A case of infant botulism with a possible link to infant formula milk powder: evidence for the presence of more than one strain of *Clostridium botulinum* in clinical specimens and food

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Infant botulism was confirmed in a 5-month-old female by both isolation of *Clostridium botulinum* type B and by detection of type B botulinum neurotoxin in rectal washout and faeces. DNA fingerprinting of nine isolates from faeces yielded two different amplified-fragment length polymorphism (AFLP) patterns. *C. botulinum* was isolated from two of 14 food and drink items from the patient's home: *C. botulinum* type A was recovered from an opened container of dried rice pudding and *C. botulinum* type B from opened infant formula milk powder. Ten *C. botulinum* type B isolates from the opened infant formula yielded four AFLP patterns, two of which were indistinguishable from the clinical isolates. Fifteen unopened foods were tested and *C. botulinum* type B of a unique AFLP pattern was recovered from one unopened infant formula of the same batch as the opened container. It is suggested that multiple *C. botulinum* were present in both food and the intestine during infant botulism.

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INTRODUCTION

Intestinal colonization botulism in infants (infant botulism) results from ingestion of spores of *Clostridium botulinum*, which germinate, colonize the intestine and produce neurotoxin *in vivo* (Arnon, 1992). The clinical effects of the neurotoxin include hypotonia and descending symmetrical paralysis. Ventilation may be required and paralysis may persist for several weeks. The majority of cases occur within the first 6 months of life (Arnon, 1992). Laboratory confirmation of a clinically diagnosed case of infant botulism requires detection of botulinum toxin in serum or faeces using the neutralization bioassay and/or the recovery of organisms that produce botulinum neurotoxin from faeces (Arnon, 1992; CDC 1997).

The source of *C. botulinum* is not known for the majority of cases of infant botulism; however, honey and corn syrup have been identified as sources of the organism in a small number of cases (Arnon *et al.*, 1979; Arnon, 1992; Midura, 1996). Additional reservoirs have been postulated since this bacterium is widespread in soil, dust and other foods (Arnon *et al.*, 1979; Arnon, 1992; Midura, 1996). Previous studies have not identified any association between infant botulism and breast milk or infant formula milk feeding (Midura, 1996). *C. botulinum* type A organisms and toxin were isolated from an opened jar of home-canned beef and peas that was epidemiologically linked to a case of botulism in an infant (Armada *et al.*, 2003). Although other tests were consistent with a diagnosis of botulism, it was

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Abbreviation: AFLP, amplified-fragment length polymorphism.

suggested that this case was foodborne botulism caused by ingestion of preformed toxin and not intestinal colonization (Armada *et al.*, 2003).

In the UK there were five reported cases of infant botulism between 1978 and 1994 (Turner *et al.*, 1978; Smith *et al.*, 1989; Jones *et al.*, 1990; CDSC, 1993, 1994); a source of infection was not identified in any of these cases. We report here the results of microbiological and molecular studies on the sixth reported UK case of infant botulism, which occurred in 2001.

METHODS

Microbiological methods. Clinical specimens were tested for the presence of botulinum neurotoxins using a neutralization mouse-bioassay (CDC, 1998). Monovalent neutralizing antisera were supplied by the Centers for Disease Surveillance and Control, Atlanta. Rectal washout, faeces and food samples were examined for *C. botulinum* organisms by enrichment in pre-reduced cooked meat medium with added glucose and starch (CDC, 1998). Enrichment cultures were incubated aerobically at 30 °C for 4–14 days and after primary inoculation with food or faeces were either untreated or heat shocked at 60 °C for 30 min. Cell-free culture supernatants were tested for neurotoxin using the neutralization bioassay as above (CDC, 1998). *C. botulinum* isolates were purified from toxin-positive enrichment cultures using solid media with and without antibiotics (Dezfulian *et al.*, 1981) and were incubated in an anaerobic cabinet at 30 °C (Don Whitley Scientific) in an atmosphere of 80 % N_2, 10 % H_2 and 10 % CO_2 for 1–3 days. The toxin type of pure cultures was confirmed by neutralization of cell-free culture supernatants of a cooked meat culture in the bioassay as above (CDC, 1998).

An estimate of the numbers of *C. botulinum* in 100 g of food, with a 95 % confidence interval, using data from a mixture of different sized samples was made by probable number using generalized linear modelling (Francis *et al.*, 1994).

DNA extraction and amplified-fragment length polymorphism analysis. For evaluation of the amplified-fragment length polymorphism (AFLP) analysis and for comparison with the cultures isolated from the case of infant botulism, unrelated *C. botulinum* from the HPA National Collection of Type Cultures (NCTC, London, UK) or the Food Safety Microbiology Laboratory (FSML) culture collection were cultured as above.

For AFLP analysis, a single colony of *C. botulinum* was subcultured onto well-dried Columbia blood agar and incubated for 48 h at 30 °C in an anaerobic cabinet as above. Growth was visually assessed and isolates showing a single colonial morphology were subcultured onto four Columbia blood agar plates. DNA was extracted from the resulting pure bacterial growth after 48 h at 30 °C in an anaerobic cabinet using a modification of the method of Boom (McLauchlin *et al.*, 1999). Briefly, bacterial growth was harvested into 900 µl of L6 buffer [10 M guanidinium thiocyanate in 0·1 M Tris/HCl pH 6·4, plus 35 mM EDTA pH 8, 2 % (w/v) Triton X-100] plus 60 µl of isoamyl alcohol and 0·3 g of 0·1 mm diameter zirconium beads (Stratech Scientific). Cells were disrupted by mechanical agitation in a Beadbeater-8 (Stratech Scientific) at maximum speed for 1 min. Tubes were left at room temperature for 5 min before centrifugation at 13 400 ***g*** for 15–30 s. The particulate material was discarded and 100 µl of size-fractionated silica (Severn Biotech) was then added to the supernatant, which was gently mixed for 10 min at room temperature. The suspension was centrifuged as above and the supernatant discarded. The silica was then washed by centrifugation, twice with 200 µl of L2 buffer (10 M guanidinium thiocyanate in 0·1 M Tris/HCl pH 6·4), twice with 200 µl of 80 % cold ethanol and once with 200 µl of cold acetone, after which the pellet was dried for 10 min at 56 °C. This was then resuspended in 100 µl of sterile distilled water, vortexed briefly and incubated for 5 min at 56 °C. The DNA (supernatant) sample was collected by centrifugation as above.

AFLP was performed using a modification of the method of Gibson *et al.* (1998) that has previously been used for other *Clostridium* species (McLauchlin *et al.*, 2002). Approximately 4 µg of DNA was added to 24 U of *Hin*dIII (Gibco Life Technologies), resuspended in water to a final volume of 20 µl and incubated overnight at 37 °C. Five microlitres of the digested DNA was added to 0·2 µg of the adapter oligonucleotides ADH1 and ADH2 (Gibco Life Technologies), 1 U of T4 DNA ligase and ligase buffer (Gibco Life Technologies) in a final volume of 20 µl and incubated at room temperature for 3 h. The sequences of the adapters ADH1 and ADH2 (5′–3′) were ACGGTATGCGACAG and GAGTGC CATACGCTGTCTCGA, respectively. The ligated DNA was heated to 80 °C for 10 min, fivefold diluted in sterile distilled water and 5 µl used for each PCR reaction.

PCR reactions were performed in 50 µl final volumes and contained: 5 µl of ligated DNA, 2·5 mM $MgCl_2$, 300 ng primer (either HI-A, HI-C, HI-G or HI-T; Gibco Life Technologies) and 1·25 U Taq DNA polymerase in 1× PCR buffer (Gibco Life Technologies). The mixture was subjected to an initial denaturing step of 94 °C for 4 min, followed by 35 cycles of 1 min at 94 °C, 1 min at 60 °C and 2·5 min at 72 °C. The base sequences of the primers HI-A, HI-C, HI-G and HI-T (5′–3′) were GGTATGCGACAGAGCTTA, GGTATGCGACAGAGCTTC, GGTATGCGACAGAGCTTG and GGTATGCGACAGAGCTTT, respectively. A no-template control was included in each batch of tests.

Banding patterns were resolved by running 13 µl of the amplified product in a 1·5 % agarose gel containing ethidium bromide, observed under UV transillumination and fluorescent bands were recorded with the Gel Doc 2000 gel documentation system (Bio-Rad Laboratories). Banding patterns of images of the ethidium-bromide-stained gels were analysed by visual inspection: minor bands were not considered and individual patterns were arbitrarily defined. AFLP types for *C. botulinum* type A were designated with lower-case letters and *C. botulinum* type B with numbers.

RESULTS AND DISCUSSION

Clinical presentation

A formula-fed previously healthy infant aged 5 months was admitted into a local hospital on 9 June 2001 with a history of several days of constipation and progressive descending neurological deterioration. A clinical diagnosis of infant botulism was made. Botulinum neurotoxin was not detected in a serum sample collected on 12 June 2001. The infant was constipated so a rectal washout was taken on 15 June 2001 and subsequently a stool specimen was collected on the same day. *C. botulinum* neurotoxin type B was detected and *C. botulinum* organisms were isolated from both the rectal washout and the faeces. Nine different pure isolates of *C. botulinum* type B were recovered (two from rectal washout and seven from the faecal specimen).

A few weeks after admission the patient developed mild cerebral atrophy (on MRI scan) and drug-resistant focal seizure activity. Three years on, although she has made gradual improvement, she still has significant global developmental delay, including language and cognitive elements as well as gross motor components (persistent hemiparesis).

The clinical diagnosis for this patient was confirmed by both the isolation of *C. botulinum* and the detection of botulinum neurotoxin and thus fulfils a case definition previously outlined (CDC, 1997). This is the sixth reported case of infant botulism in the UK. Infant botulism is a reportable disease in the UK and should be suspected in infants of less than 1 year old (usually less than 6 months) with symptoms that include constipation, lethargy, weak cry and bulbar palsies, and which may be followed by progressive weakness, impaired respiration and death. All six reported UK cases have been at the severe end of the spectrum of illness and were ventilated (Turner *et al.*, 1978; Smith *et al.*, 1989; Jones *et al.*, 1990; CDSC, 1993, 1994). This raises the possibility of under-recognition, as not all cases of infant botulism require ventilation (Arnon, 1992).

Analysis of foods

Fourteen food or drink samples obtained from the patient's home on 19 and 26 June 2001 were examined for *C. botulinum* organisms (Table 1). *C. botulinum* type A was isolated from an opened container of dried rice pudding with fruit: 17 separate enrichment cultures (11 heat-shocked and six unheated broths, each inoculated with 1–5 g of food) were examined from a total of 58 g of this food. Neurotoxin was detected in one of the heat-shocked enrichment cultures inoculated with 5 g of food, and five distinct *C. botulinum* type A cultures were purified. Ten unopened containers of the same brand of dried rice pudding were obtained from retail sources: *C. botulinum* was not isolated from a total of 50 g of food tested in 5 g aliquots (Table 1).

C. botulinum type B organisms were isolated from an opened container of infant formula milk powder collected from the patient's home. A total of 58 g was examined in 17 enrichment cultures (11 heat-shocked and six unheated, inoculated with 1·5–5 g food each). *C. botulinum* was isolated from one unheated and one heat-shocked enrichment culture, both of which were inoculated with 5 g of food: 16 pure isolates of *C. botulinum* were recovered, all were type B. Five unopened containers of infant formula milk powder of the same batch as that from the patient's home were obtained from the manufacturer and a total of 100 g of sample was examined. Five separate *C. botulinum* type B cultures were isolated from an enrichment culture inoculated from one of the five containers.

The numbers of *C. botulinum* spores present in 100 g of rice

Table 1. Results of microbiological analysis of food samples

Food and no. of samples tested	Total amount tested	No. of enrichment cultures per food sample	Results of enrichment culture for *C. botulinum* organisms
Opened food samples collected from patient's home			
Dried food			
Rice pudding with fruit* ($n = 1$)	58 g	17	Type A isolated from one enrichment culture
Oat porridge ($n = 1$)	58 g	17	Not isolated
Infant formula milk powder† ($n = 1$)	58 g	13	Type B isolated from two enrichment cultures
Sterilized shelf stable puréed food			
Mixed fruit dessert jar ($n = 1$)	3 g	2	Not isolated
Mixed fruit pudding jar ($n = 1$)	3 g	2	Not isolated
Mixed fruit purée jar ($n = 1$)	3 g	2	Not isolated
Honey ($n = 1$)	50 g	10	Not isolated
Unopened food samples collected from patient's home			
Dried food			
Infant formula milk powder ($n = 1$)	3 g	2	Not isolated
Sterilized shelf stable puréed food			
Concentrated mixed fruit juice ($n = 1$)	40 ml	2	Not isolated
Vegetables and chicken purée ($n = 1$)	3 g	2	Not isolated
Vegetable purée ($n = 1$)	3 g	2	Not isolated
Vegetable and chicken purée ($n = 1$)	3 g	2	Not isolated
Fruit purée ($n = 1$)	56 g	16	Not isolated
Rusks ($n = 1$)	56 g	16	Not isolated
Unopened food samples obtained from manufacturer or retailer			
Dried food			
Rice pudding with fruit* ($n = 10$)	50 g	16	Not isolated
Infant formula milk powder† ($n = 25$)	100 g	11	Type B isolated from one food sample

*Food of the same brand.

†Food of the same brand and batch.

pudding with fruit and the infant formula milk powder collected from the patient's home were estimated to be 0·17 (95 % confidence interval, 0·02–1·28) and 0·38 (95 % CI, 0·09–1·51), respectively. The number of *C. botulinum* spores present in 100 g of the unopened batch of infant formula milk was estimated to be 0·14 (95 % CI, 0·02–0·86).

Laboratory tests for *C. botulinum* organisms and toxins are specialized and since a single case of botulism can signal a national emergency, suspect samples should be sent urgently to specialist National Reference Centres; for the UK this is the Health Protection Agency Food Safety Microbiology Laboratory, London. Tests for the detection of neurotoxin rely on a mouse bioassay, which remains the most sensitive and specific method. *C. botulinum* are a very diverse group of organisms that have been defined on the basis of toxin production but which should be classified as at least four separate species (Collins & East, 1998). Furthermore, organisms that have been unequivocally identified as *Clostridium baratii* or *Clostridium butyricum* have caused cases of both infant and adult botulism (McCroskey *et al.*, 1991; Aureli *et al.*, 1986; Hatheway & McCroskey, 1987). There is no selective enrichment medium specific for *C. botulinum* and no phenotypic characteristic that can be used to identify toxigenic organisms on solid microbiological plate media. The use of the bioassay to identify enrichment broths that contain neurotoxin, and hence *C. botulinum* organisms, remains the most important component of the isolation procedure. For isolation of low numbers of organisms in foods such as the dried products tested here, relatively large samples should be examined, preferably with multiple replicates.

In this study, *C. botulinum* type A was isolated from an opened container of dried rice pudding with fruit, but the bacterium was not isolated from 10 unopened containers from the same manufacturer. Type A neurotoxin was not detected in any of the clinical specimens and all of the nine clinical isolates of *C. botulinum* were type B, so there is no evidence linking the dried rice pudding with fruit to the case. *C. botulinum* type B was isolated from an opened container of infant formula milk powder from the patient's home and an unopened container of the same batch obtained prior to distribution and retail sale. The infant formula powder consumed by the patient was made in a batch of 122 388 cans in October 1998, which was recalled by the manufacturer in August 2001, and was towards the end of its shelf life in October/November 2001. The parents of the patient did not report unusual preparation of the infant formula, which was reconstituted for each feed with cooled boiled water. After each feed, any reconstituted food was discarded.

From the estimate of the numbers of organisms in the unopened can and assuming a random distributed of *C. botulinum* in the batch, a 900 g can would on average have 13 *C. botulinum* spores and the probability that a tin had no organisms is very small ($<$ 1 in 100 000). If an infant's average daily consumption of milk powder is 130 g, then the probability of exposure to one or more *C. botulinum* spores is approximately 0·85 per day (this is calculated using the Poisson distribution and assuming that the tins tested represent a random selection of the batch), and if only this single case resulted from this level of contamination then the attack rate is 1 per 728 209 exposures to the bacterium. This low attack rate is also supported by the incidence of infant botulism in the UK, since the last reported laboratory-confirmed infant botulism case was 1994 (CDSC, 1994).

It is known that honey (Arnon *et al.*, 1979; Nevas *et al.*, 2002) and corn syrup (Midura, 1996; Lilly *et al.*, 1991) contain *C. botulinum* spores and that these foods are a potential source of the organism in cases of infant botulism. The patient discussed here had not eaten honey, and neither was the organism isolated from honey recovered from the patient's home. *C. botulinum* spores have a widespread distribution in the environment and this has also been postulated as a source of the organism in cases of infant botulism (Arnon *et al.*, 1979; Chin *et al.*, 1979; Istre *et al.*, 1986). There have been a small number of surveys on the presence of *C. botulinum* in infant food. *C. botulinum* type B was isolated from one of 40 dry cereals (triplicate tests on 25 g) examined by Hauschild *et al.* (1988), but was not isolated by Guilfoyle & Yager (1983) from 10 g portions of 87 samples of dry cereal foods, 26 samples of non-fat dry milk, 40 samples of canned formula food and 20 samples of bottled baby food. In a third survey, *C. botulinum* was not isolated from 25 g portions of 90 samples of dry cereal and 100 samples of commercial baby formula (Kautter *et al.*, 1982). It has been estimated that the contamination level of *C. botulinum* in milk is about 1 spore l^{-1} (Collins-Thompson & Wood, 1993), and Franciosa *et al.* (1999) suggest that a sample size of at least 50 g is needed to give a realistic estimate of the number of organisms present. Based on the result presented here from the unopened can of infant formula feed, we have estimated that samples of $>$200 g are necessary for a 95 % chance of detecting a single *C. botulinum*. This strongly suggests that previous surveys are likely to have underestimated the prevalence of *C. botulinum* in infant food. A wide range of components of dried infant foods do not undergo a 'botulinum kill', and if this bacterium were found to be widespread, this would present novel problems for food regulation. We are currently developing methods for the analysis of large food samples to detect small numbers of *C. botulinum* spores to further assist in the investigation of this disease.

There is no information on the presence of isolates of *C. botulinum* type B in the patient's home since environmental sampling was not performed. It is possible that contamination of the opened dried foods occurred before or during the factory processing, or in the patient's home. The possibility that the patient contaminated the foods should not be discounted although the similar levels estimated in the opened and unopened formula milk (2·6-fold difference with overlapping confidence intervals) is compatible with a common source of contamination. Alternatively, cross-contamination may have occurred in the laboratory, although we believe this final possibility is unlikely because the clinical and food enrichment cultures were inoculated 7

days apart, and the same AFLP pattern was observed from multiple subsamples from the original packages and these were purified on different days (see later text).

Characterization of isolates by AFLP

Initial experiments were performed with different PCR primers for AFLP analysis on 10 *C. botulinum* cultures (four type A and six type B). Satisfactory and potentially discriminatory banding patterns were obtained with primer HI-C. Overall a total of 54 *C. botulinum* cultures were tested (Table 2), 30 isolates from this incident of infant botulism and 24 unrelated isolates. Amongst the unrelated isolates, six were from the NCTC, including NCTC 11199, which is a type A isolate from one of the other UK cases of infant botulism (Turner *et al.*, 1978). To assess the reproducibility of the system, duplicate extracts were prepared from five different cultures, triplicate extracts from one culture and 42 extracts were tested at least twice. Indistinguishable banding patterns were obtained in all instances when testing DNA from the same culture. Overall, 20 distinct AFLP patterns were distinguished (Table 2) and each pattern was specific for types A or B.

Suitable DNA was extracted from 30 isolates associated with the 2001 case of infant botulism, all of which successfully generated AFLP profiles (Table 3). Seven different patterns were obtained, all of which were unique to this incident except for that generated by the type A isolate, which generated the same pattern as NCTC 9837, a *C. botulinum* isolated from fish in Mauritius in 1955. Five *C. botulinum* type A isolates from dried rice pudding with fruit were tested and all generated AFLP pattern e (Table 3). DNA from nine *C. botulinum* type B isolates from clinical specimens yielded two distinct AFLP patterns: both isolates from the rectal washout and six isolates from the faecal specimen were classified as pattern 1, and the remaining isolate from the faeces was pattern 3 (Table 3, Fig. 1). Thirteen *C. botulinum* type B isolates from the opened container of infant formula powder gave four distinct AFLP patterns: pattern 1 (one isolate), pattern 2 (one isolate), pattern 3 (10 isolates) and pattern 4 (one

Table 2. AFLP analysis of *C. botulinum* isolates

C. botulinum type	No. of cultures tested		Total no. of AFLP types
	Isolates associated with case*	Other unrelated isolates	
A	5	11†	7
B	25	13‡	13

*2001 UK case of infant botulism.

†Including NCTC 9837, NCTC 7272 and NCTC 11199.

‡Including NCTC 751, NCTC 3807 and NCTC 3815.

Table 3. Results of AFLP analysis of *C. botulinum* associated with the case of infant botulism

Specimen or sample	*C. botulinum* toxin type	Designated AFLP pattern	No. of cultures tested
Rectal washout	B	1	2
Faeces	B	1	6
	B	3	1
Rice pudding with fruit (opened)	A	e	5
Infant formula milk powder (opened)*	B	1	1
	B	2	1
	B	3	10
	B	4	1
Infant formula milk powder (unopened)*	B	5	3

*Food of the same brand and batch.

isolate). DNA from 11 of the isolates from the opened infant formula powder generated AFLP patterns 1 and 3, which were the same two patterns obtained with isolates from the infant's faeces and rectal washout (Table 3). DNA from three isolates of *C. botulinum* type B from the unopened infant formula was tested: all were AFLP pattern 5 and were distinct from all other isolates.

Using AFLP analysis, *C. botulinum* type B isolates with two distinct AFLP patterns (1 and 3) were demonstrated in both clinical specimens and in infant formula powder consumed by the infant, which suggests a link between the food and the case. Isolates from the unopened container of infant formula powder were of an AFLP pattern distinct from clinical isolates and from isolates from the opened container of infant formula powder. AFLP analysis is a generic method for DNA fingerprinting and is ideally suited to organisms where there is limited existing typing information (McLauchlin *et al.*, 2002). Analysis of *C. botulinum* found a diversity of AFLP types generated by organisms from different sources, and the data presented confirms our previous experience (McLauchlin *et al.*, 2002), suggesting that this method is both

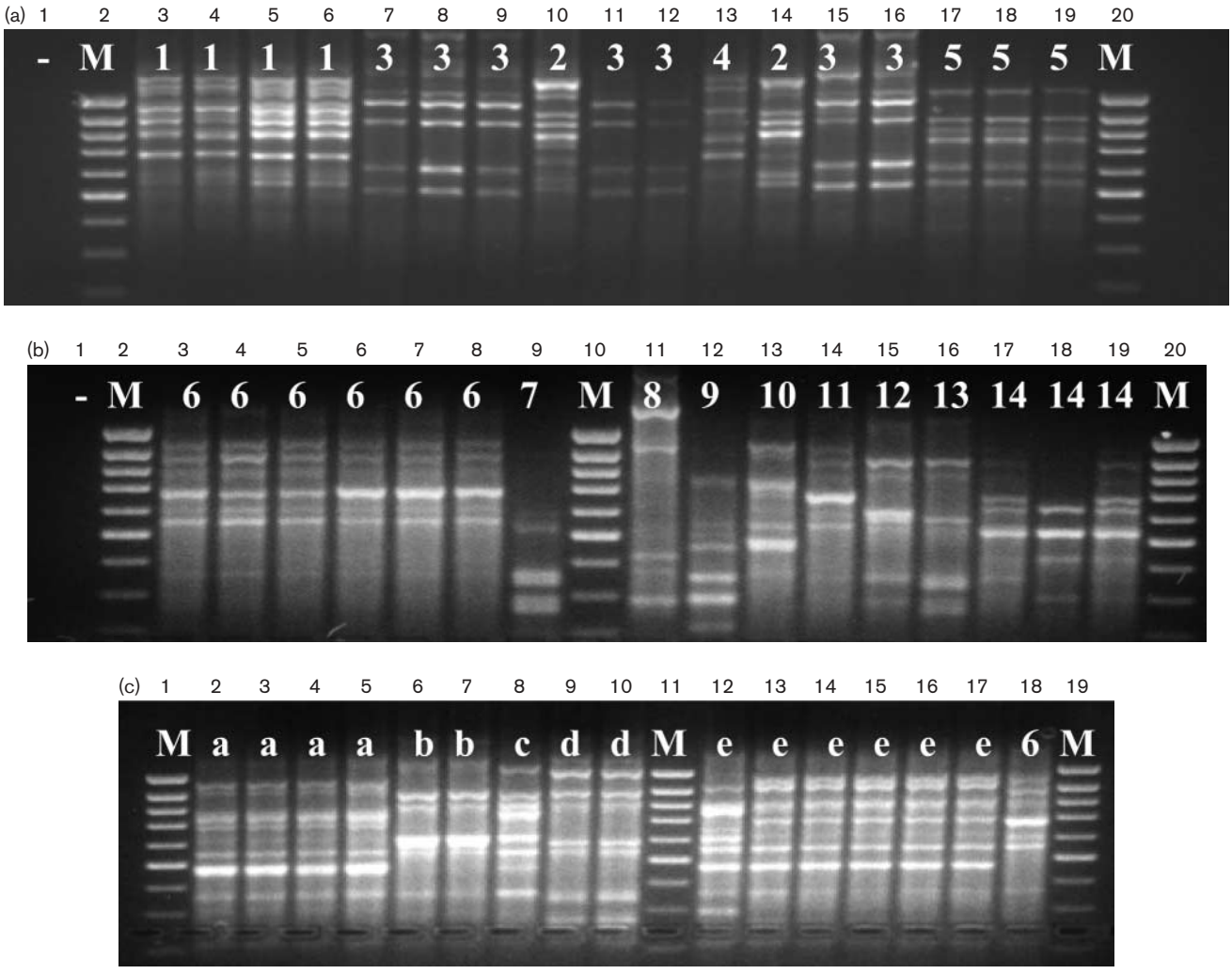

Fig. 1. Agarose gel showing AFLP patterns from *C. botulinum* cultures. AFLP patterns for *C. botulinum* type A are indicated by lowercase letters and for *C. botulinum* type B are indicated by numbers. (a) Preparations from the *C. botulinum* type B from the infant botulism case. Lane 1, no template control; lanes 2 and 20, 100 bp molecular mass markers; lanes 3–6, clinical isolates; lanes 7–19 food isolates. (b) Unrelated isolates of *C. botulinum* type B. Lane 1, no template control; lanes 2, 10 and 20, 100 bp molecular mass markers; lanes 3–8, NCTC 751, three extracts tested in duplicate; lanes 9, 11, 12, 14 and 17–19, unrelated isolates tested on a single occasion; lane 13, NCTC 3807; lanes 15 and 16, NCTC 3815 tested in duplicate. (c) Unrelated isolates of *C. botulinum* type A and B. Lanes 1, 11 and 19, 100 bp molecular mass markers; lanes 2–10 and 12–17, *C. botulinum* type A; lane 18, *C. botulinum* type B. Lanes 2–5, NCTC 751, duplicate extracts tested in duplicate; lanes 13–17 are the five *C. botulinum* type A isolates from the infant botulism case.

reproducible and discriminatory. Further studies evaluating this method for *C. botulinum* and comparing the results from both AFLP and pulsed-field gel electrophoresis are in progress.

It is known that multiple pulsed-field gel electrophoresis types of *C. botulinum* producing the same toxin type can be isolated from the environment (Hielm *et al.*, 1998) and from fish (Hyytiä *et al.*, 1999), and that foods can contain a mixed flora of anaerobic and aerobic endospore-bearing bacteria. Hence it is possible that a mixture of *C. botulinum* organisms can be present in a single food item. It is therefore possible that an individual infant can be exposed to a mixed *C. botulinum* flora through food or the environment. Amongst a series of 336 cases of infant botulism described in the USA (Hatheway & McCroskey, 1987), both type A and B neurotoxins as well as *C. botulinum* types A and B were recovered from the faeces of a single infant, hence showing that different *C. botulinum* organisms can colonize a patient's gastrointestinal tract. We produce here for the first time, we believe, evidence for the presence of multiple *C. botulinum* strains of the same toxin type in faeces from a case of infant botulism. The molecular technologies for discriminatory characterization of organisms have had limited application to this bacterium, and the analysis of large numbers of multiple isolates for comparison has probably not previously been performed as rigorously as described here.

Conclusions

In conclusion, we believe this is the first case of infant botulism in which *C. botulinum* organisms of the same toxin type have been isolated from a case of infant botulism and from a food other than honey. We also believe it is the first case of infant botulism in which more than one strain of *C. botulinum* of the same toxin type has been demonstrated in clinical specimens and a related food sample. Investigation of this case also shows the value of using molecular typing methods to clarify the relatedness of organisms and provide further evidence that multiple *C. botulinum* were present in both food and the intestine during infant botulism.

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