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Sudden Death of a Young Adult Associated with *Bacillus cereus* Food Poisoning▽

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A lethal intoxication case, which occurred in Brussels, Belgium, is described. A 20-year-old man died following the ingestion of pasta contaminated with *Bacillus cereus*. Emetic strains of *B. cereus* were isolated, and high levels of cereulide (14.8 μg/g) were found in the spaghetti meal.

CASE REPORT

On 1 October 2008, a 20-year-old man became sick after eating a meal of leftovers of spaghetti with tomato sauce, which had been prepared 5 days before and left in the kitchen at room temperature. After school, he warmed the spaghetti in the microwave oven. Immediately after eating, he left home for his sports activities, but he returned 30 min later because of headache, abdominal pain, and nausea. At his arrival, he vomited profusely for several hours and at midnight had two episodes of watery diarrhea. He did not receive any medication and drank only water. After midnight, he fell asleep. The next morning at 11:00 AM, his parents were worried because he did not get up. When they went to his room, they found him dead.

Legal examination determined the time of death, presumably at 4:00 AM, approximately 10 h after ingestion of the suspected meal. An autopsy could not be performed until 5 days later. Macroscopically, brownish and moderately softened liver and ascites (550 ml of citrine liquid) were found. The heart was macroscopically normal. A total lysis of the pancreas was also found, but it could not be excluded that this finding was due to the autopsy delay.

Microscopic findings were as follows: moderate centrolobular liver necrosis without inflammatory signs and discrete biliary stasis, significant vascular congestion of the lungs, probably due to acute cardiac insufficiency, significant necrosis from all layers of colon mucosa and submucosa alternating with better-preserved zones, and mixed intestinal flora but no evidence of invasive bacterial lesions. Significant lysis of the adrenal glands was also reported. The exact cause of death could not be determined by the autopsy because the interpretation of findings was very difficult due to the autopsy delay.

Five fecal swabs and two feces samples were taken postmortem, and samples were tested for the presence of *Bacillus cereus* by growth on mannitol egg yolk polymyxin (MYP) agar. *B. cereus* was found in only two of the five fecal swabs, and the strains isolated were named ISP321 and ISP322. No *B. cereus* was cultured from the feces samples.

Pasta and tomato sauce samples, the leftovers of the dinner, were also sent for analysis to the National Reference Laboratory for Food-borne Outbreaks (NRLFBO). For enumeration of *B. cereus* in food samples, the ISO 7932 method (16) was used. Significant *B. cereus* counts (9.5×10^7 CFU/g) were found in the pasta, while *B. cereus* was absent in the tomato sauce. The strain isolated from the pasta meal was named ISP303.

PCR assays that detect the presence of toxin genes were applied to DNA from the pasta isolate (ISP303) and the two human isolates (ISP321 and ISP322), and the results are presented in Table 1. The presence of genes encoding nonhemolytic enterotoxin (NHE) (*nheA*), phospholipase C (*plcA*), cytotoxin K (*cytK*), hemolysin II (*hlyII*), and hemolysin BL (*hblA*) was investigated (12, 13, 15). All three strains tested positive for the presence of the *nheA* and *plcA* genes. The strains were all negative for the other enterotoxin genes (*cytK*, *hlyII*, and *hblA*).

A specific cereulide-associated PCR test (7) was also used to target the *ces* genetic determinants, which are necessary for cereulide synthesis. The PCR test to detect *ces* gave positive results for all three isolates. The actual production of cereulide was also confirmed by the boar sperm assay (4; data not shown). These results suggest that all three *B. cereus* isolates, ISP303, ISP321, and ISP322, are emetic strains. The concentration of the cereulide toxin in the spaghetti was determined by liquid chromatography-tandem mass spectrometry ($LC\text{-}MS^2$) (5; L. Delbrassinne, M. Andjelkovic, A. Rajkovic, P. Dubois, E. N'Guessan, J. Mahillon, and J. Van Loco, unpublished results) to be 14.8 μg/g of pasta. The amount of cereulide determined in the spaghetti by the present study was almost 10 times higher than the cereulide concentration of 1.6 μg/g previously found in a contaminated pasta dish (18) and

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TABLE 1. Characterization of the *B. cereus* isolates[a]

Isolate	Source	*nheA*	*plcA*	*cytK*	*hlyII*	*hblA*	*ces*
ISP303	Spaghetti	+	+	−	−	−	+
ISP321	Fecal swab	+	+	−	−	−	+
ISP322	Fecal swab	+	+	−	−	−	+

[a] Toxin gene presence was assessed by PCR analysis. The "+" and "−" symbols refer to the presence and absence of the gene, respectively.

seems consequently to be within the upper range of cereulide amounts reported in foods implicated in outbreaks.

Further characterization of the three isolates was performed using repetitive sequence-based PCR (rep-PCR) and pulsed-field gel electrophoresis (PFGE) (10, 11, 25) of genomic DNA. All three strains displayed the same rep-PCR and PFGE profiles (data not shown).

B. cereus is a well-known food-poisoning organism. It can cause two types of food poisoning, described as the emetic and diarrheal syndromes (12). The emetic type is caused by a heat-stable cereulide toxin that is preformed in food (2). The symptoms are usually mild but can be severe in some cases. Four fatal cases attributed to cereulide have been reported (6, 22, 24, 26). The present results provided evidence for *B. cereus* food poisoning in a young, healthy man. Clinical data and the rapid onset of symptoms, together with microbiological and molecular study, point to *B. cereus* as the most likely cause for this fatal outcome.

Emetic strains of *B. cereus* are known to cause mitochondrial damage. Because the macroscopical methods used in autopsy could not reveal any specific cardiac symptoms, we performed further, specific microscopical staining. Immunostaining for activated caspase 3, a marker for apoptosis (1, 27), was negative, indicating the absence of mitochondrial damage. The structure of cardiac myocytes was also intact, which suggested that the sudden cardiac arrest was caused indirectly, e.g., due to acidosis.

Fulminant hepatic failure is one of the most feared complications caused by emetic *B. cereus* strains. In this case, the liver damage was mild and could not be the only explanation for the sudden death of a healthy young man. Therefore, and because of the presence of diarrhea, together with the severe colonic necrosis, PCR assays that detect the presence of enterotoxin genes were applied to the samples.

As indicated above, PCR products were found for the *nheA* and *plcA* genes but not for *cytK*, *hlyII*, and *hblA*. These observations are rather difficult to interpret, since the actual implication of putative enterotoxins in diarrhea remains a matter of debate. The absence of HBL enterotoxin in *B. cereus* strains which produce cereulide is common, whereas the emetic strains usually produce NHE (8). The pattern of toxin production for the strains isolated in the present case is presumably classical for strains implicated in emetic poisoning. Nevertheless, some emetic strains producing NHE were found to be very cytotoxic for Caco-2 cells, as previously reported (14). Furthermore, the simultaneous presence of the genetic determinants for cereulide and the genes encoding potential enterotoxins was recently demonstrated for a *B. cereus* strain (19, 20), which appeared to be particularly virulent. Could this fact have played a role in the necrosis of the colon, even though the *cytK* PCR was negative, since that this toxin is the one that has been implicated in necrotic enteritis (21)? That is an important question for which no answer can be given at the moment.

Although we cannot incriminate *B. cereus* as the direct and unique cause of death, the present case illustrates the severity of the emetic and diarrheal syndromes and the importance of adequate refrigeration of prepared food. Because the emetic toxin is preformed in food and is not inactivated by heat treatment (2, 23), it is important to prevent *B. cereus* growth and its cereulide production during storage. This toxin production is closely linked to temperature (9) and is not strictly correlated with bacterial counts, as recently demonstrated by Delbrassinne et al. (5; unpublished). The cereulide amounts produced by a *B. cereus* emetic strain inoculated at 10^6 CFU/g in cooked rice were higher at 23°C than at 30°C, whereas the opposite situation was observed for the cereulide producer counts. These results suggest that cereulide may be more actively produced at ambient temperatures. In this case, the spaghetti had been kept at room temperature for several days: this allowed *B. cereus* to grow to the previously mentioned very high concentrations and produced the high toxin concentration (14.8 μg/g) found in the pasta and which is likely responsible for the fatal outcome. According to previously published reports, toxin concentrations that induce emesis in humans, as determined by the cytotoxicity test of HEp-2 cells on the basis of several contaminated foods, extended from 0.01 to 1.28 μg cereulide/g of food (3) and from 0.005 to 32 μg cereulide/g of food (17), determined by LC-MS. Although those concentrations did not implicate lethal cases, we do not know what amounts were actually ingested. In the fatal case described by Mahler et al. (22), strains isolated from the pan residue produced NHE (detected by the BCE-VIA assay) and the emetic toxin when grown on cooked rice. This highlighted the virulence of strains capable of this simultaneous production, as reported in this study.

In conclusion, although the autopsy results were not conclusive, probably due to the delay in analysis, the large number of *B. cereus* cells and the significant cereulide concentration found in the leftovers are the most likely cause of death of the young healthy man. Further investigation of these isolates and this case may provide insights into the virulence mechanisms of emetic *B. cereus* isolates.

REFERENCES

1. **Abbate, A., et al.** 2005. Infarct-related artery occlusion, tissue markers of ischaemia, and increased apoptosis in the peri-infarct viable myocardium. Eur. Heart J. **26:**2039–2045.
2. **Agata, N., M. Ohta, M. Mori, and M. Isobe.** 1995. A novel dodecadepsipeptide, cereulide, is an emetic toxin of *Bacillus cereus*. FEMS Microbiol. Lett. **129:**17–19.
3. **Agata, N., M. Ohta, and K. Yokoyama.** 2002. Production of *Bacillus cereus* emetic toxin (cereulide) in various foods. Int. J. Food Microbiol. **73:**23–27.
4. **Andersson, M. A., R. Mikkola, J. Helin, M. C. Andersson, and M. Salkinoja-Salonen.** 1998. A novel sensitive bioassay for detection of *Bacillus cereus* emetic toxin and related depsipeptide ionophores. Appl. Environ. Microbiol. **64:**1338–1343.
5. **Delbrassinne, L., et al.** 2011. Follow-up of the Bacillus cereus emetic toxin production in penne pasta under household conditions using liquid chromatography coupled with mass spectrometry. Food Microbiol. **28:**1105–1109.
6. **Dierick, K., et al.** 2005. Fatal family outbreak of *Bacillus cereus*-associated food poisoning. J. Clin. Microbiol. **43:**4277–4279.
7. **Ehling-Schulz, M., M. Fricker, and S. Scherer.** 2004. Identification of emetic

toxin producing *Bacillus cereus* strains by a novel molecular assay. FEMS Microbiol. Lett. **232:**189–195.
8. **Ehling-Schulz, M., et al.** 2005. Emetic toxin formation of Bacillus cereus is restricted to a single evolutionary lineage of closely related strains. Microbiology **151:**183–197.
9. **Finlay, W. J. J., N. A. Logan, and A. D. Sutherland.** 2000. *Bacillus cereus* produces most emetic toxin at lower temperatures. Lett. Appl. Microbiol. **31:**385–389.
10. **Gaviria Rivera, A. M., and F. G. Priest.** 2003. Pulsed field gel electrophoresis of chromosomal DNA reveals a clonal population structure to *Bacillus thuringiensis* that relates in general to crystal protein gene content. FEMS Microbiol. Lett. **223:**61–66.
11. **Gevers, D., G. Huys, and J. Swings.** 2001. Applicability of rep-PCR fingerprinting for identification of *Lactobacillus* species. FEMS Microbiol. Lett. **205:**31–36.
12. **Granum, P. E., and T. Lund.** 1997. *Bacillus cereus* and its food poisoning toxins. FEMS Microbiol. Lett. **157:**223–228.
13. **Guinebretière, M.-H., V. Broussolle, and C. Nguyen-The.** 2002. Enterotoxigenic profiles of food-poisoning and food-borne *Bacillus cereus* strains. J. Clin. Microbiol. **40:**3053–3056.
14. **Guinebretière, M. H., et al.** 2010. Ability of Bacillus cereus group strains to cause food poisoning varies according to phylogenetic affiliation (groups I to VII) rather than species affiliation. J. Clin. Microbiol. **48:**3388–3391.
15. **Guttmann, D. M., and D. J. Ellar.** 2000. Phenotypic and genotypic comparison of 23 strains from the *Bacillus cereus* complex for a selection of known and putative *B. thuringiensis* virulence factors. FEMS Microbiol. Lett. **188:** 7–13.
16. **International Organization for Standardization.** 15 June 2004, posting date. Microbiology of food and animal feeding stuffs. Horizontal method for the enumeration of presumptive *Bacillus cereus*. Colony-count technique at 30 degrees. ISO 7932. International Organization for Standardization, Geneva, Switzerland.
17. **In't Veld, P. H., I. A. Fitz-James, M. Nuppunen, M. Kankare, and T. Johansson.** 2010. Quantification of cereulide from food poisoning outbreaks caused by *Bacillus cereus*, poster PEB1.26. Proc. FoodMicro Symp.
18. **Jääskeläinen, E., et al.** 2003. *In vitro* assay for human toxicity of cereulide, the emetic mitochondrial toxin produced by food poisoning *Bacillus cereus.* Toxicol. In Vitro **17:**737–744.
19. **Kim, J. B., et al.** 2010. Emetic toxin producing *Bacillus cereus* Korean isolates contain genes encoding diarrheal-related enterotoxins. Int. J. Food Microbiol. **144:**182–186.
20. **Kim, J. B., et al.** 2010. Comparison of enterotoxin production and phenotypic characteristics between emetic and enterotoxic *Bacillus cereus*. J. Food Prot. **73:**1219–1224.
21. **Lund, T., M. L. De Buyser, and P. E. Granum.** 2000. A new cytotoxin from *Bacillus cereus* that may cause necrotic enteritis. Mol. Microbiol. **38:**254–261.
22. **Mahler, H., et al.** 1997. Fulminant liver failure in association with the emetic toxin of *Bacillus cereus*. N. Engl. J. Med. **336:**1142–1148.
23. **Rajkovic, A., et al.** 2008. Heat resistance of *Bacillus cereus* emetic toxin, cereulide. Lett. Appl. Microbiol. **46:**536–541.
24. **Shiota, M., et al.** 2010. Rapid detoxification of cereulide in *Bacillus cereus* food poisoning. Pediatrics **125:**e951–e955.
25. **Swiecicka, I., and J. Mahillon.** 2006. Diversity of commensal *Bacillus cereus sensu lato* isolated from the common sow bug (*Porcellio scaber*, Isopoda). FEMS Microbiol. Ecol. **56:**132–140.
26. **Takabe, F., and M. Oya.** 1976. An autopsy case of food poisoning associated with *Bacillus cereus*. Forensic Sci. **7:**97–101.
27. **Zidar, N., Z. Dolenc-Stražar, J. Jeruc, and D. Štajer.** 2006. Immunohistochemical expression of activated caspase-3 in human myocardial infarction. Virchows Arch. **448:**75–79.