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Pulsotype Diversity of *Clostridium botulinum* Strains Containing Serotypes A and/or B Genes

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Abstract

Clostridium botulinum strains are prevalent in the environment and produce a potent neurotoxin that causes botulism, a rare but serious paralytic disease. In 2010, a national PulseNet database was established to curate *C. botulinum* pulsotypes and facilitate epidemiological investigations, particularly for serotypes A and B strains frequently associated with botulism cases in the United States. Between 2010 and 2014 we performed pulsed-field gel electrophoresis (PFGE) using a PulseNet protocol, uploaded the resulting PFGE patterns into a national database, and analyzed data according to PulseNet criteria (UPGMA clustering, Dice coefficient, 1.5% position tolerance, and 1.5% optimization). A retrospective data analysis was undertaken on 349 entries comprised of type A and B strains isolated from foodborne and infant cases to determine epidemiological relevance, resolution of the method, and the diversity of the database. Most studies to date on the pulsotype diversity of *C. botulinum* have encompassed very small sets of isolates; this study, with over 300 isolates, is more comprehensive than any published to date. Epidemiologically linked isolates had indistinguishable patterns, except in four instances and there were no obvious geographic trends noted. Simpson's Index of Diversity (D) has historically been used to demonstrate species diversity and abundance within a group, and is considered a standard descriptor for PFGE databases. Simpson's Index was calculated for each restriction endonuclease (*Sma*I, *Xho*I), the pattern combination *Sma*I-*Xho*I, as well as for each toxin serotype. The D values indicate that both enzymes provided better resolution for serotype B isolates than serotype A. *Xho*I as the secondary enzyme provided little additional discrimination for *C. botulinum*. *Sma*I patterns can be used to exclude unrelated isolates during a foodborne outbreak, but pulsotypes should always be considered concurrently with available epidemiological data.

Keywords

Clostridium botulinum; PFGE; surveillance; botulism; subtyping

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Disclosure Statement

No competing financial interests exist.

Introduction

Botulism is a rare, potentially fatal illness caused by botulinum neurotoxins (BoNT) that are produced by *Clostridium botulinum* and rare strains of *Clostridium butyricum* and *Clostridium baratii*. Foodborne botulism results from the ingestion of food containing preformed BoNT and infant botulism is caused by ingestion and colonization of the intestines by *C. botulinum* spores, which germinate and produce toxin in the intestinal tract of babies younger than 1 year.

There are seven serologically distinct BoNTs (designated serotypes A through G) defined by neutralization of their effects in animal models by toxin-specific polyclonal antibodies. In the United States, 67% of foodborne botulism and 99% of infant botulism cases reported from 2001 to 2012 were due to serotypes A and B (Centers for Disease Control and Prevention, 2014). Although most strains of *C. botulinum* express a single toxin serotype, some strains have been shown to produce more than one serotype: Ab, Af, Ba, and Bf (Gimenez and Ciccarelli, 1978; Hatheway *et al.*, 1981; Gimenez, 1984; Fernandez *et al.*, 1986; Hatheway and McCroskey, 1987; Franciosa *et al.*, 1997; Raphael *et al.*, 2010, 2014). Many strains designated as type A by mouse bioassay harbor nucleotide sequences for both type A and B toxins (Franciosa *et al.*, 1994). These strains have been designated A(B) to indicate the presence of a *bont/B* gene without type B-specific enzymatic activity. Since 2010, when the *bont* gene real-time PCR assay was introduced (unpublished data), 90% of *C. botulinum* type A strains isolated during botulism laboratory investigations at the Centers for Disease Control and Prevention (CDC) were determined to be A(B) strains (Raphael *et al.*, 2014).

Pulsed-field gel electrophoresis (PFGE) has been the “gold standard” for molecular subtyping of foodborne bacteria and is frequently used to assist epidemiological investigations (Tenover *et al.*, 1995; Lehmann, 1996; Popovic *et al.*, 2001; Ribot *et al.*, 2001; Schalch *et al.*, 2003; Olsen *et al.*, 2005). CDC and the Virginia Division of Consolidated Laboratory Services collaborated to develop a standardized PFGE protocol for subtyping *C. botulinum* using *Sma*I and *Xho*I as primary and secondary restriction enzymes, respectively. The procedure was approved by PulseNet in 2010 and a national database was established. PulseNet is a network of public health laboratories that formed in 1996 to share PFGE pulsotypes and epidemiologically related information to rapidly identify foodborne outbreaks in real time (Ransom and Kaplan, 1998; Swaminathan *et al.*, 2001; Barrett *et al.*, 2006; Gerner-Smidt *et al.*, 2006). Currently, 426 *Sma*I patterns and 316 *Xho*I patterns from 426 *C. botulinum* isolates have been uploaded to the PulseNet database, representing 243 unique strains over a 41-year time span.

PFGE has been used to evaluate the genetic diversity of *C. botulinum* (Hielm *et al.*, 1998a, b; Sperner *et al.*, 1999; Stolle *et al.*, 2001; Nevas *et al.*, 2005, 2006; Leclair *et al.*, 2006, 2013; Umeda *et al.*, 2009; Reddy *et al.*, 2013; Kenri *et al.*, 2014; Marshall *et al.*, 2014). Studies have included strains isolated from a variety of sources, such as food, environmental, and clinical samples associated with botulism cases in several countries; however, our knowledge regarding the diversity of *C. botulinum* types A and B associated with botulism cases in the U.S. is very limited. In this study, we present the results of a comprehensive

review of 349 serotype A and B isolates from foodborne and infant events compiled since 2010 within the PulseNet *C. botulinum* database.

Materials and Methods

Bacterial strains

Between 2010 and 2014 *C. botulinum* serotype A and B strains were isolated from clinical and food specimens in our laboratory, pulled from the historical collection for typing, or submitted for subtyping by regional public health laboratories (Table 1). All isolates were coded according to an approved CDC human subjects protocol, fully characterized, and confirmed to be pure before beginning PFGE. For the purpose of this retrospective analysis, we excluded isolates from environmental sampling or wound botulism cases and included 349 type A, B, or bivalent isolates of foodborne or infant colonization etiology (Table 1). Of the 235 type A isolates, 80 were A(B) strains (Fig. 1). Specimens or isolates received over a span of 41 years (1973–2014) originated from several countries (United States [$n = 296$], Argentina [$n = 43$], Mexico [$n = 2$], Chile, Ecuador, Rwanda, Taiwan, and Thailand each had $n = 1$).

PFGE analysis

PFGE was performed according to a PulseNet protocol (Centers for Disease Control and Prevention, 2013; Lúquez *et al.*, 2015), which recommends typing with the primary enzyme for every isolate and typing with a secondary enzyme only if further discrimination is needed. Results were analyzed using BioNumerics 6.6 software (Applied Maths, Saint-Martens-Latern, Belgium) and patterns uploaded to the PulseNet *C. botulinum* National Database. PFGE patterns were evaluated according to PulseNet criteria as described previously (Gerner-Smidt *et al.*, 2006) (See Supplementary Data for full list of strains and pattern names. Supplementary Data are available online at http://www.liebertpub.com/fpd). Retrospective data analysis was undertaken to consider the 4-year span of data collected and to calculate the overall Simpson's Index of Diversity for foodborne and infant isolates associated with these serotypes (Simpson, 1949; Hunter and Gaston, 1988; Danoff-Burg and Xu, 2005).

Results

A total of 349 type A and B strains were analyzed by PFGE with the primary restriction enzyme *Sma*I, and 73.4% of those were also analyzed with the secondary enzyme *Xho*I (Table 1). The 183 isolates from foodborne botulism cases were divided into 61 and 41 unique *Sma*I and *Xho*I patterns, respectively; the 164 isolates from infant botulism cases were divided into 77 and 72 unique *Sma*I and *Xho*I patterns, respectively. Type A isolates were divided into 59 and 41 unique *Sma*I and *Xho*I patterns, respectively; serotype B isolates were divided into 51 and 55 unique *Sma*I and *Xho*I patterns, respectively.

Within type A isolates, Simpson's Index result was 0.9204 (*Sma*I) and 0.8682 (*Xho*I); and for type B isolates, it was 0.9781 (*Sma*I) and 0.9882 (*Xho*I). For the 73.4% of the database analyzed by two enzymes, the Simpson's Index for type A was 0.8930 and for type B was 0.9922.

Overall, the top 5 *Sma*I patterns represented 54.3% of the patterns observed among type A isolates (Fig. 2a). Combination DRPS16.0001/DRPZ11.0001 (“Pattern 1/1”) represented 22.9% of the entire database and 34.0% of all type A entries. With one exception (an isolate originating from an infant case in Ecuador), all isolates with pattern 1/1 originated from U.S. cases occurring between 1973 and 2013 representing 18 states and Washington, D.C. (Fig. 1).

The second most common *Sma*I pattern among *C. botulinum* type A isolates was DRPS16.0007 (Fig. 2a). All isolates with this pattern were from botulism cases in Argentina, except one that was isolated from an infant case in Florida. The third most common *Sma*I pattern among *C. botulinum* type A was DRPS16.0027 with 17 isolates (Fig. 2a). These isolates originated from several U.S. states (Alaska, Colorado, Kentucky, Louisiana, Michigan, New Mexico, Oklahoma). The fourth most common *Sma*I pattern within serotype A was DRPS16.0022 (Fig. 2a). All were isolated from a foodborne event in New Mexico and a separate event in Mississippi. The fifth most common *Sma*I pattern in the database among *C. botulinum* serotype A was DRPS16.0008 (Fig. 2a). These originated from Arizona, Colorado, and Oklahoma.

Among serotype B isolates, the top 5 *Sma*I patterns represented 34.3% of the *Sma*I patterns observed. The two most frequent serotype B patterns were DRKS16.0020 and DRKS16.0003 (Fig. 2b). Of the isolates from pattern DRKS16.0020, one was from Hawaii, whereas 10 were from New Jersey and West Virginia. *Sma*I pattern DRKS16.0003 was seen in a much wider geographical distribution: Alabama, Colorado, Kansas, New Jersey, and Oklahoma.

The three next most common patterns among serotype B strains each had five isolates (Fig. 2b). Pattern DRKS16.0001 was composed of isolates from Washington, DC, Kentucky, and Ohio. Pattern DRKS16.0005 represented one case from Tennessee, one case from West Virginia, two cases from Kentucky, and one from Taiwan. Pattern DRKS16.0026 represented a single foodborne event in Michigan.

The PFGE national database also contained a small number of bivalent strains: one type Ab isolate from Ohio, one type Ab isolate from Utah, two type Ab isolates from Mexico, two type Ba isolates from Colorado and Florida, and one type Bf isolate from TX. Of particular interest is the type Ab isolate from Ohio that had pattern DSSS16.0002 and was indistinguishable to an A(B) pattern DRPS16.0013 associated to a foodborne botulism case. The two type Ab isolates from Mexico are from a single event and are indistinguishable to each other (DSSS16.0003), whereas the type Ab isolate from Utah has a unique pattern, DSSS16.0001. The two type Ba isolates had differing *Xho*I patterns, DXYX11.0001 and DXYX.0002, but were indistinguishable by *Sma*I (DXYS16.0001). The type Bf isolate from Texas had pattern combination DCBS16.0001/DCBZ11.0001.

The majority of epidemiologically linked isolates were indistinguishable by PFGE; only 4 out of 177 botulism events showed within-event pulsotype variations. In two events, pulsotypes varied by one to three bands (Fig. 3). These were two separate foodborne botulism cases in which the pulsotype from a food isolate varied from an epidemiologically

linked clinical isolate by only a few bands. However, in another foodborne outbreak, the pulsotype of a clinical isolate was clearly distinguishable from the pulsotype of the food isolate and the clinical isolates from other patients (Fig. 3). The other event was an infant botulism case, where *C. botulinum* type A was isolated from a stool sample and from infant formula. These two isolates had distinguishable pulsotypes (Fig. 3). Moreover, the *bont* gene sequences of the clinical and food isolates were identified as *bont*/A1(B5) and *bont*/A2, respectively (data not shown), thus they were not epidemiologically related.

Most isolates from unrelated foodborne botulism events had unique PFGE patterns. Among serotype A, 72% of *Sma*I patterns were unique to a particular botulism event (Fig. 4a). However, 10 *Sma*I patterns were associated with multiple events. Pattern 1/1 was seen in 27 distinct food-borne botulism events. Among serotype B isolates, 92% of *Sma*I patterns were unique to unrelated botulism events (Fig. 4a), and only one pattern was shared between two distinct events.

Fewer serotype A isolates from infant botulism cases shared pulsotypes between events since 85% of *Sma*I patterns were unique to a distinct botulism case (Fig. 4b). Only four pulsotypes, including the Pattern 1/1 combination, were shared among unrelated infant botulism cases. A higher number of infant botulism cases associated with serotype B shared *Sma*I patterns as 13 pulsotypes were associated with two or more unrelated botulism cases; 80% of *Sma*I patterns corresponded to unique botulism cases (Fig. 4b).

Discussion

We completed a comprehensive review of pulsotypes among *C. botulinum* serotypes A and B, compiled since 2010 within a PulseNet *C. botulinum* PFGE database. The database contained a wide distribution of representative strains across time as well as geography, etiology, and serotype. Event can refer to a single case or an outbreak of two or more cases —every botulism case is investigated by both epidemiology and laboratory. Due to the nature of the investigations, there were more isolates from foodborne botulism cases than infant botulism cases; for each infant botulism case only one or two specimens were typically sent, whereas for each foodborne botulism investigation, multiple specimens were examined from different patients as well as different suspect foods. Additionally, foodborne isolates were initially chosen for PFGE due to the immediate need to link food and patient samples. This selection bias accounts for the differences seen in the database.

Typically a primary PFGE enzyme is chosen that yields 15–20 bands and provides good discrimination for known strains, and a secondary enzyme is chosen to provide further discrimination within the primary enzyme patterns (Allardet-Servent *et al.*, 1989). The Simpson's Index (D) calculations indicate that the primary enzyme *Sma*I and secondary enzyme *Xho*I each produced better discrimination for type B isolates than type A. It is difficult to interpret the D values for the combined patterns *Sma*I-*Xho*I because not all of the isolates in the database have data for both enzymes, creating a bias in the calculation. The large proportion (34.3%) of pattern combination DRPS16.0001/DRPX11.0001 that comprises the type A group contributed to this effect, reducing the overall diversity of the group (Raphael *et al.*, 2014). Although the PulseNet database contains pulsotypes from a

highly diverse selection of isolates collected by CDC over several decades, our analysis is limited by the available patterns in the database. These patterns may not be an accurate representation of the diversity of *C. botulinum* serotype A and B strains, as historical isolates were selected based on relevance to other studies or as part of historically significant foodborne investigations. A further limitation that may contribute to these diversity values is these data represent a subset of states that contribute to the database, creating a possible geographical bias. Not all public health laboratories with botulism testing capacity submitted isolates to CDC for PGFE subtyping.

It is difficult to compare PFGE results between protocols; differing rare cutting restriction enzymes, differing switch times for the electric field, and differing run times, all produce fundamentally different pulsotypes that cannot be compared with each other. However, previous reports have suggested extensive diversity among Group I strains from Finland (Nevas *et al.*, 2005), Group II strains from Finland (Hielm *et al.*, 1998a), serotype E isolates from Finland (Hielm *et al.*, 1998b; Hyytia *et al.*, 1999), serotype E isolates from Canada (Leclair *et al.*, 2006), and serotype B isolates from Japan (Umeda *et al.*, 2009). Few publications have described pulsotype diversity of A and B strains from the United States, and those that do are small studies (Raphael *et al.*, 2008; Luquez *et al.*, 2012; Marshall *et al.*, 2014). We are not aware of any other study, which provides a calculation of Simpson's Index of Diversity for a comprehensive database of *C. botulinum* pulsotypes. Simpson's Index of Diversity is commonly used to determine the diversity of a group, and in the context of PFGE represents the probability that two pulsotypes selected at random will belong to different patterns.

Most epidemiologically linked isolates had indistinguishable PFGE fingerprints. In two cases there was a slight variation that could be due to *in vivo* genetic changes during the course of an outbreak. In a third case, isolates resulted in very different PFGE patterns, even though they were epidemiologically linked suggesting that unique strains may be isolated during some foodborne outbreaks. However, pulsotypes can be useful for excluding geographically or temporally linked strains from an outbreak investigation, which is useful for linking foodborne patient isolates to each other or to a suspect food. Overall, no one pattern seemed linked to a particular geographical location, except *Sma*I pattern DRPS16.0007, from Argentina. This pattern represents a subset of subtype A2 strains which are rare (Luquez *et al.*, 2012). However, this geographical link could be explained by sampling bias since most strains producing the subtype A2 originated from Argentina.

Considering the top *Sma*I patterns for serotype B, it can be concluded that pulsotypes are of little use in characterizing infant cases. As these colonization events are thought to be acquired from the environment, this is not unexpected. Specific patterns are seen sporadically across a wide geographical distribution and are often shown to be unrelated by secondary enzyme *Xho*I in addition to having no linking epidemiological information. These examples reinforce the need for epidemiological context when using pulsotypes to link cases.

The seven bivalent strains analyzed here represented just 0.02% of the national PFGE database. It is interesting that a *C. botulinum* type Ab strain isolated from a botulism patient

in Ohio had a pulsotype indistinguishable from two isolates [subtype A(B)] from Florida: one of which was from a foodborne case and the other one from an infant botulism case. Additionally, these three strains were isolated over a wide temporal range (1976, 2006, and 2010). The *Sma*I/*Xho*I patterns of this *C. botulinum* type Ab isolate did not match the patterns of the other three type Ab isolates in the database, suggesting diversity rather than a single clone capable of producing both toxins. *Sma*I/*Xho*I patterns of two *C. botulinum* type Ba were indistinguishable from each other. Although *C. botulinum* type Ba also produces both toxins A and B, these two isolates had patterns distinct from the type Ab strains. A single *C. botulinum* type Bf strain was available in the database and its pattern did not match the patterns of any of the other bivalent strains. Although our study was not designed to assess genealogical lineage of these strains, it appears from the pulsotypes that each is emerging separately from the other bivalent strains as well as from its original dominant serotype.

Conclusions

As has been reported previously (Luquez *et al.*, 2012; Raphael *et al.*, 2014), PFGE may not be discriminatory enough to be useful in excluding isolates during an outbreak caused by A(B) isolates. The isolates that produced pulsotype pattern 1/1 appear to be widely distributed geographically within the United States. Several methods have been used historically to discriminate between these A(B) strains with little success. Focused microarrays, multilocus sequence typing, multiple-locus variable-number tandem repeat analysis, and amplified fragment length polymorphism all indicate that this group has a high degree of homology, and while they cluster apart from other type A strains, they are nearly indistinguishable among the A(B) group (Raphael *et al.*, 2014). Franciosa's group had some success separating a small number of A(B) strains using PFGE, but used different method than PulseNet's protocol precluding comparison here (Franciosa *et al.*, 2004). Raphael reported some success with a reference-free single-nucleotidepolymorphism-based approach for whole genome sequence analysis of this group (Raphael *et al.*, 2014). Bioinformatics analyses combined with the quickly evolving whole genome sequencing methods promise to help discriminate between outbreak- and nonoutbreak-related strains in future epidemiological investigations for *C. botulinum*. As we develop and optimize sequencing procedures, this historical PFGE data can provide guidance on prioritization of historical samples to sequence as well as guidelines for how isolates can be expected to cluster.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

Allardet-Servent A, Bouziges N, Carles-Nurit MJ, Bourg G, Gouby A, Ramuz M. Use of low-frequency-cleavage restriction endonucleases for DNA analysis in epidemiological investigations of nosocomial bacterial infections. J Clin Microbiol. 1989; 27:2057–2061. [PubMed: 2550517]

Barrett TJ, Gerner-Smidt P, Swaminathan B. Interpretation of pulsed-field gel electrophoresis patterns in foodborne disease investigations and surveillance. Foodborne Pathog Dis. 2006; 3:20–31. [PubMed: 16602976]

Centers for Disease Control and Prevention. Clostridium botulinum [Protocol]. 2013. Available at: https://www.cdc.gov/pulsenet/pathogens/pfge.html/, accessed October 10, 2014

Centers for Disease Control and Prevention. Botulism Annual Summary, 2012. US Department of Health and Human Services C; Atlanta, GA: 2014.

Danoff-Burg, J., Xu, C. Biodiversity calculator. 2005. Available at: http://www.columbia.edu/itc/cerc/danoff-burg/Biodiversity%20Calculator.xls, accessed September 1, 2014

Fernandez RA, Ciccarelli AS, Arenas GN, Gimenez DF. First outbreak of botulism caused by *Clostridium botulinum* subtype Af. Rev Argentina Microbiol. 1986; 18:29–31.

Franciosa G, Fenicia L, Pourshaban M, Aureli P. Recovery of a strain of *Clostridium botulinum* producing both neurotoxin A and neurotoxin B from canned macrobiotic food. Appl Environ Microbiol. 1997; 63:1148–1150. [PubMed: 9055430]

Franciosa G, Ferreira JL, Hatheway CL. Detection of type A, B, and E botulism neurotoxin genes in *Clostridium botulinum* and other Clostridium species by PCR: Evidence of unexpressed type B toxin genes in type A toxigenic organisms. J Clin Microbiol. 1994; 32:1911–1917. [PubMed: 7989542]

Franciosa G, Floridi F, Maugliani A, Aureli P. Differentiation of the gene clusters encoding botulinum neurotoxin type A complexes in *Clostridium botulinum* type A, Ab, and A(B) strains. Appl Environ Microbiol. 2004; 70:7192–7199. [PubMed: 15574917]

Gerner-Smidt P, Hise K, Kincaid J, et al. PulseNet USA: A five-year update. Foodborne Pathog Dis. 2006; 3:9–19. [PubMed: 16602975]

Gimenez DF. *Clostridium botulinum* subtype Ba. Zentralbl Bakteriol Mikrobiol Hyg A. 1984; 257:68–72. [PubMed: 6380157]

Gimenez DF, Ciccarelli AS. New strains of *Clostridium botulinum* subtype Af. Zentralbl Bakteriol Orig A. 1978; 240:215–220. [PubMed: 77604]

Hatheway CL, McCroskey LM. Examination of feces and serum for diagnosis of infant botulism in 336 patients. J Clin Microbiol. 1987; 25:2334–2338. [PubMed: 3323228]

Hatheway CL, McCroskey LM, Lombard GL, Dowell VR Jr. Atypical toxin variant of *Clostridium botulinum* type B associated with infant botulism. J Clin Microbiol. 1981; 14:607–611. [PubMed: 7037830]

Hielm S, Bjorkroth J, Hyytia E, Korkeala H. Genomic analysis of *Clostridium botulinum* group II by pulsed-field gel electrophoresis. Appl Environ Microbiol. 1998a; 64:703–708. [PubMed: 9464411]

Hielm S, Bjorkroth J, Hyytia E, Korkeala H. Prevalence of *Clostridium botulinum* in Finnish trout farms: Pulsed-field gel electrophoresis typing reveals extensive genetic diversity among type E isolates. Appl Environ Microbiol. 1998b; 64:4161–4167. [PubMed: 9797260]

Hunter PR, Gaston MA. Numerical index of the discriminatory ability of typing systems: An application of Simpson's index of diversity. J Clin Microbiol. 1988; 26:2465–2466. [PubMed: 3069867]

Hyytia E, Hielm S, Bjorkroth J, Korkeala H. Biodiversity of *Clostridium botulinum* type E strains isolated from fish and fishery products. Appl Environ Microbiol. 1999; 65:2057–2064. [PubMed: 10224001]

Kenri T, Sekizuka T, Yamamoto A, et al. Genetic characterization and comparison of *Clostridium botulinum* isolates from botulism cases in Japan between 2006 and 2011. Appl Environ Microbiol. 2014; 80:6954–6964. [PubMed: 25192986]

Leclair D, Fung J, Isaac-Renton JL, et al. Foodborne botulism in Canada, 1985–2005. Emerg Infect Dis. 2013; 19:961–968. [PubMed: 23735780]

Leclair D, Pagotto F, Farber JM, Cadieux B, Austin JW. Comparison of DNA fingerprinting methods for use in investigation of type E botulism outbreaks in the Canadian Arctic. J Clin Microbiol. 2006; 44:1635–1644. [PubMed: 16672387]

Lehmann DM. Reengineer the process first. Semin Nurse Manag. 1996; 4:8. [PubMed: 8696844]

Lúquez, C., Joseph, LA., Maslanka, SE. Molecular subtyping of *Clostridium botulinum* by pulsed field gel electrophoresis. In: Jordan, K., Dalmasso, M., editors. Methods and Protocols. Clifton, NJ: Springer; 2015.

Luquez C, Raphael BH, Joseph LA, Meno SR, Fernandez RA, Maslanka SE. Genetic diversity among *Clostridium botulinum* strains harboring bont/A2 and bont/A3 genes. Appl Environ Microbiol. 2012; 78:8712–8718. [PubMed: 23042179]

Marshall KM, Nowaczyk L II, Raphael BH, Skinner GE, Rukma Reddy N. Identification and genetic characterization of *Clostridium botulinum* serotype A strains from commercially pasteurized carrot juice. Food Microbiol. 2014; 44:149–155. [PubMed: 25084657]

Nevas M, Lindstrom M, Hielm S, Bjorkroth KJ, Peck MW, Korkeala H. Diversity of proteolytic *Clostridium botulinum* strains, determined by a pulsed-field gel electrophoresis approach. Appl Environ Microbiol. 2005; 71:1311–1317. [PubMed: 15746333]

Nevas M, Lindstrom M, Horman A, Keto-Timonen R, Korkeala H. Contamination routes of *Clostridium botulinum* in the honey production environment. Environ Microbiol. 2006; 8:1085–1094. [PubMed: 16689729]

Olsen SJ, Patrick M, Hunter SB, et al. Multistate outbreak of Listeria monocytogenes infection linked to delicatessen turkey meat. Clin Infect Dis. 2005; 40:962–967. [PubMed: 15824987]

Popovic T, Schmink S, Rosenstein NA, et al. Evaluation of pulsed-field gel electrophoresis in epidemiological investigations of meningococcal disease outbreaks caused by *Neisseria meningitidis* serogroup C. J Clin Microbiol. 2001; 39:75–85. [PubMed: 11136752]

Ransom G, Kaplan B. USDA uses PulseNet for food safety. J Am Vet Med Assoc. 1998; 213:1107.

Raphael BH, Choudoir MJ, Luquez C, Fernandez R, Maslanka SE. Sequence diversity of genes encoding botulinum neurotoxin type F. Appl Environ Microbiol. 2010; 76:4805–4812. [PubMed: 20511432]

Raphael BH, Luquez C, McCroskey LM, et al. Genetic homogeneity of *Clostridium botulinum* type A1 strains with unique toxin gene clusters. Appl Environ Microbiol. 2008; 74:4390–4397. [PubMed: 18502928]

Raphael BH, Shirey TB, Luquez C, Maslanka SE. Distinguishing highly-related outbreak-associated *Clostridium botulinum* type A(B) strains. BMC Microbiol. 2014; 14:192. [PubMed: 25031122]

Reddy NR, Marshall KM, Morrissey TR, et al. Combined high pressure and thermal processing on inactivation of type A and proteolytic type B spores of *Clostridium botulinum*. J Food Prot. 2013; 76:1384–1392. [PubMed: 23905794]

Ribot EM, Fitzgerald C, Kubota K, Swaminathan B, Barrett TJ. Rapid pulsed-field gel electrophoresis protocol for subtyping of *Campylobacter jejuni*. J Clin Microbiol. 2001; 39:1889–1894. [PubMed: 11326009]

Schalch B, Bader L, Schau HP, et al. Molecular typing of *Clostridium perfringens* from a food-borne disease outbreak in a nursing home: Ribotyping versus pulsed-field gel electrophoresis. J Clin Microbiol. 2003; 41:892–895. [PubMed: 12574310]

Simpson EH. Measurement of diversity. Nature. 1949; 163:688.

Sperner B, Schalch B, Eisgruber H, Stolle A. Short protocol for pulsed field gel electrophoresis of a variety of Clostridia species. FEMS Immunol Med Microbiol. 1999; 24:287–292. [PubMed: 10397313]

Stolle A, Sperner B, Schalch B, Eisgruber H. Comparison of protocols for pulsed-field gel electrophoresis of Clostridia. Electrophoresis. 2001; 22:1585–1589. [PubMed: 11386674]

Swaminathan B, Barrett TJ, Hunter SB, Tauxe RV, Force CDCPT. PulseNet: The molecular subtyping network for foodborne bacterial disease surveillance, United States. Emerg Infect Dis. 2001; 7:382–389. [PubMed: 11384513]

Tenover FC, Arbeit RD, Goering RV, et al. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: Criteria for bacterial strain typing. J Clin Microbiol. 1995; 33:2233–2239. [PubMed: 7494007]

Umeda K, Seto Y, Kohda T, Mukamoto M, Kozaki S. Genetic characterization of *Clostridium botulinum* associated with type B infant botulism in Japan. J Clin Microbiol. 2009; 47:2720–2728. [PubMed: 19571018]

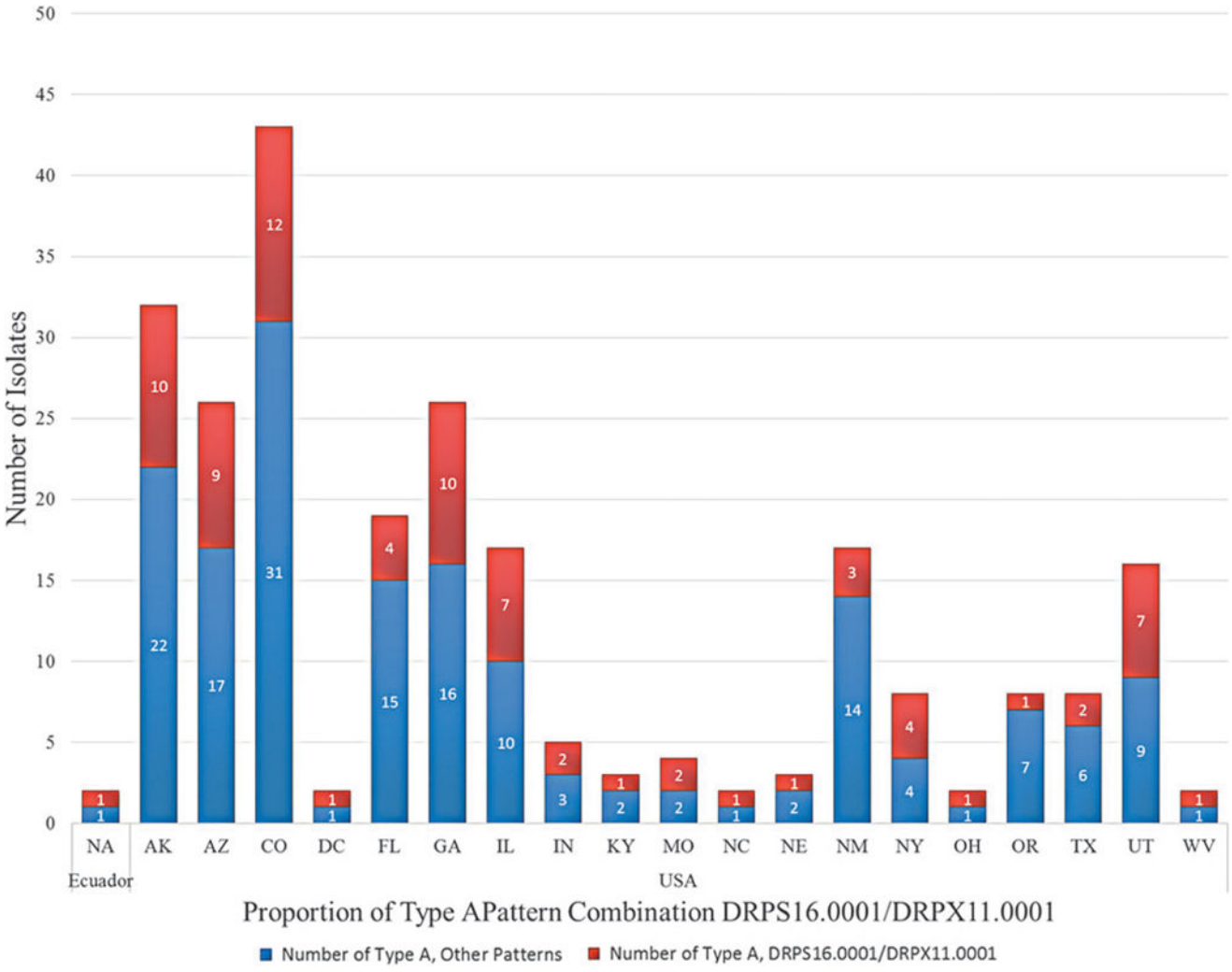

FIG. 1.

For locations with pattern combination DRPS16.0001/DRPX11.0001: total number of *Clostridium botulinum* type A cases by country and state, with proportion of pattern combination DRPS16.0001/DRPX11.0001 shown in red. All 80 of this pattern combination are A(B).

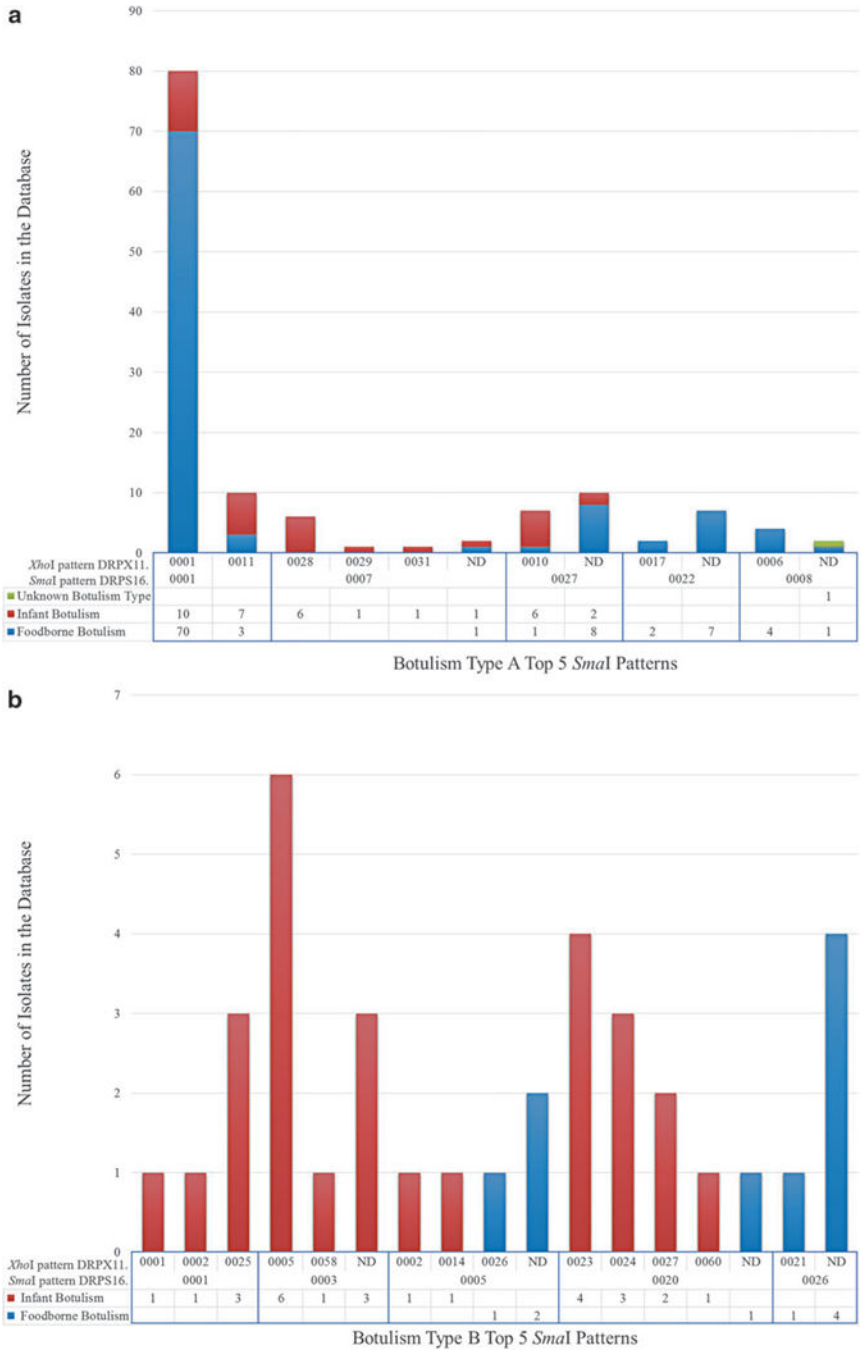

FIG. 2.

(a) Top 5 type A *Sma*I patterns by botulism type and *Xho*I pattern. **(b)** Top 5 type B *Sma*I patterns by botulism type and *Xho*I pattern.

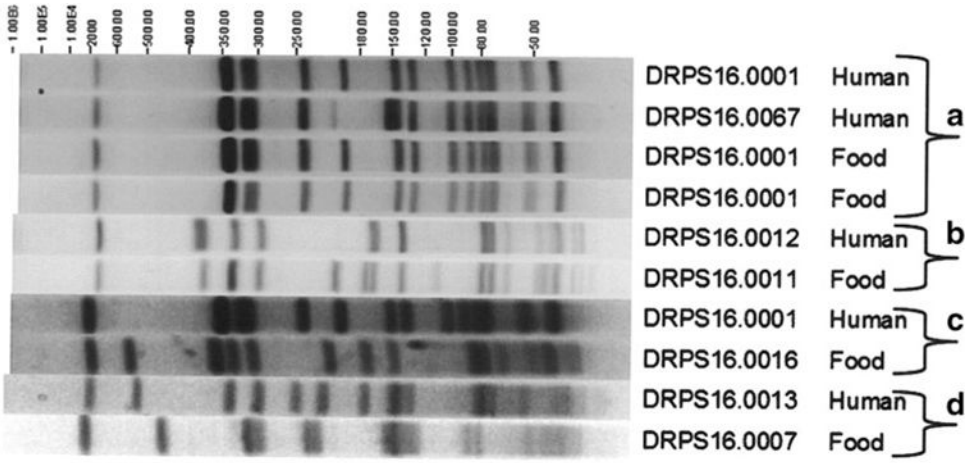

FIG. 3.

Two groups of epidemiologically linked human and food *Clostridium botulinum* isolates with *Sma*I fingerprints that vary by one to three bands **(a, b)**; one group of epidemiologically linked human and food isolates with *Sma*I patterns that vary by more than three bands **(c)**; one infant isolate and infant formula isolate from an opened container **(d)**.

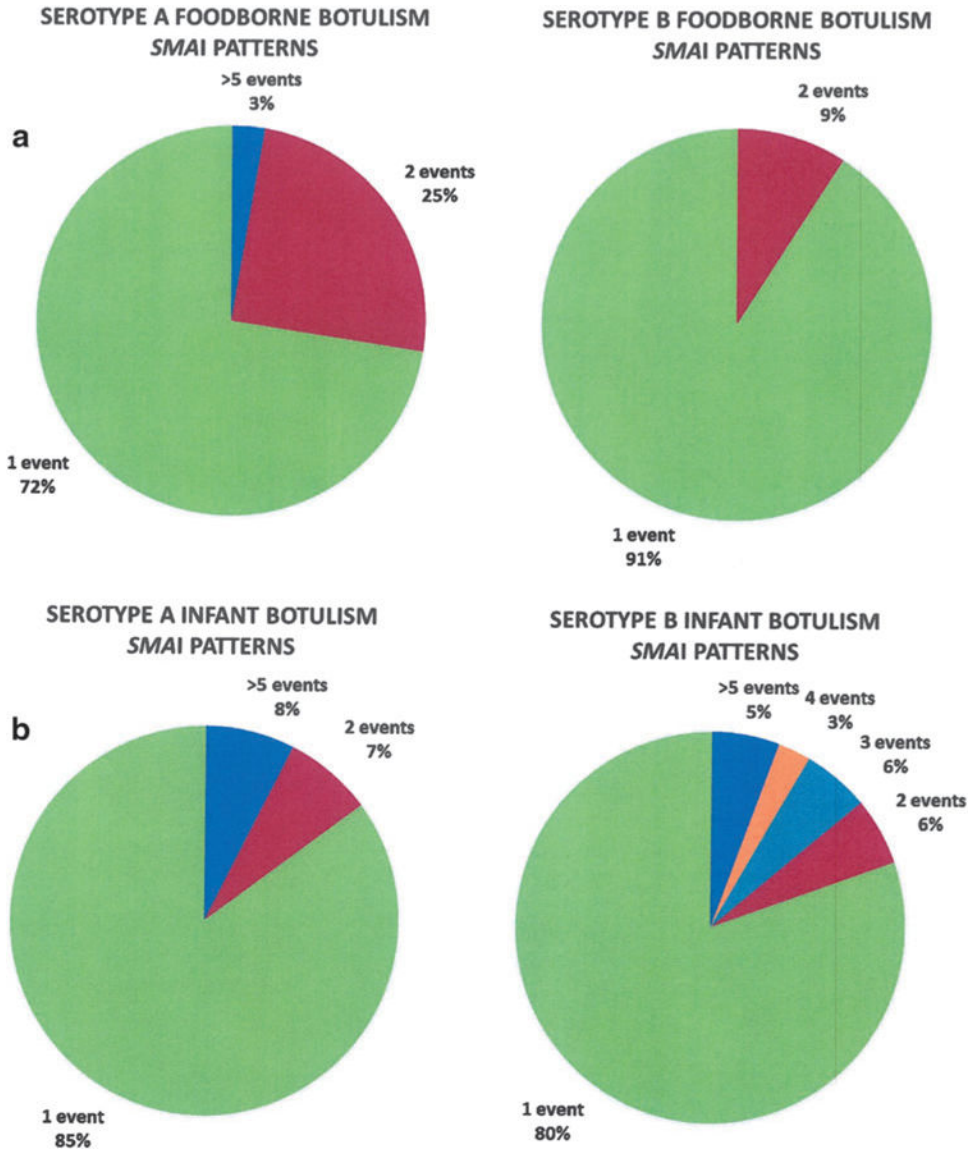

FIG. 4.

(a) Percentage of unique serotype A or B *Clostridium botulinum Sma*I patterns shared by multiple foodborne events. **(b)** Percentage of unique serotype A or B *Clostridium botulinum Sma*I patterns shared by multiple infant botulism events.

Table 1

Summary of Botulism Types Included in the *Clostridium botulinum* Pulsed-Field Gel Electrophoresis National Database: By Serotype, Restriction Enzyme Analysis, and Botulism Type

No. of strains analyzed	Restriction enzyme		Botulism type		
	***Sma*I**	***Xho*I**	**Foodborne**	**Infant**	**Unknown**
Type A	235	166	157	76	2
Type B	107	86	22	85	0
Bivalent (Ab, Ba, Bf)	7	4	4	3	0
Total	349	256	183	164	2

Of the type A strains, 80 were A(B): 68 foodborne, 11 infant, and 1 of unknown source.