Letters to the Editor

Use of Pulsed-Field Gel Electrophoresis for a Pseudoepidemic of *Clostridium difficile* Infections in a Pediatric Oncology and Hematology Department

To the Editor:

Pediatric oncology and hematology clinicians at the Strasbourg University Hospitals were alarmed in January 2000 by a marked increase in *Clostridium difficile*–associated diarrhea among their patients. Six cases involving five children suffering from acute lymphoblastic leukemia and one suffering from neuroblastoma had been identified in 4 months, despite application of strict isolation and disinfection measures including the use of NP30Ter aldehyde spray (1.8% formaldehyde and 1.5% glutaraldehyde; Phagogene, Carros, France) for terminal disinfection of a hospital room after a case-patient was discharged. Diagnosis was based on clinical signs, isolation of *C. difficile*, testing for cytotoxin in stool samples, and the absence of any other pathogen.

In response to the possibility of an epidemic, the Strasbourg Institute of Health decided to compare isolated *C. difficile* strains by pulsed-field gel electrophoresis (PFGE) to verify the cross-transmission of the infection. No samples were taken from the children's environment at the time of the clinical signs. Seven *C. difficile* strains were isolated from the six patients; one of the children had had two cultures 1 week apart. Isolation and identification were conducted by the Strasbourg Bacteriology Institute according to methods routinely used. Stool samples were cultured on pre-reduced cycloserine–cefoxitin–fructose agar plates in an anaerobic atmosphere. Characteristic colonies were confirmed as *C. difficile* by study of indole production and sugar fermentation. The presence of *C. difficile* toxin B was tested for by its specific cytopathogenic effect on MacCoy cells and its neutralization by an anti–toxin B serum produced by the Strasbourg Bacteriology Institute for its own use. The seven strains were compared by PFGE after DNA macrorestriction by enzyme *Sma*I, using Bio-Rad PFGE kits and the GenePath electrophoretic system (Bio-Rad, Marnes-la-Coquette, France) according to the manufacturer instructions. The seven strains were typable by the conventional PFGE method. It revealed a different electrophoretic profile for each patient, with the pulsotypes of the two isolates from the same child being identical (strains 5 and 5'; Figure).

The involvement of *C. difficile* in pediatric disease is the subject of much debate, as symptom-free carriage of sometimes toxinogenic strains is frequent before the acquisition of adult colonic flora. However, several authors tend to favor the involvement of this bacterium in nosocomial diarrhea in children, particularly in pediatric oncology.[1,2] Because these patients are frequently hospitalized, are subjected to enteric decontamination protocols, and receive frequent treatments with wide-spectrum antibiotics and anti-cancer chemotherapy, they are at risk for colonization by this bacterium. According to Schuller et al.,[2] the gastrointestinal symptoms correlate with chemotherapy-induced phases of immunosuppression, particularly in children suffering from leukemia or lymphoma. Our series of cases, in which five of the six children suffered

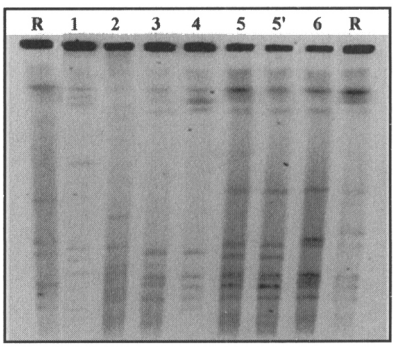

FIGURE. Pulsed-field gel electrophoresis of the *Clostridium difficile* strains (1 to 6). Strains 5 and 5' were isolated from the same patient. R is the "reference strain."

TABLE
DESCRIPTION OF THE CASES

Patient No.	Age	Date of Strain Isolation	Underlying Disease	Immune Status
1	7 y	10/19/1999	B-cell ALL (relapse)	Aplasia for 9 days
2	20 mo	11/15/1999	T-cell ALL (post-transplant)	Immunosuppressant treatment
3	14 y	11/20/1999	T-cell ALL (consolidation)	Aplasia for 14 days
4	2 y	12/9/1999	Metastatic neuroblastoma	Aplasia for 6 days
5	8 y	1/21/1999 1/27/2000	T-cell ALL (at diagnosis)	Aplasia for 10 days
6	11 y	1/31/2000	B-cell ALL (Burkitt's lymphoma)	Aplasia for 9 days

ALL = acute lymphoblastic leukemia.

from leukemia, is in concurrence with this observation (Table). However, as Schuller et al. note, malignant blood diseases and lymphoproliferative syndromes are also linked to longer hospitalizations and more frequent use of antibiotics. Although to date there have been few studies on aplasia as a risk factor for *C. difficile* infections, these are not uncommon among patients treated in oncology and hematology departments.[3] Studies concerning immunity of the host in *C. difficile* infections and their recurrences suggest that a prominent part is played by the capacity to produce an effective humoral response against toxin A.[4,5]

In our study, the marked increase in cases of diarrhea was not related to cross-infection between children because each child carried a totally different clone. The high level of genetic diversity in strains infecting patients of oncology units, both adults and children, has been reported by others.[2,6-8] This situation contrasts with that in other hospital settings, which often involve one to two predominant epidemic clones.[8] The extreme example of this is the epidemic strain known as "PCR ribotype 1," which was isolated in 58% of cases of *C. difficile* infection identified in United Kingdom hospitals, according to the United Kingdom Anaerobe Reference Unit.[9] The strict protective isolation of patients with chemotherapy-induced aplasia may play a role in preventing cross-infection with *C. difficile*. Nevertheless, oncology patients have an increased risk of coming into contact with *C. difficile* spores because they are frequently hospitalized. There is a need for longitudinal studies of the course of the infection to determine whether treatment-induced aplasia and the onset of diarrheal symptoms follow a period of asymptomatic colonization. The current study cannot support a hypothesis of endogenous origin of *C. difficile* infection, as there were no data about the children being colonized by *C. difficile* before the onset of infection. On the contrary, Shim et al. identified prior colonization as a factor protective against *C. difficile*–associated disease, although there was no mention of the immunity status of the studied populations.[10]

The absence of cross-contamination in the course of our cluster suggests the effectiveness of infection control measures in the unit. No modification in patient care was made, particularly regarding antibiotics used for enteral decontamination prior to anti-cancer chemotherapy, that could explain the increased incidence of infections during this period. Regardless of the presumed source of a case, rapid diagnosis, isolation, and sporicidal disinfection of equipment and room surfaces are necessary to limit the risk of spread. On an individual level, primary prevention of *C. difficile* infections seems difficult, as little can be done to avoid important risk factors.

The complexity of the epidemiology of nosocomial infection with *C. difficile* is the result of parameters such as the strain in question, the receptiveness of the host, and the infection control measures implemented. As we have illustrated, caution must be exercised before reaching the conclusion that an epidemic exists, particularly in oncology departments. A cluster of epidemiologically unrelated cases cannot be eliminated without the use of particularly discriminating typing techniques such as PFGE.

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Infections Due to Group B Streptococci in Neonates Are Not Associated With Higher Mortality Than Infections Due to Other Organisms

To the Editor:

Group B streptococci (*S. agalactiae*) are known to be common perinatally transmitted infectious disease agents among neonates and may cause sepsis, meningitis, or both[1] associated with substantial mortality (10% to 15%).[2] We investigated all group B streptococci infections among 246 infections in neonates hospitalized in a national referral neonatal center in Bratislava, Slovak Republic, from January 1, 1999, to January 1, 2001.

On comparison of the group of 18 neonates infected with group B streptococci with the 228 neonates infected with other organisms in univariate analysis (Epi-Info, version 2.1; Centers for Disease Control and Prevention, Atlanta, GA), the single important risk factor for group B streptococci infections was an umbilical catheter (Table). Umbilical swabs positive for group B streptococci were the only isolates associated with this type of infection. There were no other significant risk factors