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Nosocomial CDC Group IV c-2 Bacteremia: Epidemiological Investigation by Randomly Amplified Polymorphic DNA Analysis

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The CDC group IV c-2 bacterium is a gram-negative bacillus rarely isolated from clinical specimens. This organism caused catheter-related bacteremia in five immunocompromised children hospitalized in two distinct wards of our institution between November 1993 and October 1994. Three patients recovered on empiric antibacterial chemotherapy combining ceftazidime and amikacin, and a fourth patient required imipenem instead of ceftazidime. The fifth patient recovered without treatment. Catheter removal was never necessary. The randomly amplified polymorphic DNA technique with three different primers was applied to nine isolates recovered by culturing blood from the five children and showed that all of the patients harbored isolates of the same genotype. The source of the outbreak could not be determined.

CDC group IV c-2 (C IV-2) is an oxidase-positive, nonfermenting, gram-negative environmental bacillus seldomly isolated from clinical specimens. Since 1985, seven cases of human infection have been reported in the world literature, with three cases of septicemia, two cases of peritonitis, one plantar abscess complicated by septicemia, and one case of tenosynovitis (2, 6, 7, 9, 12, 13, 17). Two cases were nosocomial (6, 13). Between November 1993 and October 1994, we cultured C IV-2 from blood obtained via the catheters of five children hospitalized in two distinct wards of our institution. We carried out a retrospective epidemiological study by DNA analysis using the randomly amplified polymorphic DNA (RAPD) method (15, 16) to determine the relationship between the nine isolates (three from two patients each and one from each of the other three patients).

The five patients were 1 month to 14 years old (Table 1). All patients were immunocompromised and had central venous catheters. Three children were hospitalized in the gastroenterology-nutrition ward in November 1993, February 1994, and March 1994, respectively. All patients had severe diseases (Table 1). Two patients had hematologic malignancies (leukemia and lymphoma) and were admitted to the hematology unit in September 1994 and October 1994, respectively. Bacteremia lasted for 2 days or more in four children. One patient, with only a single positive blood culture, was not treated. Empiric antibacterial chemotherapy combining ceftazidime and amikacin was effective in three patients, while imipenem instead of ceftazidime was necessary in the last patient. Catheter removal was never necessary. All of the patients recovered.

The nine C IV-2 clinical isolates are described in Table 1. On Gram staining, the bacteria were short, gram-negative, coccobacillary rods. Biochemical identification was performed with the ID-32-GN system (bioMérieux, Marcy-l'Etoile, France) (1). Antibiotic susceptibility was determined by the disk diffusion method on Mueller-Hinton medium (Diagnostics Pasteur, Marnes-la-Coquette, France) (5). MICs were determined by the E-test (AB Biodisk, Solna, Sweden). Four reference strains (strains G6817, G3900, G608, and F4862) obtained from the Centers for Disease Control and Prevention (CDC) were tested for comparison: three were isolated from blood (two in the United States and one in Argentina) and one was isolated from a bronchial specimen (in the United States).

Bacterial DNA was studied by the randomly amplified polymorphic DNA (RAPD) procedure adapted from that described by Williams et al. (16). C IV-2 isolates were grown overnight at 37°C on sheep blood agar plates. Several colonies were suspended in 600 μl of distilled water to obtain a dense suspension. After rapid bacterial lysis by boiling and centrifugation, the pellet was discarded and the supernatant was diluted in distilled water at an optimal dilution of 1/10 for DNA amplification. We used three primers, 5′-TCACGATGCA-3′ (primer I), 5′-GCCCCCAGGGGCACAGT-3′ (primer II), and 5′-TTATGTAAAACGACGGCCAGT-3′ (primer III) (Unité de Chimie Organique, Institut Pasteur, Paris, France), for each RAPD procedure (15, 16). DNA was amplified in 50 μl of a solution containing 50 ng of DNA, 3 μM (each) primer, 2.5 U of AmpliTaq DNA polymerase (Boehringer, Meylan, France), the four deoxynucleoside triphosphates (400 μM each; Boehringer), 4 mM $MgCl_2$, 10 mM Tris-HCl (pH 8.3), and 50 mM KCl. The mixtures were overlaid with 60 μl of mineral oil and were subjected to 35 cycles of amplification (95°C for 1 min, 35°C for 1 min [primer I] or 55°C for 1 min [primers II and III], and 72°C for 1 min) in a thermocycler (Perkin-Elmer Cetus, Norwalk, Conn.). In a further cycle, the first denaturation step at 95°C lasted for 3 min and the incubation at 72°C lasted for 5 min. The products of the RAPD assay were resolved by electrophoresis on a 2% agarose gel and were visualized after staining with ethidium bromide.

Two biochemical ID-32-GN patterns were observed among the nine clinical isolates, with eight isolates exhibiting the same pattern (Table 1). All isolates were resistant to ticarcillin, aztreonam, and aminoglycosides and were susceptible to ticarcillin-clavulanate, piperacillin, cefotaxime, ceftazidime, imipenem, trimethoprim-sulfamethoxazole, and ciprofloxacin

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TABLE 1. Origins and characteristics of the nine C IV c-2 clinical isolates

Strain[a]	Ward	Age of patient	Underlying disease	Date of isolation (mo/day/yr)	Biochemical ID-32-GN pattern
A1	Gastroenterology	1 mo	Jejunal atresia	11/23/93	00730066022
B1	Gastroenterology	11 yr	Nasopharynx carcinoma	2/27/94	00730066022
C1	Gastroenterology	3 mo	Immunodeficiency	3/5/94	00730066022
D1	Hematology	11 yr	Lymphoma	9/6/94	00730066022
D2				9/28/94	00730066022
D3				9/29/94	00770067122
E1	Hematology	14 yr	Leukemia	10/10/94	00730066022
E2				10/17/94	00730066022
E3				10/17/94	00730066022

[a] In the strain designations, identical letters refer to the same patient and the numbers correspond to different isolates from the same patient; all were isolated from blood cultures obtained via catheter. All isolates had the following antibiotic susceptibility pattern, defined as susceptibility (S) or resistance (R) to ticarcillin, ticarcillin-clavulanate, piperacillin, piperacillin-tazobactam, cefotaxime, ceftazidime, imipenem, aztreonam, gentamicin, trimethoprim-sulfamethoxazole, and ciprofloxacin: RSSSSSSRRSS, respectively. All isolates had RAPD patterns α, β, and γ with primers I, II, and III, respectively.

(Table 1). The mean MICs of ceftazidime, cefotaxime, and imipenem were 2, 0.5, and 0.25 mg/liter, respectively. RAPD analysis revealed a single pattern (α, β, and γ, respectively) with each of the three primers (I, II, or III); the four reference strains each produced distinct patterns (Fig. 1).

The ecology of C IV-2 is poorly documented; 7 of the 37 isolates (19%) identified by CDC were thought to derive from water (12). The clinical rarity and apparent absence of mortality reflect its low level of pathogenicity (13). Most patients with C IV-2 infection have underlying diseases (leukemia, lymphoma, vascular disease, diabetes mellitus, or chronic renal failure) (2, 6, 7, 9, 13, 17). C IV-2 appears to be an opportunistic pathogen and can cause nosocomial infections (6, 13). Bacteremia caused by this organism is often associated with central venous catheters (2, 13). Cefotaxime, ceftriaxone, imipenem, tetracycline, and ciprofloxacin are regularly active in vitro, while the activities of cefoxitin and co-trimoxazole are variable; resistance to ampicillin and aminoglycosides is common (2, 6, 7, 9, 13, 17). Among the beta-lactams, cefotaxime and imipenem had the best in vitro activities against our isolates. One patient was empirically treated with ceftazidime, but the patient's fever continued until imipenem was added; this could be explained by the MICs (2 mg/liter for ceftazidime and 0.25 mg/liter for imipenem).

To our knowledge, no molecular epidemiological studies of C IV-2 have been reported. Among the available molecular typing methods, we chose RAPD for its simplicity and the rapidity conferred by the PCR procedure and for the good resolving power of the RAPD procedure (3, 4, 15, 16). This technique has already been used to type several different organisms (3, 4, 8, 10, 11, 14). Single short primers with arbitrary nucleotide sequences are used to amplify genomic DNA. Rapid bacterial lysis by heating circumvents lengthy DNA extraction-purification steps and speeds the analysis. The RAPD method is particularly useful when a rapid comparison of bacterial strains is necessary. Four reference strains were included in the present study to evaluate the discriminatory power of the method for C IV-2.

Biochemical typing and antibiotic susceptibility are conventional methods based on the analysis of phenotypic characteristics; the biochemical patterns of our C IV-2 isolates were relatively uniform. The results obtained by DNA analysis allowed us to conclude that all of the children in both wards harbored the same strain. The infections were hospital acquired and occurred between November 1993 and October 1994. No new cases occurred after October 1994. Patient-to-patient strain transfer may be excluded, because none of the children were hospitalized during overlapping periods. Unfortunately, the epidemiological investigation was conducted retrospectively in January 1995, and the origin of the outbreak could not be determined.

In conclusion, DNA analysis by the RAPD method proved

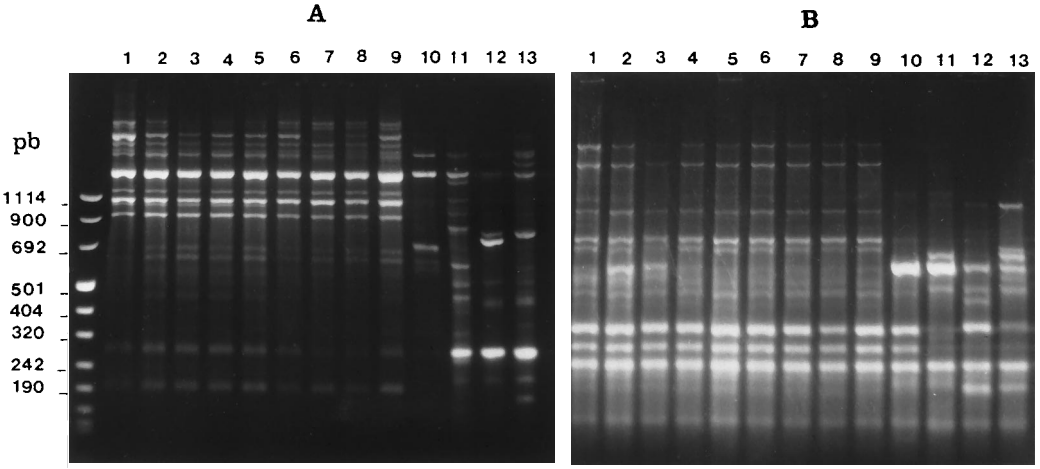

FIG. 1. DNA fingerprinting of C IV-2 by RAPD analysis with primers I (A) and III (B). Lanes 1, strain A1; lanes 2, B1; lanes 3, C1; lanes 4, D1; lanes 5, D2; lanes 6, D3; lanes 7, E1; lanes 8, E2; lanes 9, E3; lanes 10 through 13, CDC reference strains G6817, G3900, G608, and F4862, respectively. pb, base pairs.

to be useful for the epidemiological investigation of a hospital outbreak of C IV-2 infection.

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