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Case Report

First confirmed case of infant botulism caused by *Clostridium botulinum* type A(B) in a 10-month-old infant in Hanoi, Vietnam

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ABSTRACT

Infant botulism is a rare but sometimes life-threatening toxemia caused by ingestion of *Clostridium botulinum* spores. Although cases of infant botulism have probably occurred in Vietnam in the past, they have never been diagnosed and reported. Herein, we report the isolation of *C. botulinum* type A(B) from the stool of a 10-month-old infant during hospitalization.

Introduction

Clostridium botulinum is an obligate anerobic bacterium classified into four distinct metabolic groups (I–IV) based on phylogenetic and physiological properties, or into seven types (A–G) based on the botulinum neurotoxin (BoNT) produced (Peck, 2009). Infant botulism is a rare and underdiagnosed disease caused by BoNT-producing clostridia that can temporarily colonize the intestinal lumen of infants less than 1 year of age (Dilena et al., 2021). It occurs when spores of *Clostridium botulinum* are accidentally ingested by swallowing microscopic dust particles that carry the spores. The source of spores is usually unknown, although some risk factors have been proposed, including breastfeeding and consumption of honey (Spika et al., 1989).

In Vietnam, *C. botulinum* has been isolated from honey, infant foods (Vu, 2006), and home-canned pâté; a case of foodborne illness in adults was described recently (Hoang et al., 2022). However, infant botulism has not yet been reported in Vietnam. The disease is likely to be underdiagnosed because of its low index of suspicion or overlap of symptoms with other neurological syndromes. This study describes the first laboratory-confirmed infant botulism case in Hanoi, Vietnam. *C. botulinum* carrying *bont/A1* and silent *bont/B* [*bont/(B)*] genes was isolated.

Case Presentation

A 10-month-old female with no underlying diseases was admitted to the intensive care unit of Vietnam National Children's Hospital on April 28, 2021, after 2 days of weak crying, excessive sleep, and poor head control, as well as 1 day of decreased oral food intake and breathing difficulties. She had several additional clinical symptoms, including loss of consciousness, hypotonia, and dropped eyelids. However, she had no fever, vomiting, or facial paralysis. She presented some of the following clinical signs and symptoms, which are published by the US CDC: constipation, poor feeding, ptosis, sluggish pupils, flattened facial expression, diminished suck and gag reflexes, weak and altered cry, respiratory difficulty, and possible respiratory arrest) (CDC, 2022). Written informed consent for the use of clinical details was obtained from the patient's caregiver.

A magnetic resonance imaging scan of the head was performed, and a cerebrospinal fluid sample was examined. No abnormalities were ob-

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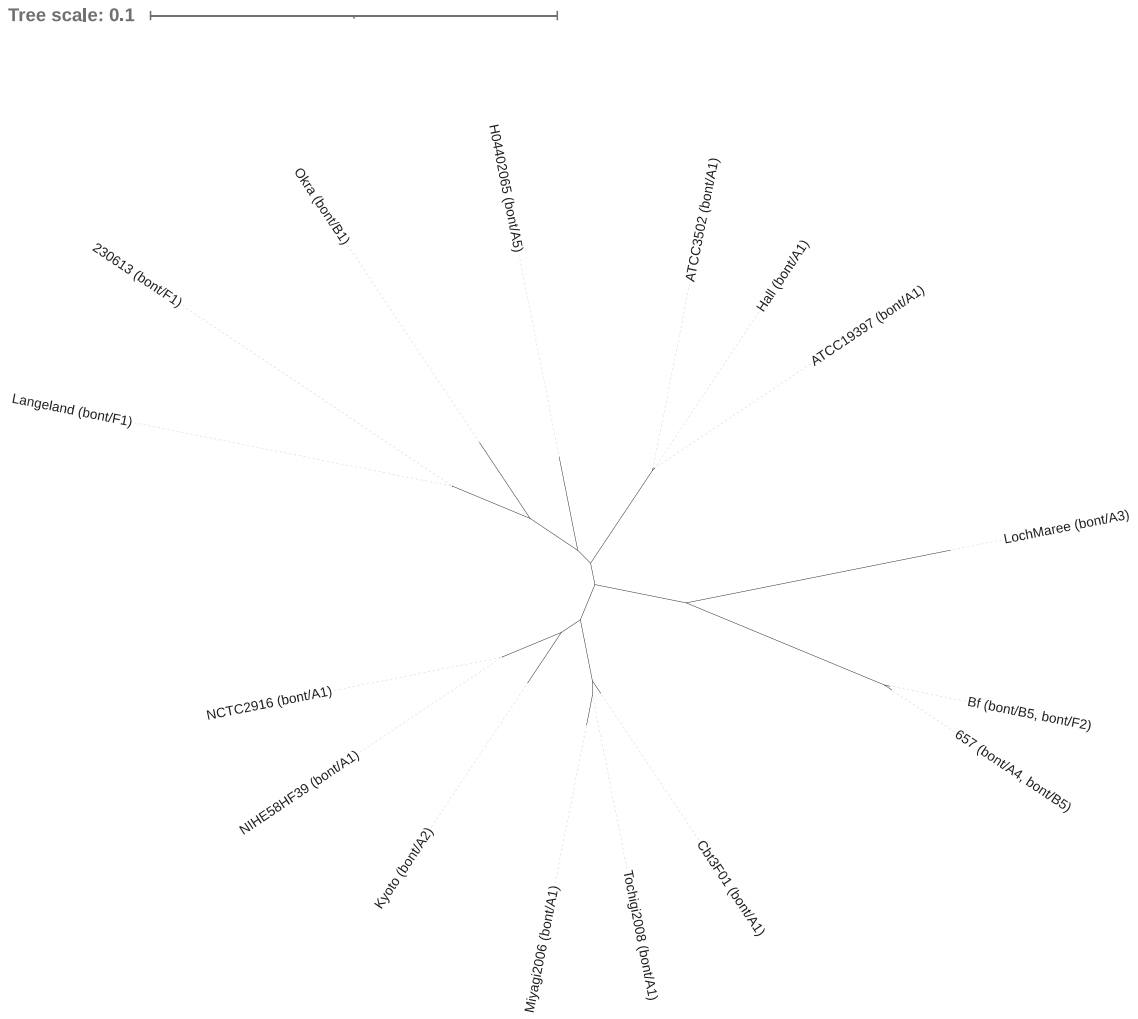

Figure 1. Phylogenetic relationships among 16 *Clostridium botulinum* strains. In total, 135 210 single-nucleotide variants were identified in 2365 core genes. Types of *bont* gene, which code active BoNT, are indicated in parentheses.

served. Electromyography showed low-voltage compound motor units consistent with axonal neuropathy. After consulting a medical doctor who had previously worked with two cases of adult botulism at the poison control center of Bach Mai Hospital, foodborne botulism was suspected. On May 3, a stool sample was collected and sent to the National Institute of Hygiene and Epidemiology (NIHE) for laboratory diagnosis. The patient was administered prednisolone at 2 mg/kg/day, mestinon at 6 mg/kg/day, and digestive enzymes for 5 days, but no improvement was observed. The patient also received supportive nutrition through a nasogastric tube.

In the Laboratory of Anerobic Bacteria, Department of Bacteriology, NIHE, the stool samples were inoculated with *C. botulinum* isolation (CBI) agar (Dezfulian et al., 1981). Colonies that grew on the CBI agar were observed for the presence of lipase-positive (Lip+), morphology, after which they were analyzed by multiplex PCR for the detection of *C. botulinum* type A, B, E, and F, following the method described by Lindström et al. (2001). On May 5, the PCR results confirmed the presence of botulinum toxin genes (*bont/A* and *bont/B*), and the isolated strain was designated as NIHE58HF39. On May 7, botulism antitoxin heptavalent (BAT) from Cho Ray Hospital, Ho Chi Minh City, was administered at 1/10 of the adult dose after consultation with the Bach Mai Hospital poison control center. The patient's symptoms still did not improve. Stool samples were collected several times until May 16. Lip+ colonies carrying *bont/A* and *bont/B* genes were still present in these stool samples. On May 18, some of the patient's clinical signs improved; specifically, she cried loudly with tears, was able to swallow food, and had improved muscle tone. The patient was discharged from the hospital on May 20. *C. botulinum* was not isolated in the two follow-up stool samples collected on June 1 and July 2.

Whole-genome sequencing of the NIHE58HF39 strain was performed as described previously (Mazuet et al., 2016). The nucleotide sequence data were submitted to the NCBI Sequence Read Archive (accession number: SRR19523447). Genome assembly was performed in SPAdes v.3.15.4 using the '–careful' option and a read coverage cutoff value of 10. Annotation was performed using the DDBJ Fast Annotation and Submission Tool (https://dfast.ddbj.nig.ac.jp/) (Tanizawa et al., 2018). To perform phylogenetic analysis, the genomes of 15 members of *C. botulinum* group I were obtained from the public database, and core gene alignments were constructed in Roary v.3.13.0 using the '-i 80' and '-e –mafft' options (Page et al., 2015). Single-nucleotide variants were extracted from the core gene alignment using SNP-sites v.2.5.1 (Page et al., 2016), after which they were used for deciphering the phylogenetic relationships by reconstructing a phylogenetic tree using IQ-TREE v.1.6.12 with 1000 ultrafast bootstrap replicates (Nguyen et al., 2015). The results indicated that the isolated strain carried *bont/A1* and *bont/(B)* genes, and was relatively close to the NCTC2916 strain (Figure 1). The nucleotide sequence of *bont/B* of this strain was found to be 100% identical to that of strain Iwate2007 (accession number: AB665556), which carries truncated botulinum neurotoxin type B.

Discussion and Conclusion

Infant botulism was first described in 1976 (Midura and Arnon, 1976). It is caused by *C. botulinum* colonizing the large intestine; the bacteria then produce neurotoxins (BoNTs) which spread through the bodies of infants under 1 year of age. BoNTs A, B, E, and rarely F cause botulism in humans, while types C and D cause botulism in animals and birds. Infant botulism is mainly caused by types A and B (Armada et al., 2003). With regard to our described case, we report that the genome of strain *C. botulinum* NIHE58HF39 contains the neurotoxin *bont/A1* and *bont/(B)* genes, that is, BoNT serotype A.

In 1997, BabyBIG (botulism immune globulin) intravenous (human) trials in California demonstrated the safety and efficacy of human-derived BIG, which reduced the mean hospital stay from 5.5 to 2.5 weeks (Payne et al., 2018). Unfortunately, BabyBIG was not available in Vietnam at the time of our case of infant botulism in Hanoi. Thus, a single dose (1/10 vial) of heptavalent botulism antitoxin was administered to the patient. Within 11 days of intravenous botulinum antitoxin administration, the child could swallow easily and muscle strength had improved significantly. The patient was discharged after 3.5 weeks of hospitalization and currently shows normal development; all symptoms have been fully resolved, with no sequelae.

C. botulinum spores are widespread in soil and dust. Therefore, objects that could have entered the mouth of the patient should be checked carefully to identify the source. In contrast to the findings of previous studies, in which honey consumption was significantly associated with type B infant botulism (Nevas et al., 2006), our patient had no history of honey consumption. Moreover, *C. botulinum* was not isolated from the food available to the patient (breast milk, porridge, yogurt, unsalted butter, cake), or the stool samples of family members. Environmental factors, such as soil or dust, within the patient's daily living area may have been related to this case of *C. botulinum* infection, but no environmental samples were obtained to confirm this. The neurotoxicity mouse bioassay, which detects BoNTs in samples with high sensitivity, was not conducted in this study due to technical problems. The introduction of this mouse bioassay helps to detect *C. botulinum* in samples, which can be useful in identifying the source.

This study reported the first confirmed case of infant botulism in Vietnam, as a consequence of infection with *C. botulinum* type A(B). Even though the risk factors for the case of infant botulism reported here remain unknown, further studies of environmental samples will allow us to better understand the epidemiology of infant botulism in Vietnam.

Author contributions

Sample analysis: TTN and NTT; genomic analysis: LHH, LTT, MM, TK, and MS; manuscript writing: NTT, LHH, MM, and MS; revision and supervision: NTT, LHH, NTT, NTHG, DTTD, PBY, BTT, TAT, and MS.

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Conflicts of interest

None.

Ethical approval

Written informed consent for the use of clinical details was obtained from the patient's caregiver.

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