specific tests like real-time PCR, immune PCR, *etc*. have been developed for detection of *C. botulinum* cells as well as the neurotoxin genes. Our group[10] described a multiplex PCR for the simultaneous detection of botulinum neurotoxin and perfringens toxin genes. These methods however, can be made available at reference centres only. Therefore, ELISA developed by Jain *et al*[8] is an important step towards rapid diagnosis of botulinum toxin in food and clinical samples.

The number of cases of foodborne and infant botulism has changed little in recent years, but wound botulism has increased because of the use of black tar heroin, especially in California[11]. Although the botulinum toxin is destroyed by thorough cooking over the course of a few minutes, the spore itself is not killed by the temperature reached with normal sea-level-pressure boiling, leaving it free to grow and produce the toxin when conditions are right. The only known prevention measure for infant botulism is to avoid feeding honey to infants less than 12 months of age. Treatment may include antitoxins, intensive medical care or surgery of infected wounds. Therefore, timely diagnosis can be life saving.

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