Use of Pulsed-Field Gel Electrophoresis To Investigate a Pseudo-Outbreak of *Bacillus cereus* in a Pediatric Unit

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*Bacillus cereus* is a well-known cause of food poisoning. It also causes rare systemic infections, usually in immunocompromised patients. Dissemination of this species in hospitals has been reported. Most of these episodes were pseudo-outbreaks and were usually secondary to equipment or environmental contamination. We report here on the use of pulsed-field gel electrophoresis (PFGE) to analyze a pseudo-outbreak of *B. cereus* in a pediatric unit. Different restriction endonucleases had been tested, and *SmaI* was found to give the best result for PFGE. Among the 26 clinical isolates of *B. cereus* and the type strain of the species, 15 distinct PFGE patterns were distinguished. PFGE after DNA macrorestriction with *SmaI* could clearly differentiate between the epidemiologically related isolates and the unrelated isolates. Because the same epidemic strain of *B. cereus* was isolated from the settle plates which were exposed near the outlet of the ventilation system, the source of this pseudo-outbreak was suspected to be the unit’s air filtration system. This is one of the first reports of the application of PFGE to the study of *B. cereus*, and this method is useful for epidemiological investigation.

*Bacillus cereus*, a gram-positive, rod-shaped, spore-forming aerobe is widely distributed in nature and is frequently considered a saprophyte or contaminant when recovered from cultures of different sources. Only in relatively recent times has the potential of *B. cereus* as a systemic pathogen come to be more widely recognized and of concern (5, 16). The most common infection caused by this species is food poisoning (1, 4). The rare nongastrointestinal infections seen are associated with wound injuries and burns, hemodialysis, immunocompromised hosts, parenteral drug abuse, blood transfusion, and spinal anesthesia (5). Dissemination of this species in maternity units (18) and intensive care units (2) has been reported previously. Most of these episodes were pseudo-outbreaks and were usually secondary to equipment or environmental contamination (6, 8, 11, 13, 17). A pseudo-outbreak is a situation in which an organism is recovered in culture at a rate that is greater than expected and that cannot be correlated clinically with the supposed infection implied by the culture results (9). Recognizing and tracking the source of such pseudo-outbreaks can be a difficult task (9, 11). This situation may result from systematic extrinsic contamination during specimen collection or processing or intrinsic contamination at the time that the culture medium is manufactured or prepared (12). For tracing the source of contamination, molecular analysis, such as pulsed-field gel electrophoresis (PFGE), has been applied successfully to investigations of pseudo-outbreaks (3, 10, 12). PFGE had been tried by Harrell et al. (7) to differentiate *Bacillus anthracis* from related species and among strains of *B. anthracis*. They found that PFGE may be useful in separating *B. anthracis* from closely related species, but more sensitive methods are needed for identification of strains of this species.

We report here on the use of PFGE as an adjunct in a clinical epidemiological investigation of a pseudo-outbreak due to *B. cereus*.

**Materials and Methods**

**Background and pseudo-outbreak investigation.** In early August 1996, the infection control committee in a 450-bed tertiary care hospital became aware that *B. cereus* strains were being isolated at an unusual frequency from the pediatric unit. Between August and October, the proportion of positive blood cultures that were identified as *B. cereus* was 50% and was higher than that identified in the previous 6 months (11%). Accordingly, an investigation was begun. Epidemiological and clinical data on culture-positive patients was gathered as soon as an increase in the number of positive specimens was noted, and the clinical isolates of *B. cereus* were stored in the laboratory. Examination of each patient’s clinical status through consultations with their attending physicians suggested contamination rather than true infection with this organism. Because the unusual frequency of isolation of *B. cereus* occurred only in the pediatric unit, it was speculated that the contamination might occur within the pediatric unit, not the microbiology laboratory. In view of the widespread dispersal of the organism, extensive environmental sampling (including patients’ skin, bedding, disinfectants, and the tops of blood culture bottles) was performed in the pediatric unit. Settle plates were exposed for 4 h in different areas of the pediatric unit, especially near the outlet of the ventilation system. They were also placed in the microbiology laboratory and randomly selected general wards in this hospital.

**Bacterial strains.** Eleven clinical isolates of *B. cereus* isolated from the blood, pus, and sputum of 11 pediatric patients during the pseudo-outbreak period which occurred between August 1996 and October 1996 were selected for this study. Representative *B. cereus* isolates collected from environmental investigations were also included. Seven isolates collected from different wards of this hospital and five isolates collected from another hospital were selected as epidemiologically unrelated controls. The type strain of the species ATCC 11778 was also included in this study for comparison. Suspected *B. cereus* isolates were subcultured onto phenol red-mannitol-egg yolk medium (Oxoid Ltd., Basingstoke, United Kingdom). Strains found to be lecithinase positive and mannitol negative after overnight incubation at 37°C were confirmed to be *B. cereus* by conventional biochemical tests (15).

**PFGE.** Genomic DNA was prepared by the technique of Harrell et al. (7), with some modifications. A 5-ml early-log-phase culture was grown in Trypticase soy broth (Difco Laboratories, Detroit, Mich.) (shaking at 37°C for 3 h). Fifteen milliliters of SE buffer (75 mM NaCl, 25 mM EDTA [pH 7.5]) was added, and the tubes were centrifuged at 3,000 × g to pellet the cells. The cell pellet was resuspended in 0.5 ml of SE buffer containing 1 mg of lysozyme (Sigma Chemical Co., St. Louis, Mo.) and 10 U of lysostaphin (Sigma). This bacterial suspension was then mixed with 0.5 ml of 2% Low-Melting agarose (Bio-Rad Laboratories, Richmond, Calif.), dispensed in a plug mold (Bio-Rad Laboratories), and allowed to solidify. For lysis, the resulting plugs were then placed in a mixture of 10 mM Tris-EDTA (pH 7.6), 100 mM EDTA, 1 mM NaCl, 0.2% sodium deoxycholate, 0.5% sodium lauryl sarcosine, 0.5 mg of lysozyme per ml, and 5 U of lysostaphin per ml. Following overnight incubation at 37°C, the plugs were transferred to a solution which contained 1% sodium lauryl sarcosine, 0.5 M EDTA (pH 9.5), and 500 μg of proteinase K per ml, and the mixture was incubated for 2 days at 56°C under gentle shaking. The plugs were washed once for 1 h at room temperature in TE buffer (10 mM Tris-EDTA [pH 7.5], 10 mM EDTA), dried, and embedded in 1% agarose gel.
In this study, we found that SmaI is the most suitable restriction endonuclease for digestion of B. cereus DNA in a PFGE typing system. Minimal cutting of B. cereus DNA by DraI, SspI, ApaI, and SpeI and overactive cutting of B. cereus DNA by XbaI were found. Macrogenome restriction of B. cereus DNA by either NotI or SfiI produced fewer than seven bands by PFGE. The discriminatory power of PFGE provided by these two enzymes thus was as poor as expected (data not shown).

Table 1 lists the epidemiological data and the PFGE patterns for the isolates of B. cereus included in this study. Colonies of B. cereus were only found on the settle plates that had been exposed to the pediatric unit. Isolates from three of these plates, which had been put near the outlet of the ventilation system in the pediatric unit, were selected for the molecular analysis. No B. cereus was isolated from settle plates that had been put in other hospital units or the microbiology laboratory. B. cereus was also isolated from the skin of most patients hospitalized in the pediatric unit. Some of the bedding was also contaminated with this microorganism, but the disinfectants and the tops of the blood culture bottles were not.

The fingerprints generated by macrorestriction with SmaI comprised approximately 15 to 25 bands of approximately 5 to 500 kb (Fig. 1 and 2). According to the interpretive criteria of Tenover et al. (14), 15 distinct PFGE patterns were distinguished among these 26 clinical isolates of B. cereus and the type strain of the species. PFGE after macrorestriction of the DNA with SmaI could clearly differentiate between the epidemiologically related isolates and the unrelated isolates. Hence, the discriminatory power of PFGE typing for B. cereus is satisfactory. Minor band differences (fewer than three bands) were noted among the epidemic strains (isolates S1, S4 to S6, and S8 to S14). However, according to the criteria of Tenover et al. (14), they were considered to be epidemiologically closely related. The PFGE patterns of each isolate on 3 different days were consistent. Hence, the reproducibility of PFGE analysis of B. cereus is good. The problem of preparation of chromosomal DNA for macrorestriction is the sporulation of B. cereus.

**RESULTS AND DISCUSSION**

In this study, we found that SmaI is the most suitable restriction endonuclease for digestion of B. cereus DNA in a PFGE typing system. Minimal cutting of B. cereus DNA by DraI, SspI, ApaI, and SpeI and overactive cutting of B. cereus DNA by XbaI were found. Macrogenome restriction of B. cereus DNA by either NotI or SfiI produced fewer than seven bands by PFGE. The discriminatory power of PFGE provided by these two enzymes thus was as poor as expected (data not shown).

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However, this can be prevented by using a fresh early-log-phase culture for DNA extraction. Harrell et al. (7) mentioned the use of ampicillin-sulbactam to lyse the cell walls of Bacillus species during extraction of chromosomal DNA. However, we found that this step is not really essential.

The predominant PFGE genotype was type A in the pediatric unit. This epidemic strain was also isolated from the three settie plates which had been exposed to the outlet of the ventilation system in the pediatric unit. It was possible that the epidemic strain of B. cereus had contaminated the unit’s air filtration system. The spores of this strain might then disseminate in the air of the unit and contaminate the skin of the patients. Youngs et al. (18) reported a similar B. cereus outbreak in a maternity unit. The primary source of the organism, although suspected to be contamination of the unit’s air filtration system, could not be positively be established (18). We tried to control the dissemination of this epidemic strain by cleaning the ventilation system of the pediatric unit. This was difficult to achieve because the system in this unit was old, and the filters did not work very well and could not be changed. However, the isolation of B. cereus decreased after November when we encouraged hand washing and the use of aseptic procedures in this pediatric unit.

In conclusion, the PFGE technique with SmalI for DNA macrorestriction was found by this study to be a highly discriminatory and reproducible method for the epidemiological investigation of B. cereus infection. To our knowledge, this is one of the first reports of the use of PFGE for typing this species. This typing method can facilitate the reliable evaluation of the clonal relationship of B. cereus isolates and the identification of the common sources of outbreaks or pseudo-outbreaks. However, since the results of this study are based on an analysis of only a small number of isolates, further studies involving much larger numbers of epidemiologically unrelated strains will be required in order to validate the results presented above.

REFERENCES