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Molecular Study of Nosocomial Nocardiosis Outbreak Involving Heart Transplant Recipients

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Randomly amplified polymorphic DNA analysis and rRNA gene restriction patterns (ribotyping) were compared as methods of investigating a nosocomial outbreak of nocardiosis involving three heart transplant recipients. No clear distinctions between three clinically related isolates and four unrelated strains were obtained by ribotyping. On the contrary, randomly amplified polymorphic DNA analysis with two selected primers, primers 2650 and DKU49, showed one pattern for the three related isolates and four patterns for the unrelated strains.

Nocardiae are gram-positive, partially acid-fast, branching filamentous, soil-inhabiting, aerobic actinomycetes (2). Species of the *Nocardia asteroides* complex (25, 26), which includes *Nocardia asteroides* sensustricto, *Nocardia farcinica*, and *Nocardia nova*, can cause acute or chronic, suppurative, or granulomatous diseases. They are generally associated with localized pulmonary and disseminated involvement. The infection frequently disseminates to the central nervous system and soft tissues and, more rarely, to other organs, such as the kidney (3, 6). Nocardial infections have mostly been reported for immunocompromised hosts (13), including AIDS patients. Nosocomial outbreaks of nocardiosis have been reported for such immunocompromised patients, more commonly in renal transplant recipients (28) than in heart transplant recipients (11, 22). Evidence for a possible nosocomial transmission was based on antigenic characters and fatty acid patterns of *N. asteroides* strains from patients (10, 18, 23) and the environment, but none of them was studied by genetic methods. Here we report the application of three genotyping techniques (DNA restriction patterns, rRNA gene restriction pattern [ribotyping], and randomly amplified polymorphic DNA [RAPD] analysis) to the laboratory evaluation of a suspected outbreak of nocardiosis.

Three men were admitted to a cardiological hospital in October 1991 following heart transplantation. Within the first month, while receiving prednisone, azathioprine, and cyclosporin A, they developed pulmonary infection requiring bronchoscopy. The first patient, a 41-year-old man, was transferred to the cardiological center on 8 October. On 5 November, he developed a febrile syndrome with a focus on the left lung base. A chest X ray showed a node in the left posterior lower lobe. Antibiotic treatment with roxithromycin was initiated, and the fever soon disappeared. However, on 18 November he spiked a fever to 38.5°C with a productive cough. A chest X ray revealed infiltrates in both upper and lower lobes. Two painful subcutaneous abscesses were detected. A brain tomography scan showed cerebral localizations. On 22 November, bronchial aspirate (BA) and bronchoalveolar lavage (BAL) staining showed filamentous, branching, gram-positive rods whose appearance was suggestive of the genus *Nocardia*. *N. farcinica* was isolated from both samples. Blood cultures were negative. Despite treatment with imipenem and amikacin, the patient died on 26 November. During the same period (9 November), the second patient, a 52-year-old man, displayed cough and purulent expectoration without fever. A chest X ray revealed a round opacity in the left lower base. Gram's staining of BAL and BA were negative, but *N. farcinica* was cultured from both samples. He recovered after a 2-month treatment with amoxicillin-clavulanate. Eleven days later (20 November), the third patient, a 53-year-old man, developed a febrile syndrome for 2 days without pulmonary clinical features. A chest X ray revealed an opacity in the right base. Gram's staining of BAL and BA on 22 November showed branching gram-positive rods, and both yielded *N. farcinica*. Trimethoprim-sulfamethoxazole was initiated, and the fever disappeared within 4 days. The patient was treated successfully for 2 months.

The occurrence of three cases of nocardiosis within a short time period in the same care unit strongly suggested a common source of contamination. To verify this hypothesis, we have analyzed the three isolates, isolates N1, N2, and N3, in parallel with four other *N. farcinica* strains and compared their genetic patterns. The unrelated strains came from the Research Institute of Tuberculosis, Tokyo, Japan (strains KK73-14 [R1] and KK73-15 [R2]), and from the National Reference Center for Mycosis and Antifungal Agents, Institut Pasteur, France (strains R3 and R4, isolated from two French patients with systemic nocardiosis). Each strain was identified as *N. farcinica* by standard chemotaxonomic and physiologic properties (7, 8). The enzymatic characteristics of the seven strains were studied with the API ZYM system (bioMérieux, La Balmes les Grottes, France) by the method of Boiron and Provost (4). Testing of the organisms' susceptibilities to antibiotics was done as described previously (5) by the disk diffusion method in Mueller-Hinton agar (Sanofi Diagnostics Pasteur, Marnes la Coquette, France). DNA extraction was performed by a mycobacterial DNA extraction protocol (19). Chromosomal DNA (2 μg) was digested with 10 U of *Pvu*II and *Eco*RI (Boehringer GmbH, Mannheim, Germany) and with 10 U of *Bam*HI, *Kpn*I, and *Bgl*II (Gibco-BRL, Cergy Pontoise, France) in appropriate buffer for 4 h at 37°C. The DNA fragments were separated in a 0.8% agarose gel in 1× TBE (Tris-borate-EDTA) buffer and were transferred to a nylon membrane (Boehringer GmbH) in 20× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate). For ribotyping, hybridization and washes were carried

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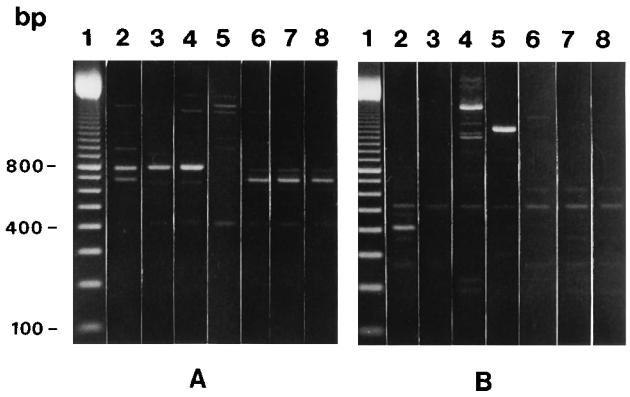

FIG. 1. RAPD patterns. (A) Primer 2650; (B) primer DKU49. Lanes 1, DNA ladder (100 bp); lanes 2 through 8, strains R1, R2, R3, R4, N1, N2, and N3, respectively.

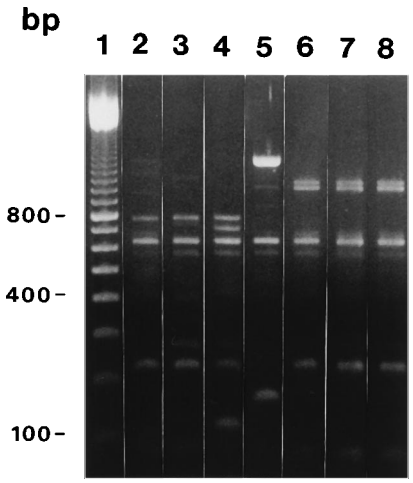

FIG. 2. RAPD patterns with primers 2650 and DKU49 combined. Lane 1, DNA ladder (100 bp); lanes 2 through 8, strains R1, R2, R3, R4, N1, N2, and N3, respectively.

out at 60 and 55°C, respectively, in a hybridization chamber (Techne, Cambridge, United Kingdom). Ribotypes were assessed with a cDNA 16S + 23S *Escherichia coli* RNA (12) digoxigenin labeled by the manufacturer's protocol (Boehringer GmbH). For RAPD analysis, the 10-mer primer 2650 (5′-CAATCGCCGT-3′) (24) and the 12-mer primer DKU49 (5′-CCGCCGACCGAG-3′) (14) were used, alone and in combination, with a DNA thermal cycler (Techne PHC-2). The 50-μl reaction mixture contained 50 mM KCl, 10 mM Tris-HCl (pH 8.8), 2 mM $MgCl_2$, 0.01% (vol/vol) gelatin, 200 μM each deoxyribonucleotide (Pharmacia, Uppsala, Sweden), 50 pmol of primer, 2 U of *Taq* DNA polymerase (Gibco-BRL), and 20 ng of genomic DNA. Thirty-five amplification cycles (denaturation at 94°C for 30 s, annealing at 40°C for 30 s, and extension at 72°C for 1 min) were performed. Fifteen microliters of the PCR products, along with a 100-bp ladder, was subjected to electrophoresis in a 2% agarose gel (Pharmacia). The gels were stained with ethidium bromide (1 mg/ml) and were visualized with UV transilluminator.

Enzymatic activity profiles and antibiotic susceptibility patterns were used to confirm the identification of the seven bacterial strains studied as *N. farcinica*. These phenotypes were not useful for strain typing because of the large extent of homogeneity from strain to strain. The DNA restriction patterns with the five enzymes tested did not show significant differences between each related and unrelated strain (data not shown). The ribotypes obtained with *Pvu*II, *Bgl*II, and *Kpn*I were identical for all strains. The ribotypes of strains R2 and R3 obtained with *Bam*HI were identical to those of strains N1, N2, and N3, as were those of strains R1 and R4 obtained with *Eco*RI (data not shown). Nevertheless, only two or three hybridation fragments were observed. By RAPD analysis with the two primers used alone or in combination, we observed five different DNA patterns: one pattern was common to the three related strains and four different patterns were found for the four unrelated strains (Fig. 1 and 2).

Nocardiosis outbreaks are uncommon and are difficult to investigate (1, 18, 20). Using the DNA restriction patterns obtained with all enzymes tested, we did not find any discrepancies between our seven *N. farcinica* strains. However, using *Pvu*II, Patterson et al. (15) observed one DNA pattern with a pseudoepidemic *N. asteroides* strain and a different pattern with a single unrelated strain. Ribotyping was initially described as a taxonomic tool by Grimont and Grimont (9). This method has been used for epidemiological studies of *Rhodococcus equi* (12) and *Corynebacterium* spp. (16). Despite the use of five different enzymes, related and unrelated strains could not be separated. As a hypothesis, we suggest that *N. farcinica*, like *Mycobacterium leprae*, possesses few rRNA operons (21), exhibiting few hybridization fragments. The RAPD method was initially described by Williams et al. (27) for use in differentiating eucaryotes and procaryotes. Selection of the appropriate primers is of the most importance for increasing the efficiency of RAPD analysis (24). Our RAPD protocol produced sufficiently highly polymorphic patterns to distinguish our clonal strain from four unrelated strains. RAPD analysis is more rapid and convenient than ribotyping (1 day versus 3 to 4 days). These advantages must be confirmed, first, by studying a larger number of strains and, second, by comparing RAPD analysis with another genotyping method, such as pulsed-field gel electrophoresis. Pulsed-field gel electrophoresis may be suitable for analyzing a large number of strains and appears to be more effective than ribotyping (17).

In conclusion, we report here the first molecular description of a nosocomial outbreak caused by *N. farcinica* in immunodepressed heart transplant recipients. The application of RAPD analysis demonstrates that a great deal of genetic heterogeneity exists at the subspecies level, which could be of help in providing evidence of a nosocomial nocardiosis outbreak and in formulating effective infection control measures.

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