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Use of a Repetitive DNA Probe To Type Clinical and Environmental Isolates of *Aspergillus flavus* from a Cluster of Cutaneous Infections in a Neonatal Intensive Care Unit

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***Aspergillus flavus* is second to *A. fumigatus* as a cause of invasive aspergillosis, but no standard method exists for molecular typing of strains from human sources. A repetitive DNA sequence cloned from *A. flavus* and subcloned into a pUC19 vector, pAF28, was used to type 18 isolates from diverse clinical, environmental, and geographic sources. The restriction fragment length polymorphisms generated with *Eco*RI- or *Pst*I-digested genomic DNA and probed with digoxigenin-labeled pAF28 revealed complete concordance between patterns. Eighteen distinct fingerprints were observed. The probe was used to investigate two cases of cutaneous *A. flavus* infection in low-birth-weight infants in a neonatal intensive care unit (NICU). Both infants were transported by the same ambulance and crew to the NICU on the same day. *A. flavus* strains of the same genotype were isolated from both infants, from a roll of tape used to fasten their umbilical catheters, from a canvas bag used to store the tape in the ambulance, and from the tape tray in the ambulance isolette. These cases highlight the need to consider exposures in critically ill neonates that might occur during their transport to the NICU and for stringent infection control practices. The hybridization profiles of strains from a second cluster of invasive *A. flavus* infections in two pediatric hematology-oncology patients revealed a genotype common to strains from a definite case patient and a health care worker. A probable case patient was infected with a strain with a genotype different from that of the strain from the definite case patient but highly related to that of an environmental isolate. The high degree of discrimination and reproducibility obtained with the pAF28 probe underscores its utility for typing clinical and environmental isolates of *A. flavus*.**

Members of the genus *Aspergillus* are among the most widespread fungi in the environment, being found in the soil, on plants, in dust, on food, and in the air. In nonimmunocompromised persons, these molds can cause localized infections of the lungs, sinuses and other sites. In immunocompromised individuals, inhalation of conidia gives rise to invasive infection of the lungs or sinuses, often followed by dissemination to other organs. Involvement of the skin is uncommon, but two forms of cutaneous aspergillosis have been described in compromised patients (26; T. J. Walsh, Editorial Response, Clin. Infect. Dis. **27:**453–457, 1998). In primary cutaneous aspergillosis, lesions arise at or near intravenous catheter insertion sites, at surgical wound sites, and at sites associated with occlusive dressings or burns. In secondary cutaneous aspergillosis, the lesions result either from extension to the skin from underlying infected structures or from hematogenous dissemination. Most reported cases of cutaneous aspergillosis have been caused by *Aspergillus fumigatus*, but a number have been associated with *A. flavus*, the second most common etiologic agent of human aspergillosis.

The evidence incriminating different environmental sources of *A. flavus* infection has always been circumstantial because only recently have molecular typing methods been developed to trace the spread of particular subspecific strains. Among the typing methods that have been applied to *A. flavus* are restriction endonuclease analysis and the detection of restriction fragment length polymorphisms (RFLPs) by Southern hybridization and probing with ribosomal or other repetitive sequences (19, 20). The application of randomly amplified polymorphic DNA (RAPD) analysis as a genotyping method has also been reported (14).

Restriction endonuclease analysis of total cellular DNA has not proved to be a suitable method for discrimination of strains of *A. flavus* (2). Moody and Tyler (19) identified mitochondrial DNA RFLPs and used them to propose the occurrence of species within the *A. flavus* group: *A. flavus*, *A. parasiticus*, and *A. nomius*. RFLP analysis of *A. flavus* nuclear DNA probed with recombinant DNA clones from *A. nidulans* and *Neurospora crassa* supported the results obtained with mitochondrial DNA but revealed limited geographic correlations among *A. flavus* strains (20). RAPD analysis has been used to type strains of *A. flavus*, but the profiles may suffer from a lack of reproducibility (14). Moreover, RAPD analysis may not be appropriate as a tool for epidemiologic tracking of isolates or for surveying the genetic variation in natural populations because of its inability to discriminate artifactual variation from actual polymorphism (24, 25). Buffington et al. (2) combined the products from RAPD analysis-RFLP analysis of a tester strain of *A. flavus* to produce a DNA probe for Southern blot analysis. Although a high degree of discrimination among strain types was achieved, the probe and target sequences are anonymous and the probe is available in only a single laboratory.

In an effort to develop an improved method for molecular typing of clinical and environmental isolates of *A. flavus*, we evaluated a DNA fingerprinting procedure that uses a repetitive DNA sequence cloned from *A. flavus* var. *flavus* to probe RFLPs of genomic DNA (16). The method was evaluated with

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TABLE 1. Origins of *A. flavus* isolates collected from patients with aspergillosis and the hospital environment and strains of diverse geographic and clinical origins[a]

Isolate no.	Geographic origin	Source	Culture no.	Genotype by Southern blot analysis
AFL1	Fort Worth, Tex.	Abdominal skin lesion, patient A	98-019777	A
AFL2	Fort Worth, Tex.	Abdominal skin lesion, patient B	98-019778	A
AFL3	Fort Worth, Tex.	Adhesive tape	98-019779	A
AFL4	Fort Worth, Tex.	Blood culture, patient C	98-019781	A
AFL5	Fort Worth, Tex.	Transport isolette	98-019782	A
AFL6	Fort Worth, Tex.	Canvas tape bag	98-019783	A
AFL7	Fort Worth, Tex.	TDOH	98-019784	B
AFL8	Los Angeles, Calif.	Ward 4 West vent	B5105	C
AFL9	Los Angeles, Calif.	BMT nursing station vent	B5106	D
AFL10	Los Angeles, Calif.	Nasal sinus of HCW	B5107	E
AFL11	Los Angeles, Calif.	Probable IA, primary blood culture, patient 2	B5108	D
AFL12	Los Angeles, Calif.	Probable IA, secondary blood culture, patient 2	B5109	F
AFL13	Los Angeles, Calif.	Definite IA, blood culture, patient 1	B5110	E
AFL14	Los Angeles, Calif.	Ward 3 West vent (adjacent NICU)	B5117	D
AFL15	Savannah, Ga.	Nasal sinus	AFS1	G
AFL16	Savannah, Ga.	Nasal sinus	AFS2	H
AFL17	Savannah, Ga.	Nasal sinus	AFS3	I
AFL18	Savannah, Ga.	Nasal sinus	AFS4	J
AFL19	Ohio	Human sputum	ATCC 64025	K
AFL20	New Jersey[b]	Proteinase production	ATCC 11497	D
AFL21	Thailand	Soy sauce, Koji	ATCC 44310	L
AFL22	Virginia	Prosthetic mitral valve	ATCC 34896	M
AFL23	Unknown	Unknown clinical isolate	96001	N
AFL24	Maryland	Amputation stump, 1959	NIH 5239	O
AFL25	Maryland	Unknown clinical isolate, 1959	NIH 5251	P
AFL26	Maryland	Unknown clinical isolate, 1960	NIH 5250	Q
AFL27	Brazil	Lung tissue	B4955	R
AFL28	Pennsylvania	Prosthetic heart valve	B5096	S
AFL29	Dawson, Ga.	Peanut	NPRL1 (high aflatoxin)	T
AFL30	Dawson, Ga.	Peanut	NPRL2 (low aflatoxin)	U
AFL31	Dawson, Ga.	Peanut	NPRL3 (no aflatoxin)	V

[a] Abbreviations; HCW, health care worker; TDOH, Texas State Department of Health; IA, invasive aspergillosis; NPRL, National Peanut Research Laboratory, Dawson, Ga.; ATCC, American Type Culture Collection, Rockville, Md.; NIH, National Institutes of Health, Bethesda, Md.
[b] Culture was originally deposited in 1929 by Selman Waksman, New Brunswick, N.J.

a panel of clinical and environmental isolates of *A. flavus* from different sources and then was applied to strains from two small clusters of pediatric cases of invasive aspergillosis: cutaneous *A. flavus* infection in low-birth-weight infants from a neonatal intensive care unit (NICU) and invasive pulmonary aspergillosis in a hematology-oncology unit (2).

MATERIALS AND METHODS

Isolates. A total of 31 isolates of *A. flavus* were studied (Table 1). These included six clinical and environmental isolates (isolates AFL1 to AFL6) obtained from a cluster of cutaneous aspergillosis cases in the NICU at Cook Children's Medical Center, Fort Worth, Tex. A single control isolate of *A. flavus* unrelated to this cluster (isolate AFL7) was provided by the Texas Department of Health. The remainder of the collection consisted of seven *A. flavus* isolates (isolates AFL8 to AFL14) collected during an outbreak of invasive aspergillosis in the hematology-oncology and bone marrow transplant (BMT) units at Los Angeles Children's Hospital, Los Angeles, Calif. (2); four nasal sinus isolates (isolates AFL15 to AFL18) obtained from patients with allergic fungal sinusitis at the Memorial Medical Center in Savannah, Ga.; four isolates (isolates AFL19 to AFL22) obtained from the American Type Culture Collection; six isolates from human sources (isolates AFL23 to AFL28) obtained from the culture collections of the Mycotic Diseases Branch, Centers for Disease Control and Prevention, Atlanta, Ga., or the National Institutes of Health, Bethesda, Md.; and two aflatoxin-producing isolates (isolates AFL29 and AFL30) and one non-aflatoxin-producing isolate (isolate AFL31) obtained from the National Peanut Research Laboratory, Dawson, Ga.

Isolates were confirmed as *A. flavus* on the basis of their macroscopic and microscopic characteristics in culture (12). Isolates were maintained on 2.5% malt extract agar slants and were stored at 4°C. In preparation for DNA extraction and molecular typing, monoconidial isolates of each *A. flavus* culture were produced by the limiting dilution method. Conidial suspensions were harvested from a 7-day growth of a single conidium on potato dextrose agar and were stored in 50% (vol/vol) sterile glycerol–phosphate-buffered saline at −20°C.

Epidemiologic investigation. Two preterm infants delivered by emergency cesarean section at separate outlying hospitals were transported to the NICU, Cook Children's Medical Center in an isolette by the same ambulance and crew on March 10, 1998. Baby A (patient A in Table 1) weighed 792 g at birth. On day 3 of life, the infant developed black abdominal skin lesions under adhesive tape fastening umbilical catheters. Culture of the lesions resulted in the isolation of *A. flavus*, and biopsy of the site revealed scattered septate, branching fungal hyphae consistent with an *Aspergillus* species. The baby lived for 12 days, and the immediate cause of death was respiratory arrest. No autopsy was performed.

Baby B (patient B in Table 1) weighed 420 g at birth. On day 7 of hospitalization, the infant developed black abdominal skin lesions at the site of umbilical catheters secured with tape from the same roll used for baby A. *A. flavus* grew from cultures of these lesions. The infant was treated with intravenous amphotericin B for 7 days prior to his death on day 17. No autopsy was performed, but cutaneous aspergillosis was considered to be a contributing cause of death.

A third child, a 4-year-old male (patient C in Table 1), was admitted to the same hospital and was treated for brucellosis during the same week that the two low-birth-weight infants were treated. The hospital laboratory recovered *A. flavus* from a culture of a single blood sample from this child. This patient received no antifungal therapy and recovered, and he was discharged from the hospital.

Construction was in progress to replace double doors at the entrance to the NICU during the time that the infected infants were being treated in the unit. Environmental samples were obtained from various sources, including the NICU itself, the ambulance, and materials from the ambulance. The environmental samples culture positive for *A. flavus* included a roll of adhesive tape, a canvas bag used to store rolls of tape, the transport isolette, and a roll of clear plastic film stored in a closet in the NICU. The roll of tape kept in the ambulance was used to fasten umbilical intravascular catheters to each infant.

Isolation of *A. flavus* genomic DNA. A 10-μl loopful of conidia from each monoconidial isolate was used to seed 50 ml of yeast-peptone-dextrose (YPD) broth (Difco Laboratories, Detroit, Mich.) supplemented with 50 μg of ampicillin (FisherBiotech, Fair Lawn, N.J.) per ml in a 250-ml Erlenmeyer flask. Cul-

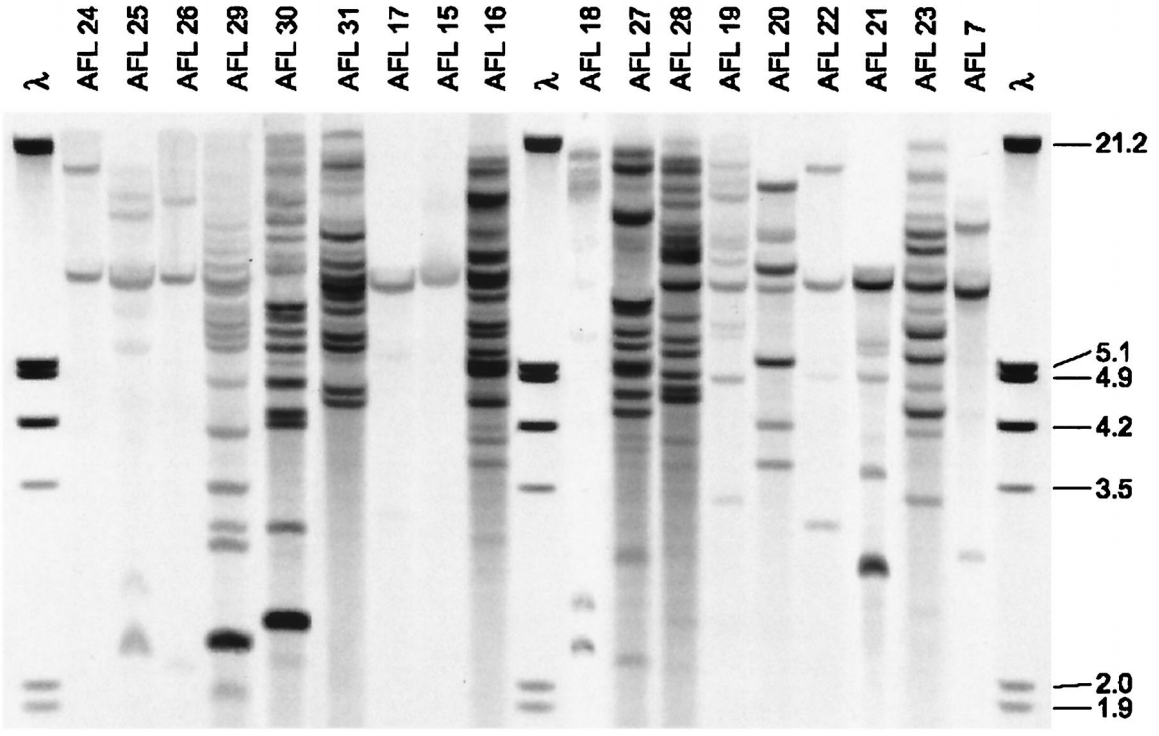

FIG. 1. Southern blot patterns of *Eco*RI-digested DNAs of 18 unrelated isolates of *A. flavus* hybridized with pAF28. Molecular size markers of *Eco*RI- and *Hin*dIII-digested bacteriophage λ DNA (in kilobases) are shown on the right. Strains AFL29, AFL30, AFL31, and AFL20 were from environmental sources.

tures were incubated in a rotary shaker at 175 rpm and 35°C for 36 h. The mycelia were harvested by filtration through a Whatman no. 1 filter cup (Whatman International, Ltd., Maidstone, United Kingdom) and rinsed with sterile distilled water. The mycelial mat was then transferred to a precooled porcelain mortar containing 0.5-mm-diameter sterile glass beads, frozen with liquid N_2, and ground to a fine powder in a biological safety cabinet. A total of 10 ml of the following lysis buffer equilibrated at 65°C was added: 20 mM EDTA, 10 mM Tris, 1% Triton X-100, 500 mM guanidine thiocyanate, and 250 mM NaCl (pH 8.0). The suspension was incubated at 65°C for 10 min to inactivate nuclease activity and to induce protein denaturation. Proteinase K was added to a concentration of 1.0 mg/ml, and the suspension was incubated at 55°C for 3 h with gentle agitation. Potassium acetate was added to a final concentration of 1 M, the mixture was incubated in an ice bath for 30 min, and the suspension was centrifuged for 15 min at 4°C to remove precipitated materials. The cleared lysate was transferred to a 30-ml Oak Ridge tube, and nucleic acids were precipitated with 1 volume of isopropanol and centrifuged at 7,500 × *g* for 15 min. The nucleic acid pellet was dried and dissolved in TE buffer (0.01 M Tris, 1 mM EDTA [pH 8.0]) and diluted with 5 to 10 volumes of QBT buffer (Qiagen Inc., Chatsworth, Calif.) for purification on a Qiagen tip of the appropriate capacity. *A. flavus* genomic DNA was purified according to the manufacturer's recommendations (3; Qiagen Genomic DNA Handbook, Qiagen GmbH, Qiagen Inc., Hilden, Germany, 1995). Purified DNA was washed with 70% ethanol, dried, resuspended in an appropriate volume of TE buffer, and stored at −20°C. DNA concentrations were determined with Hoechst 33258 fluorescent dye (Sigma Chemical Co., St. Louis, Mo.) in a fluorometer (Fluorescence Concentration Analyzer; IDEXX Laboratory, Westbrook, Maine).

Restriction endonuclease digestion and Southern hybridization. Restriction endonuclease digestion of 3 μg of *A. flavus* genomic DNA with *Eco*RI or *Pst*I (Roche Molecular Systems, Indianapolis, Ind.) was performed according to the manufacturer's specifications. After digestion, restriction endonuclease fragments were resolved by electrophoresis through a 0.9% agarose (I.D.NA agarose; FMC Bioproducts, Rockland, Maine) gel immersed in TBE buffer (100 mM Tris, 90 mM borate, 1 mM EDTA [pH 8.3]) for 18 h at 1.2 V/cm. DNA was transferred to positively charged nylon membranes (Roche Molecular Systems) by standard procedures (15). The probe, plasmid pAF28, contains a 6.2-kb *Eco*RI fragment containing a repetitive DNA sequence isolated from *A. flavus* var. *flavus* NRRL 6541 (16). The nucleotide sequence contained in pAF28 is unknown, so at present, any homology to transposons remains to be determined. Plasmid pAF28 was labeled with digoxigenin by a random priming method according to the manufacturer's directions (DIG DNA Labeling and Detection kit; Roche Molecular Systems). Prehybridization and hybridization were carried out at 65°C in DIG Easy Hyb buffer (Roche Molecular Systems). Digoxigenin-labeled molecular size markers consisting of *Eco*RI- and *Hin*dIII-digested bacteriophage λ DNA were used (Roche Molecular Systems). After posthybridization washing and blocking, the membranes were developed chromogenically and photographed. The Southern blot patterns of different *A. flavus* strains were compared visually for the presence or absence of bands. Two isolates were considered identical when individual DNA fingerprints could be superimposed.

Analysis of data. Cluster analysis was conducted with Diversity Database fingerprinting software (Bio-Rad Laboratories, Hercules, Calif.). Similarity values were computed between *A. flavus* strains by using the Jaccard coefficient formula. Most of the Southern hybridization patterns are shown in Fig. 1, 3, and 4. The similarity matrix values were converted into a Euclidean squared distance matrix by unweighted pair group analysis with arithmetic mean (UPGMA) and were displayed as a dendrogram. The tolerance limit of ±2% was used to determine if two bands were shared or unshared between two different *A. flavus* strains. A numerical index of discrimination, *D*, based on the probability that two unrelated strains sampled from a test population will be characterized as different molecular types, was used to assess the method described in this report. Discriminatory power was calculated as described by Hunter and Gaston (11) by using Simpson's index of diversity as given in the following equation:

$$D = 1 - \frac{1}{N(N-1)} \sum_{j=1}^{s} n_j(n_j - 1)$$

where N is the total number of strains in the test population, s is the number of strain types observed, and n_j is the number of strains in the population that belong to the jth strain type.

RESULTS

DNA fingerprinting. In order to establish whether the repetitive DNA sequence probe pAF28 might be suitable for the subspecific discrimination of *A. flavus*, 18 isolates from diverse clinical, environmental, and geographic sources (isolates AFL7 and AFL15 to AFL31) were hybridized with the probe. Eighteen distinct DNA fingerprints were observed (Fig. 1). The size markers did not hybridize with pAF28; the strong signal in the hybridizations is because in the final round of experiments digoxigenin-labeled size markers were used which were stained

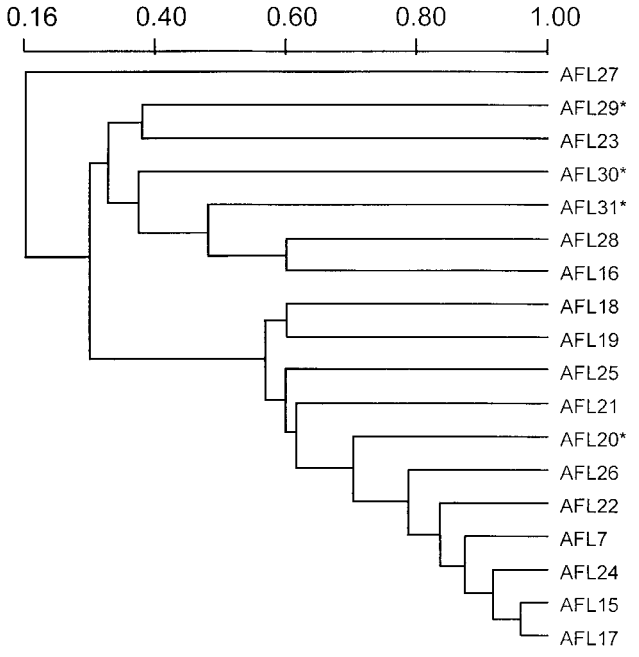

FIG. 2. UPGMA-based dendrogram of the 18 unrelated *A. flavus* isolates based on the Jaccard similarity coefficient of the hybridization patterns generated with the repetitive DNA sequence probe, pAF28. See Table 1 for descriptions of individual isolates. Asterisks indicate strains from environmental sources.

in the immunoperoxidase reaction. Cluster analysis of the fingerprints resulted in the dendrogram shown in Fig. 2, which suggests that the isolates can be divided into two distinct clusters or groups, each of which comprises nine isolates composed of both clinical and environmental isolates (isolates AFL20 and AFL29 to AFL31). With *Eco*RI digestion, 2 to 17 fragments were produced that bound to the probe in the molecular mass range of 2 to 23 kb. *Pst*I digestion produced a greater number of fragments, up to 21, ranging in mass from 1 to 24 kb (data not shown).

Molecular typing of *A. flavus* isolates from Cook Children's Medical Center. Figure 3 shows the Southern blot patterns of *Eco*RI-digested DNAs from the six *A. flavus* isolates (isolates AFL1 to AFL6) obtained from Fort Worth, Tex. The hybridization patterns observed with the *Eco*RI and *Pst*I digests were identical. The isolates obtained from the infants' abdominal lesions were indistinguishable from each other and appeared to be identical to the isolates recovered from the roll of adhesive tape used to anchor their umbilical catheters, the canvas bag used to store the tape in the ambulance, and the tape tray in the ambulance isolette (Fig. 3; Table 1). The DNA fingerprint of an *A. flavus* isolate (isolate AFL4) recovered in a culture of blood from a 4-year-old child treated during the same week as the two low-birth-weight infants was identical to those of the isolates from the two case infants (isolates AFL1 and AFL2). A seventh *A. flavus* isolate (isolate AFL7), obtained from the Texas State Department of Health, had a distinctly different fingerprint unrelated to the fingerprints of the isolates in the cluster (Fig. 3).

Molecular typing of *A. flavus* isolates from the Los Angeles Children's Hospital. Figure 4 shows the Southern blot patterns of *Eco*RI- and *Pst*I-digested DNAs from seven clinical and environmental isolates of *A. flavus* (isolates AFL8 to AFL14) from the hematology-oncology and BMT units of the Los Angeles Children's Hospital. The results are summarized in Table 1. Analysis of this cluster entailed the isolation in culture of monoconidia from the primary stock culture. Consequently, two monoconidial subcultures from the probable invasive aspergillosis case isolate were produced (isolates AFL11 and AFL12). The result indicated the presence of two infecting strains from the same patient. Four distinct strain types were detected, but none of these were identical to the genotype observed in the Cook Children's Medical Center cluster.

Discriminatory power. Cluster analysis of the DNA fingerprints of the entire set of 31 *A. flavus* isolates is illustrated in the dendrogram in Fig. 5. We observed 22 distinct DNA fingerprints among these *A. flavus* isolates. Of these, 19 strain types were unique, but the remaining three types (types A, D, and E) comprised six, four, and two identical isolates, respectively (Table 1).

To assess the discriminatory index of the pAF28 repetitive DNA sequence probe, the 31 *A. flavus* isolates were evaluated on the basis of the method of Hunter and Gaston (11). The discriminatory power was calculated, and the index obtained by this method was shown to be highly discriminatory (D = 0.9526). The reproducibility of the method was assessed in triplicate with different DNA preparations isolated from *A. flavus* cultures. The reproducibility of our experiments was 100%.

DISCUSSION

Previous studies with the pAF28 repetitive DNA sequence probe distinguished among 29 individual *A. flavus* strains obtained from agricultural sources representing 22 different vegetative compatibility groups by using *Pst*I to digest *A. flavus* DNA (16). This study demonstrated that DNA fingerprinting with the pAF28 repetitive probe is a highly reproducible and discriminatory method for tracing the transmission of human cases of *A. flavus* infection, expanding its utility beyond the agricultural purpose for which it was developed (16). These findings are consistent with the successful findings obtained with the *Afu*tI repetitive DNA sequence probe in investigations

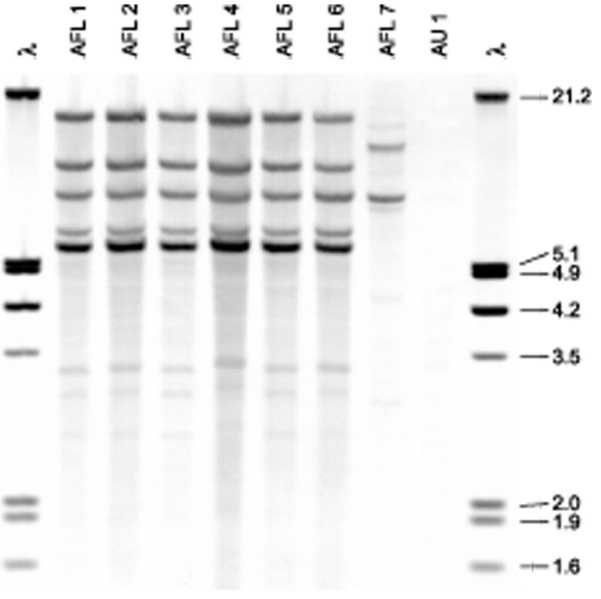

FIG. 3. Southern blot patterns of *Eco*RI-digested DNAs of six isolates of *A. flavus* collected at Cook Children's Medical Center. See Table 1 for descriptions of individual isolates. Molecular size markers (in kilobases) are shown in lanes 1 and 9 and contain *Eco*RI- and *Hin*dIII-digested DNA of bacteriophage λ.

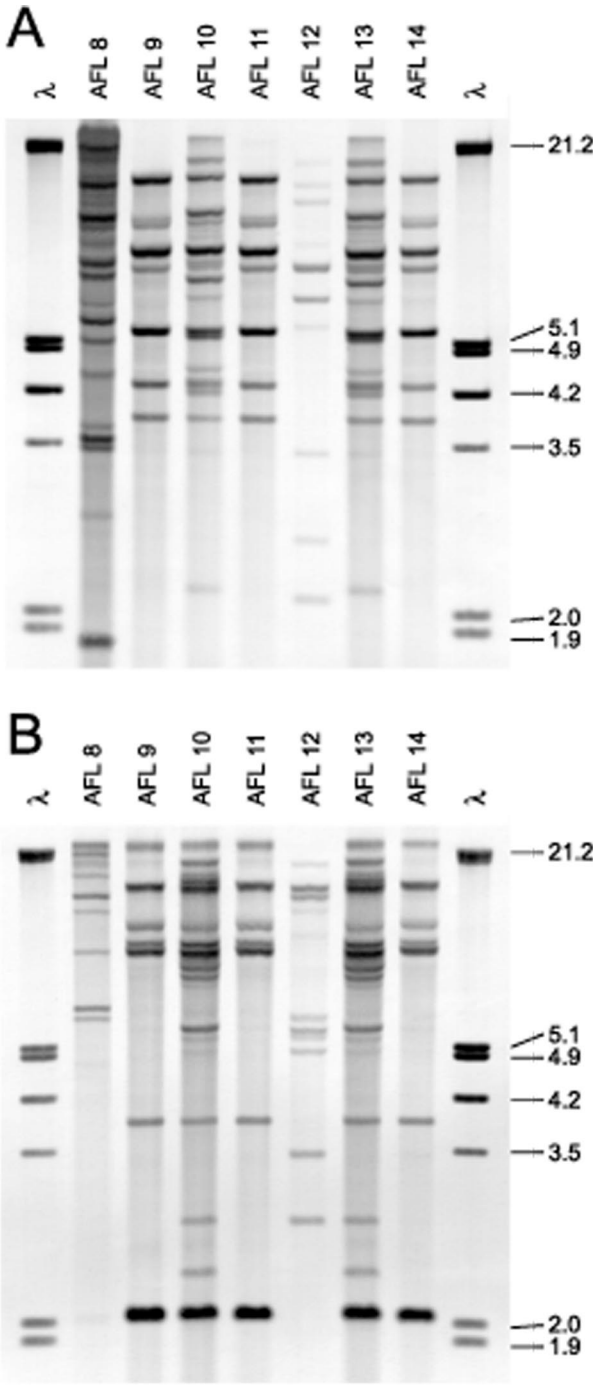

FIG. 4. Southern blot patterns of *Eco*RI-digested (A) and *Pst*I-digested (B) DNAs of clinical and environmental strains of *A. flavus* from the hematology-oncology and BMT units of the Los Angeles Children's Hospital. See Table 1 for descriptions of individual isolates. Molecular size markers (in kilobases) are shown in lanes 1 and 9 of each panel and contain *Eco*RI- and *Hin*dIII-digested DNA of bacteriophage λ.

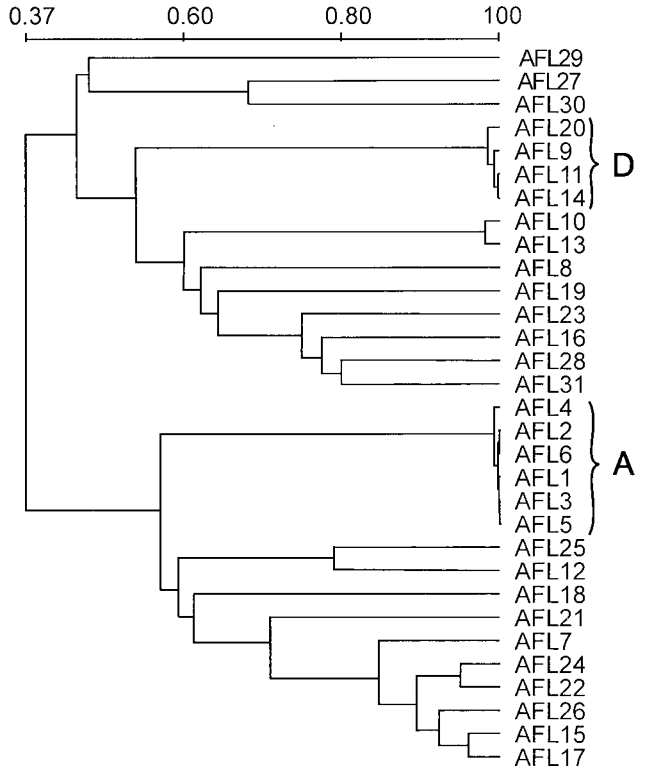

FIG. 5. UPGMA-based dendrogram of the 31 *A. flavus* isolates based on the Jaccard similarity coefficient of the hybridization patterns generated with the repetitive DNA sequence probe, pAF28. See Table 1 for descriptions of individual strains and letter designations of two clusters of identical strain genotypes (genotypes A and D).

of the nosocomial transmission of *A. fumigatus* infection (7, 8, 21). We compiled an early indication of the degree of strain diversity within *A. flavus* var. *flavus*; however, this database needs to be expanded to obtain a more complete appreciation of the population genetics of the group. A better understanding of the nature of the probe is needed after sequence analysis, and its behavior with respect to other species in *A. flavus* group should be determined. Pending the availability of such information, phylogenetic analysis could proceed with a larger collection of isolates of *A. flavus* involved in human infections. Information in such a database would be analogous to that from the characterization of clinical and environmental isolates of *A. fumigatus* (4, 5).

Geiser et al. (6) conducted PCR-RFLP analysis of 11 nuclear genes and concluded that a collection of *A. flavus*, *A. parasiticus*, and *A. oryzae* strains could be divided into two reproductively isolated clades, groups I and II, with group I comprising isolates of *A. oryzae*. Cluster analysis of 18 unrelated *A. flavus* isolates in this study also revealed two clades (Fig. 2). The clade distribution observed in this study may be a reflection of the probe, which results in two general hybridization profiles: strains with several pAF28-binding fragments and other strains with few such fragments. The *A. flavus* strains from agricultural sources, strains AFL20 and AFL29 to AFL31, were distributed in both clades. The occurrence of pAF28 sequences and the number of copies per strain was confirmed with a small number of strains representative of closely related species or varieties of the *A. flavus* group (16): *A. oryzae* (strong hybridizer, several copies), *A. parasiticus* (lower hybridization signal, several copies), *A. sojae* (low signal, small copy number), and *A. nomius* (single intense copy). A larger number of strains representing these species should be analyzed by Southern blotting with pAF28 and phylogenetic analysis to confirm the occurrence of these clades.

The extent to which other members of the *A. flavus* group

may occur in human infections is unknown, and a clarification of the taxonomic status of a larger collection of *A. flavus* clinical isolates may contribute to a better understanding of whether there is a dichotomy in the pathogenic potential of *A. flavus*. Molecular typing of an expanded collection of clinical and environmental isolates is required to address this question and to determine if there is segregation of clinical from environmental isolates within phylogenetic groups I and II of *A. flavus* reported by Geiser et al. (6). Similar approaches involving *A. fumigatus* have already yielded important information about strain diversity in that species (5).

As a fungus which primarily propagates asexually, *A. flavus* is limited in its ability to exchange genetic material between compatible strains. Barriers to vegetative fusions are widespread among individual strains of *A. flavus* (16). Limited parasexual recombination may occur, however, within isolated populations. This interpretation, if correct, is promising for the application of genomic typing to trace epidemiologically significant clonal transmission. The contribution of the airborne distribution of *A. flavus* conidia to strain diversity in a particular geographic region or hospital environment has been suggested but not confirmed by molecular typing (4).

Heterogeneity within the *A. flavus* group is under investigation (6). In this study we found that some strains weakly hybridized to the repetitive DNA sequence, consistent with earlier observations that some varieties within the *A. flavus* group possess smaller numbers of copies of the repetitive sequence. A previous study conducted to determine the percent relatedness of varieties within the *A. flavus* group on the basis of DNA-DNA reassociation showed that *A. oryzae* exhibited 100% DNA homology with *A. flavus* var. *flavus*, whereas the DNA-DNA homology of *A. sojae* was 74% and that of *A. parasiticus* was 70% (13).

Molecular typing of clinical and environmental isolates of *A. flavus* from Cook Children's Medical Center indicated that a roll of adhesive tape was the probable source of the infecting strain of *A. flavus* recovered from both low-birth-weight infants. The isolate of *A. flavus* from a culture of blood from a third child, who did not develop aspergillosis and who could not be epidemiologically linked to the NICU infants, had an DNA fingerprint identical to those of the isolates from the NICU infants (Fig. 3; Table 1). This relationship was confirmed by cluster analysis (Fig. 5). Possibly, this genotype may have been introduced from the contaminated tape into the hospital ventilation system. Alternatively, this *A. flavus* genotype could have been an endemic strain in the hospital ventilation system antedating the tape problem. Both of these possibilities allow an isolate of this genotype to be a laboratory contaminant in the culture of blood from the third patient. A more thorough environmental survey of this hospital would have been required in order to determine the wider prevalence of the *A. flavus* genotype implicated in these infections.

In 1994 Buffington et al. (2) reported one definite and one probable case of invasive infection with *A. flavus* among immunosuppressed children with underlying hematologic diseases in the Los Angeles Children's Hospital. Environmental isolates of *A. flavus* were obtained from air exhaust vents in three areas: the BMT unit nursing station, an area adjacent to Ward 4 West, and Ward 3 West adjacent to the NICU on the floor below. *A. flavus* was also isolated from the nasal sinus of a health care worker who visited patients in all units. Buffington et al. (2), using hybridization profiles obtained by RAPD analysis-RFLP analysis, concluded that the isolates recovered from the health care worker, the probable case patient, and the BMT unit air vent were identical. The *A. flavus* isolate from the definite case patient was shown to be different from all the other clinical and environmental isolates. In the present study, the pAF28 hybridization profiles of *A. flavus* isolates from the definite case patient and from the health care worker were highly related (Table 1; Fig. 4). The probable case patient was infected with a strain with a genotype different from that of the strain from the definite case patient, but the pattern was highly related to those observed for the *A. flavus* isolates from the BMT nursing station and from Ward 3 West. Moreover, the probable case patient appeared to be infected with two different *A. flavus* genotypes (isolates AFL11 and AFL12).

One explanation for the disparity between the two methods of strain typing, RAPD analysis-RFLP analysis and Southern blotting with pAF28, is that the RAPD analysis-RFLP analysis blots from the original published investigation at Los Angeles Children's Hospital were not divided by limiting dilution into cultures of monoconidial strains. In the present investigation with the pAF28 probe, all cultures were derived from each primary culture as monoconidial strains. This explains the recovery of two strains from the primary isolate from the blood of the case patient with probable invasive aspergillosis, who appears to be an example of a patient infected or colonized with two *A. flavus* genotypes. Another reason is that the two independent probes, pAF28 and the RAPD analysis-RFLP analysis probes (consisting of *A. flavus* genomic DNA amplified by PCR with two different RAPD analysis primers), may be scanning different regions of the genome or specific chromosomal regions, such that the resulting patterns are not in agreement. Further comparison of the two molecular typing methods would be desirable.

One of the reference isolates, *A. flavus* AFL20 (a 1946 New Jersey isolate), had a Southern blot genotype identical to those of three of the *A. flavus* isolates from the Los Angeles Children's Hospital (Table 1; Fig. 5). Identical genotypes were observed in two different situations: (i) a strain from the Los Angeles Children's Hospital was highly related to a culture collection strain from New Jersey and (ii) strains isolated from a patient with cutaneous aspergillosis at Cook Children's Medical Center were highly related or identical to an *A. flavus* strain isolated as a possible contaminant from a culture of blood from a pediatric patient (patient C) who was in a different ward of the hospital. These examples suggest that the population of *A. flavus* is not geographically isolated and that typing of a larger number of *A. flavus* isolates, including sampling at intervals of hospital air and solid surfaces, is warranted.

With one exception, the *Pst*I hybridization patterns observed with the Los Angeles isolates exhibited an intense anonymous DNA fragment with a molecular mass of approximately 2 kb (Fig. 4B) that was also observed with *A. flavus* AFL20. None of the *A. flavus* isolates from the Cook Children's Medical Center displayed this particular anonymous fragment or had any distinctive feature in that range of molecular mass.

This investigation has highlighted the risk for cutaneous fungal infection in preterm infants arising from the use of nonsterile adhesive tape to secure umbilical intravascular catheters. The cases reported here emphasize the need to consider exposures in critically ill neonates that might occur during their transport outside the NICU setting and highlight the need for strict adherence to established infection control practices. The use of nonsterile items which may become contaminated with environmental molds poses a special risk to low-birth-weight infants.

We hypothesize that the cases of cutaneous *A. flavus* infection described in this report arose as a result of direct contact of the skin with contaminated adhesive tape. Contaminated dressings associated with intravascular catheters have previ-

ously been described as a cause of cutaneous infection with *Aspergillus* spp. and *Rhizopus* spp. in preterm infants, human immunodeficiency virus-infected patients, and other immunocompromised individuals (9, 10, 17, 22). Bryce et al. (1) reported a cluster of four surgical and burn wound aspergillosis cases that were traced to packages of dressings contaminated during construction in the hospital's central supply area. Another report of four preterm infants with cutaneous aspergillosis which subsequently developed into fatal, disseminated infection identified the source of the infection as contaminated latex finger stalls (23). Recently, a cluster of four infants in the NICU of a United Kingdom hospital developed cutaneous mucormycosis due to *Rhizopus microsporus*. An environmental investigation isolated the fungus from contaminated wooden tongue depressors used as splints to immobilize infants' cannulated limbs (18). Infection control personnel should be aware that critically ill infants and other high-risk patients may be at significant risk for the development of aspergillosis.

The implications of this study underscore the need to avoid the use of such nonsterile items in hospital units housing patients at high risk for the development of invasive fungal infections. In particular, a policy regarding the use of sterile medical supplies by hospital staff, including ambulance transport teams, to avoid this type of exposure should be considered when managing high-risk neonates.

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