JOURNAL OF CLINICAL MICROBIOLOGY, Dec. 1997, p. 3032–3036 Vol. 35, No. 12
0095-1137/97/$04.00+0


# Molecular Tracking of *Candida albicans* in a Neonatal Intensive Care Unit: Long-Term Colonizations versus Catheter-Related Infections

BEATRIZ RUIZ-DIEZ,[1] VENANCIO MARTINEZ,[2]† MARIA ALVAREZ,[2] JUAN L. RODRIGUEZ-TUDELA,[1] AND JOAQUIN V. MARTINEZ-SUAREZ[1]*

*Unidad de Micología, Centro Nacional de Microbiología, Instituto de Salud Carlos III, 28220 Majadahonda, Madrid,*[1] *and Hospital Central de Asturias, Oviedo,*[2] *Spain*

Received 27 December 1996/Returned for modification 22 April 1997/Accepted 12 September 1997

**Nosocomial neonatal candidiasis is a major problem in infants requiring intensive therapy. The subjects of this retrospective study were nine preterm infants admitted to the neonatal intensive care unit of the Hospital Central de Asturias between March 1993 and August 1994. The infants were infected with or colonized by *Candida albicans*. Five patients developed *C. albicans* bloodstream infections. A total of 36 isolates (including isolates from catheters and parenteral nutrition) were examined for molecular relatedness by PCR fingerprinting and restriction fragment length polymorphism (RFLP) analysis. The core sequence of phage M13 was used as a single primer in the PCR-based fingerprinting procedure, and RFLP analysis was performed with *C. albicans*-specific DNA probe 27A. Both techniques were evaluated with a panel of eight *C. albicans* reference strains, and each technique showed eight different patterns. With the 36 isolates from neonates, each technique enabled us to identify by PCR and RFLP analysis seven and six different patterns, respectively. The combination of these two methods (composite DNA type) identified eight different profiles. A strain with one of these profiles was present in three patients and in their respective catheters. Patients infected with or colonized by this isolate profile were clustered in time. Among the other patients, each patient was infected over time and at multiple anatomic sites with a *C. albicans* strain with a distinct DNA type. We conclude that *C. albicans* was most commonly producing long-term colonizations, although horizontal transmission probably due to catheters also occurred.**

Fungal infections are important causes of morbidity and mortality in some groups of immunocompromised patients. Premature infants are a high-risk group, and reports of serious infections in many geographical locations are becoming more frequent (15).

Nosocomial neonatal candidiasis is the major fungal infection in infants requiring intensive care therapy (8, 15). Acquisition of *Candida* species may be vertically acquired from the mother or may be nosocomially acquired in the neonatal intensive care unit (NICU) (26). The key to proving horizontal transmission lies in the ability to type yeast isolates. This can be done only with a reproducible typing system. Several molecular epidemiology-based typing methods have been developed to allow for the discrimination of strains of yeast species: restriction endonuclease digestion of chromosomal DNA, Southern hybridization analysis with DNA probes, electrophoretic karyotyping with pulsed-field gel electrophoresis, etc. (14). Recently, PCR has been adapted to the detection of interstrain variation in eukaryotic microorganisms. The procedures may be based on the detection of genomic regions known to be variable among eukaryotes, that is, minisatellite DNA (PCR-based fingerprinting) (11, 12).

The present study describes the use of PCR fingerprinting and DNA hybridization for distinguishing different strains of *Candida albicans* in a retrospective analysis of the epidemiology of nosocomial infections caused by this species in a NICU.

(Part of this work was presented at the 36th Interscience Conference on Antimicrobial Agents and Chemotherapy, New Orleans, La., 15 to 18 September 1996 [18].)

* Corresponding author. Phone: 34-1-5097961. Fax: 34-1-5097966. E-mail address: jmartinz@isciii.es.

† Present address: Centro de Salud El Llano, Gijón, Asturias, Spain.

## MATERIALS AND METHODS

**Isolates from the NICU.** The charts for all patients in the NICU for whom at least one sample was culture positive for *C. albicans* during the period from January 1993 to December 1994 were reviewed. Case finding was accomplished by a review of both microbiology records and pediatric records of nosocomial infections.

Thirty-six clinical isolates of *C. albicans* obtained from different clinical samples in the NICU were studied (Table 1). Additional isolates of *C. tropicalis* from different specimens from the same group of patients were not included in the study due to their low numbers ($n = 3$). Yeast isolates were identified as *C. albicans* on the basis of germ tube formation in horse serum and the production of chlamydospores on cornmeal agar. The results were verified by carbohydrate assimilation and fermentation tests (1). The isolates were stored in 30% glycerol at −20°C. Before genotyping, cells were grown for 48 h at 35°C on Sabouraud dextrose agar.

**Unrelated isolates.** Eight isolates of *C. albicans* from various anatomical sites of four unrelated patients in distinct units of the same hospital were collected and typed (Table 2). This group of isolates was studied to evaluate our techniques and the distribution of the characterized molecular types in the general patient population. The control strains of *C. albicans* used in the study were WO-1 and 3153a (kindly provided by D. Soll, University of Iowa) and ATCC 64551, ATCC 64548, ATCC 64550, ATCC 90028, ATCC 24433, and ATCC 76615 (Table 2).

**Antifungal susceptibility testing.** The MICs of amphotericin B (AmB), flucytosine (5FC), fluconazole (FLZ), and itraconazole (ITZ) were determined by a broth microdilution adaptation of the reference method (13) with RPMI–2% glucose medium and by spectrophotometric reading of the MICs (17). The preparation of RPMI–2% glucose medium has previously been described in detail (9). The MIC endpoints for each antifungal agent were defined according to the reference method (13) (inhibitory concentrations of 100% for AmB and 80% for 5FC, FLZ, and ITZ). Despite the numerous differences between this broth microdilution method and the reference broth macrodilution method of the National Committee for Clinical Laboratory Standards (13), there is close agreement between the results obtained by the two methods (10).

TABLE 1. Summary of results of PCR-based fingerprinting and Southern hybridization of 36 *C. albicans* isolates from neonates

| Patient[a] | Isolate no. | Source | Date of culture (mo.day.yr) | DNA type | | Composite DNA type |
|---|---|---|---|---|---|---|
| | | | | By PCR | With the 27A probe | |
| N-1 | 1 | Urine | 3.18.1993 | A | I | 1 |
| | 2 | Blood | 3.18.1993 | A | I | |
| | 3 | Urine | 3.18.1993 | A | I | |
| | 4 | Blood | 3.20.1993 | A | I | |
| | 5 | Urine | 3.21.1993 | A | I | |
| | 6 | Urine | 3.21.1993 | A | I | |
| | 7 | Urine | 3.21.1993 | A | I | |
| | 8 | Feces | 4.5.1993 | A | I | |
| | 9 | Oral cavity | 6.7.1993 | A | I | |
| | 10 | Anus | 6.28.1993 | A | I | |
| N-2 | 11 | Urine | 5.2.1993 | B | II | 2 |
| N-3 | 12 | Blood | 9.2.1993 | C | II | |
| | 13 | Skin | 9.2.1993 | C | II | |
| | 14 | Urine | 9.2.1993 | C | II | |
| | 15 | Blood | 9.2.1993 | C | II | |
| | 16 | Endotracheal tube | 9.9.1993 | C | II | |
| N-4 | 17 | Blood | 10.17.1993 | D | III | 4 |
| | 18 | Urine | 10.21.1993 | E | III | 5 |
| | 19 | Blood | 11.2.1993 | E | III | |
| | 20 | Catheter | 11.2.1993 | E | III | |
| N-5 | 21 | Catheter | 10.24.1993 | E | III | 5 |
| | 22 | P. nutrition[b] | 11.3.1993 | E | III | |
| | 23 | Oral cavity | 11.10.1993 | E | III | |
| | 24 | Blood | 12.15.1993 | E | III | |
| | 25 | CSF[c] | 12.18.1993 | E | III | |
| | 26 | CSF | 12.24.1993 | E | III | |
| | 27 | CSF | 12.27.1993 | E | III | |
| N-6 | 28 | P. nutrition | 10.24.1993 | E | III | 5 |
| | 29 | Catheter | 11.2.1993 | E | III | |
| N-7 | 30 | Urine | 2.2.1994 | F | IV | 6 |
| N-8 | 31 | Urine | 7.26.1994 | G | ND[d] | 7 |
| | 32 | Blood | 7.29.1994 | G | V | |
| | 33 | Catheter | 8.1.1994 | G | ND | |
| | 34 | Blood | 8.11.1994 | G | V | |
| N-9 | 35 | Urine | 8.6.1994 | D | VI | 8 |
| | 36 | Urine | 8.18.1994 | D | VI | |

[a] N, neonate.
[b] P. nutrition, parenteral nutrition.
[c] CSF, cerebrospinal fluid.
[d] ND, not done.

**DNA extraction.** DNA was isolated by a previously described method (3). Briefly, cells were inoculated from slants into YEPD medium (1% yeast extract, 2% peptone, 2% dextrose), and the vessels were rotated overnight at 100 rpm at 37°C. The cells were harvested by centrifugation (12,000 × *g* for 10 min at room temperature). The pellet was resuspended in the 1 ml of cold SE buffer (20 mM citrate-phosphate buffer, [pH 5.6], 50 mM EDTA, 0.9 M sorbitol), and the mixture was then centrifuged as described above. This process was repeated twice. Cells were resuspended in 400 μl of lysis buffer (50 mM Tris-HCl [pH 7.2], 50 mM EDTA, 3% sodium dodecyl sulfate, 1% β-mercaptoethanol). The resulting mixture was incubated at 65°C for 60 min, and the DNA was further purified by treatment with RNase A (final concentration, 100 μg/ml) (28) and proteinase K (final concentration, 500 μg/ml) and repeated phenol-chloroform-isoamyl alcohol extractions as described previously (3). Finally, the DNA was dissolved in deionized water and was stored at −20°C. The concentration of DNA was estimated by comparing the intensities of the ethidium bromide-stained samples with those samples containing known amounts of bacteriophage lambda DNA on 0.8% (wt/vol) agarose gels (19).

**Primer.** The core sequence of phage M13 (5′→3′; GAGGGTGGCGGTTCT) (25) was used as a primer in these experiments. This was prepared commercially (Pharmacia Biotech, S.A., Barcelona, Spain).

**PCR-based fingerprinting.** PCR-based fingerprints were generated essentially as described by Schönian et al. (21), with some modifications. The reaction mixture contained 25 to 50 ng of genomic DNA; 1 μM oligonucleotide primer; 2.5 U of *Taq* DNA polymerase (Amplitaq; Perkin-Elmer Cetus, Madrid, Spain); 200 μM (each) dATP, dCTP, dGTP, and dTTP; 3.5 mM $MgCl_2$; 10 mM Tris-HCL (pH 8.3); and 50 mM KCl in a total volume of 100 μl. The mixture was overlaid with 60 μl of light mineral oil. Samples were amplified in a DNA thermocycler (Gene ATAQ Controller; Pharmacia) for 30 cycles of 20 s at 95°C, 60 s at 50°C, and 20 s at 72°C, preceded by a 1-min incubation at 94°C and followed by a final 6-min extension at 72°C. Negative control reactions (containing all reaction components except template DNA) were performed for each experiment. The reaction products were analyzed by electrophoresis through 1.3% agarose gels in Tris-acetate-EDTA buffer for 18 h at 35 V and were detected by staining with ethidium bromide (1 μg/ml). The molecular sizes of the

TABLE 2. Genotyping results for *C. albicans* strains used as controls.

| Strain | Origin | Patient | Source | Date of culture (mo.day.yr) | DNA profile | | Composite DNA type |
|---|---|---|---|---|---|---|---|
| | | | | | By PCR | With the 27A probe | |
| Epidemiologically unrelated strains | | | | | | | |
| CNML[a] 851 | HCA[b] | A[c]-1 | Oral | 7.1.1993 | H | VII | 9 |
| CNML 852 | HCA | A-1 | Blood | 7.1.1993 | I | VIII | 10 |
| CNML 901 | HCA | A-1 | Blood | 7.1.1993 | I | VIII | |
| CNML 902 | HCA | A-1 | Urine | 7.1.1993 | I | VIII | |
| CNML 936 | HCA | A-2 | Vaginal | 8.12.1993 | J | IX | 11 |
| CNML 1278 | HCA | A-3 | Vaginal | 3.4.1994 | J | X | 12 |
| CNML 1613 | HCA | A-4 | Blood | 7.14.1994 | K | XI | 13 |
| CNML 1717 | HCA | A-4 | Blood | 9.12.1994 | L | XII | 14 |
| Reference strains | | | | | | | |
| ATCC 64551 | | | | | M | XIII | 15 |
| ATCC 64548 | | | | | N | XIV | 16 |
| ATCC 90028 | | | | | O | XV | 17 |
| ATCC 24433 | | | | | P | XVI | 18 |
| 3153a | | | | | Q | XVII | 19 |
| WO-1 | | | | | R | XVIII | 20 |
| ATCC 64550 | | | | | S | XIX | 21 |
| ATCC 76615 | | | | | T | XX | 22 |

[a] CNML, Centro Nacional de Microbiología/Levaduras collection of fungi.
[b] HCA, Hospital Central de Asturias.
[c] A, adult.

DNA fragments relative to those of molecular size standards derived from Kilobase DNA Marker and 100 Base-Pair Ladder (Pharmacia Biotech) were determined.

To study the reproducibility of these PCR-based fingerprints, the control strains were included in each set of reactions, and all of them rendered identical and reproducible PCR patterns. Although the bands that were produced were sometimes less intense, their positions and their presence or nonpresence were highly consistent. Slight variations in electrophoresis conditions may explain the occasional variations in band intensities.

**Southern hybridization.** *Eco*RI (Pharmacia Biotech)-digested yeast DNA (2 μg) was electrophoresed through a 0.8% agarose gel for 4 h at 40 V/cm. The gel was stained, photographed, and vacuum blotted onto a nylon membrane (Hybond-N$^+$; Amersham, Madrid, Spain) by standard procedures (19). Following linking of the DNA to the membrane at 80°C for 2 h, the filter was hybridized overnight with approximately 2 ng of fluorescein-labelled (ECL System; Amersham) 27A probe DNA per ml (20). The probe consisted of an *Eco*RI fragment specific for *C. albicans* DNA cloned in vector plasmid pUC18. The recombinant plasmid was kindly provided by S. Scherer (Minneapolis, Minn.). Detection of the chemiluminescent signal was performed according to the manufacturer's (Amersham) directions. Band positions were identified by comparison with those of fluorescein-labelled *Hin*dIII digests of bacteriophage lambda, which were used as molecular size markers (Amersham).

The patterns obtained by each technique were read blindly by two persons, without regard to the patient's identity, so that the observers' expectations could not bias the choice of pattern.

## RESULTS

**Pediatric patients and isolates.** During the study period nine patients were found to have samples that were positive for *C. albicans* by culture (Table 1). All patients were prematurely delivered infants (gestational ages, 22 to 34 weeks; birth weights, 565 to 2,340 g), and five of them developed candidemia. All had prior treatment with antibiotics, were intubated, and received intravenous hyperalimentation, and none of them died.

The *C. albicans* isolates from patients N-2 to N-9 were all susceptible to the four antifungal agents tested, Amb with MICS being between 0.12 and 1.0 μg/ml, 5FC MICs being between 0.06 and 0.25 μg/ml, FLZ MICs being between 0.06 and 0.25 μg/ml, and ITZ MICs being between 0.015 and 0.12 μg/ml. Isolates 1 to 10 from patient N-1 were resistant to FLZ (MICs, >128.0 μg/ml) and ITZ (MICs, >8.0 μg/ml) but were susceptible to AmB (MICs, 1.0 μg/ml) and 5FC (MICs, 0.12 μg/ml).

**Molecular typing.** A total of 36 isolates from the nine infants colonized by or infected with *C. albicans* were typed by PCR-based fingerprinting and Southern hybridization. Each technique enabled us to identify seven and six different patterns, respectively (Table 1 and Fig. 1 and 2).

Identical PCR patterns (pattern E) were obtained for isolates 18 to 29 from patients N-4, N-5, and N-6, including the strains from catheters and parenteral nutrition (Table 1 and Fig. 1). Additionally, the isolate in the first culture of blood from patient N-4 (isolate 17) and two isolates from patient N-9 35 (isolates 35 and 36) had the same PCR pattern (pattern D). In contrast, distinctly different PCR profiles were observed for isolates from patients N-1, N-2, N-3, N-7, and N-8 (Table 1). For the seven infants infected with or colonized by two or more isolates of *C. albicans*, the same PCR pattern was observed for isolates obtained on more than one occasion and/or for isolates obtained in specimens from more than one anatomic site.

The relationship among isolates determined by Southern hybridization generally supported that determined by PCR analysis (Table 1). By testing with probe 27A, all isolates from patients N-4, N-5, and N-6 had the same basic profile (type III), allowing for minor band differences (Fig. 1). Southern hybridization also found the blood and urine isolates from infants N-4 (isolate 17) and N-9 (isolates 35 and 36) to be different (types III and VI, respectively), but the profiles for isolates from patients N-2 and N-3 (isolates 11 to 16) were found to be identical. As with PCR analysis, the isolates from infants N-1, N-7, and N-8 were all different, as determined by Southern hybridization. Likewise, the profiles obtained with probe 27 included in each set of reactions demonstrated that multiple isolates from the same patient generally had the same DNA banding pattern (Table 1 and Fig. 1 and 2).

All reference strains were different by both PCR-based fingerprinting and Southern hybridization (Table 2). The results for reference strains ATCC 64551, ATCC 64548, and ATCC 90028 are presented in Fig. 2. Eight strains from four different

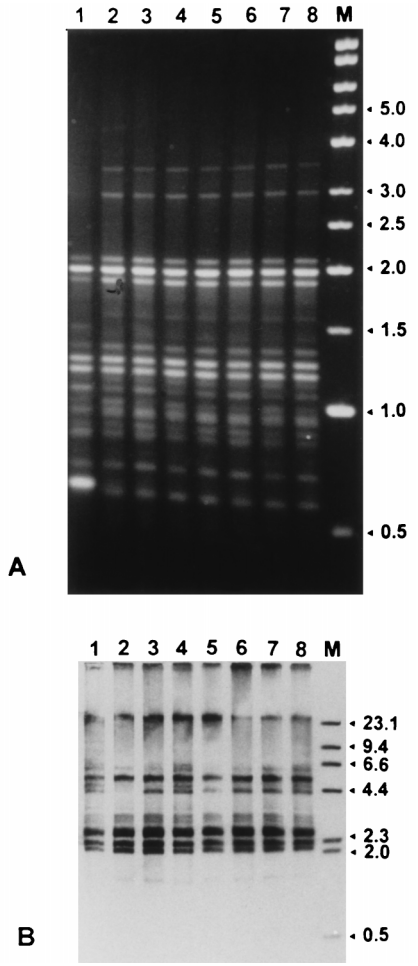


FIG. 1. Genotypic characterization of epidemiologically related *C. albicans* isolates from neonates N-4, N-5, and N-6 (described in Table 1). Lanes M, size markers; sizes are denoted in kilobase pairs; lanes 1 to 3, isolates 17 to 19, respectively, from patient N-4; lanes 4 and 5, isolates 28 and 29, respectively, from patient N-6; lanes 6 to 8, isolates 21, 25, and 27, respectively, from patient N-5. (A) PCR-based fingerprinting analysis with the core sequence of phage M13 as primer (size markers, 100-bp ladder; kilobase DNA marker [Pharmacia]). (B) Hybridization of chromosomal DNA with the fluorescein-labelled 27A probe (size markers, fluorescein-labelled *Hin*dIII digest of lambda phage [Amersham]).

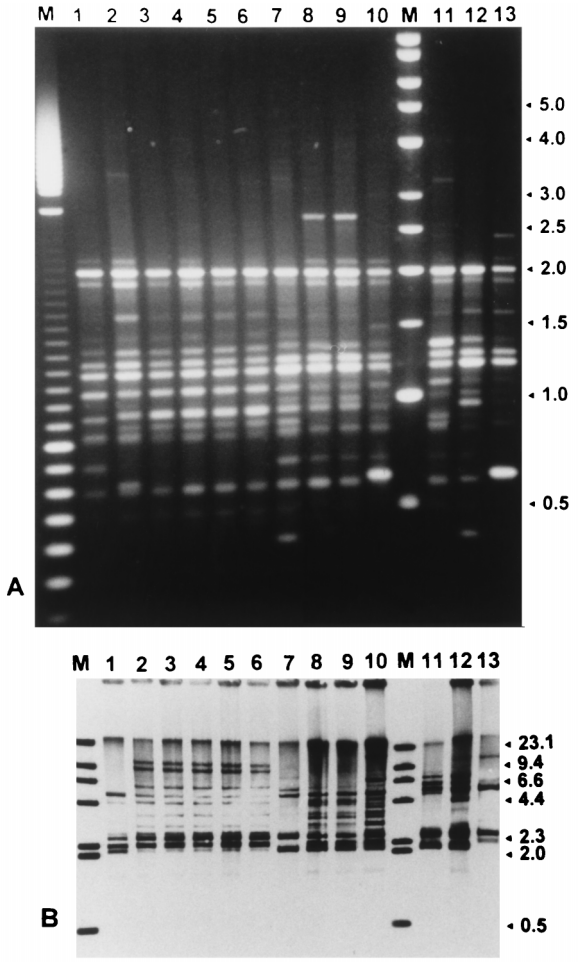


FIG. 2. Genotypic characterization of some selected *C. albicans* isolates from neonates (described in Table 1) and control strains (described in Table 2). Lanes M, size markers; sizes are denoted in kilobase pairs; lanes 1 to 10, isolates 1, 11, 12, 13, 14, 15, 30, 32, 34, and 35, respectively; lanes 11 to 13, strains ATCC 64551, ATCC 64548, and ATCC 90028, respectively. (A) PCR fingerprinting analysis with the core sequence of phage M13 as primer (size markers, 100-bp ladder; kilobase DNA marker [Pharmacia]). (B) Hybridization of chromosomal DNA with the fluorescein-labelled 27A probe (size markers, fluorescein-labelled *Hin*dIII digest of lambda phage [Amersham]).

adult patients had unique molecular types (Table 2). In some cases, multiple isolates from different body sites may be different (patient A-1; Table 2), as we have described previously (2), but in other cases the same patient was successively infected with different strains (patient A-4; Table 2).

**Distribution of molecular types.** When the results obtained by the two typing methods were considered together (composite DNA type), a total of eight DNA types were identified among 36 isolates from nine infants (Table 1). Generally, by this typing methodology, multiple isolates from the same patient had the same DNA banding patterns (Fig. 1 and 2).

A type 1 isolate (which was resistant to FLZ and ITZ) was carried by patient N-1 for a long period (4 months). The DNA type of the *C. albicans* strains from each of patients N-3, N-5, N-8, and N-9 was also maintained over time and, in most cases, among isolates from multiple anatomic sites. On the other hand, patient N-4 was infected with or colonized by two different isolates within the same time period.

Isolates of composite DNA type 5 were the only ones detected over a period of 2 months (Table 1). The fact that 12 of 13 isolates of *C. albicans* from three NICU patients had the same molecular type suggests a nosocomial source for the infections. This suggestion is supported by the observation that catheter tips from all infants were positive for the strains with the same molecular type.

## DISCUSSION

Fungal infections occur in about 5% of premature infants with low birth weights, with *C. albicans* being the species isolated most frequently (15). *C. albicans* may be most commonly transmitted vertically, and then these unique strains can be long-term colonizers of infants (6, 26). Nevertheless, horizontal transmission can also occur, usually in association with central venous catheters or endotracheal tubes (15, 16, 22, 23). With regard to the source of the candidemia, the best-recognized possibilities are also the colonized gut and intravascular catheters (27).

In our study, six patients were infected or colonized with a *C. albicans* isolate with a distinct DNA type over time and among

isolates from multiple anatomic sites. Although infants N-1 and N-2 as well as infants N-8 and N-9 were in the NICU at the same times, their infections appeared to be coincidental and not nosocomial because the molecular types of their respective isolates were different. This is in contrast to patients N-4, N-5, and N-6 (Table 1), who were also clustered in the NICU at the same time and who were infected with or colonized by multiple isolates with the same molecular type, suggesting nosocomial exposure to the yeast. The presumed mechanism for the nosocomial spread of pathogens within the NICU is via the hands of health care workers (7, 14, 24). Unfortunately, because of the retrospective nature of this study, we were unable to obtain additional samples from potentially colonized health care workers in the unit. We are therefore unable to further define the mode of transmission of *C. albicans* in this outbreak. However, the isolation of the same *C. albicans* strain from the parenteral nutrition and catheters of three patients on different dates (Table 1) suggests that the mechanism for the outbreak that we investigated is the extrinsic contamination of catheters and/or infusions. It is noteworthy that this strain was present in the NICU for 2 months. Attention to simple hand-washing procedures and scrupulous care of intravascular devices should minimize the risk of nosocomial transmission and the incidence of candidemia in the NICU. The use of molecular biology-based methods to investigate potential outbreaks should also help to define the extent of the problem.

The molecular epidemiology of infections caused by *C. albicans* has been studied by different techniques (14). In order to corroborate the typing results, it seems necessary to combine several techniques that are different in their molecular targets, allowing for the more accurate identification of *C. albicans* strains. In the present study we have used two different molecular biology-based typing methods for strain identification. Both techniques have proven to be highly reproducible and applicable to a large number of *C. albicans* isolates (2, 4, 5). Only eight different types were identified among the 36 isolates from nine infants, whereas six types were identified among isolates from four unrelated patients in other hospital units. None of the molecular types of the organisms isolated in the NICU was present in the 16 control strains (Table 2), confirming the discriminatory value of these techniques.

Although this study included only a limited number of documented cases of fungemia, larger studies of fungemia in NICUs are warranted.

## ACKNOWLEDGMENTS

The work at the Instituto de Salud Carlos III was supported in part by grants BIO94-0181 from the Comisión Interministerial de Ciencia y Tecnología and 96/0598 from the Fondo de Investigaciones Sanitarias, both of Spain. B.R.-D. is a fellow of the Instituto de Salud Carlos III (grant 96/4027).

## REFERENCES

1. **Barnett, J. A., R. W. Payne, and D. Yarrow.** 1990. Yeasts: characteristics and identification, 2nd ed. Cambridge University Press, Cambridge, United Kingdom.
2. **Berenguer, J., T. M. Díaz-Guerra, B. Ruiz-Díez, J. C. Bernaldo de Quirós, J. L. Rodríguez-Tudela, and J. V. Martínez-Suárez.** 1996. Genetic dissimilarity of two fluconazole-resistant *Candida albicans* strains causing meningitis and oral candidiasis in the same AIDS patient. J. Clin. Microbiol. **34:** 1542–1545.
3. **Bowman, B. H.** 1993. A model PCR/probe system for the identification of fungal pathogens, p. 423–430. *In* D. H. Persing, T. F. Smith, F. C. Tenover, and T. J. White (ed.), Diagnostic molecular microbiology: principles and applications. American Society for Microbiology, Washington, D.C.
4. **Díaz-Guerra, T. M., J. V. Martínez-Suárez, F. Laguna, and J. L. Rodríguez-Tudela.** 1997. Comparison of four molecular typing methods for evaluating genetic diversity among *Candida albicans* isolates from human immunodeficiency virus-positive patients with oral candidiasis. J. Clin. Microbiol. **35:** 856–861.
5. **Díaz-Guerra, T. M., B. Ruiz-Díez, J. L. Rodr V. Martínez-Suárez.** 1996. Comparison of PCR-fingerprinting *versus* RFLPs for strain delineation of *Candida albicans* from clinical sources, abstr. J22, p. 222. *In* Program and abstracts of the 36th Interscience Conference on Antimicrobial Agents and Chemotherapy. American Society for Microbiology, Washington, D.C.
6. **Faix, R. G., D. J. Finkel, R. D. Andersen, and M. K. Hostetter.** 1995. Genotypic analysis of a cluster of systemic *Candida albicans* infections in a neonatal intensive care unit. Pediatr. Infect. Dis. J. **14:**1063–1068.
7. **Finkelstein, R., G. Reinhartz, N. Hashman, and D. Merzbach.** 1993. Outbreak of *Candida tropicalis* fungemia in a neonatal intensive care unit. Infect. Control Hosp. Epidemiol. **14:**587–590.
8. **Johnson, D. E., T. R. Thompson, T. P. Green, and P. Ferrieri.** 1984. Systemic candidiasis in very low-birth-weight infants (<1,500 grams). Pediatrics **73:** 138–143.
9. **Martínez-Suárez, J. V., and J. L. Rodríguez-Tudela.** 1995. Patterns of in vitro activity of itraconazole and imidazole antifungal agents against *Candida albicans* with decreased susceptibility to fluconazole from Spain. Antimicrob. Agents Chemother. **39:**1512–1516.
10. **Martínez-Suárez, J. V., and J. L. Rodríguez-Tudela.** 1996. In vitro activities of semisynthetic pneumocandin L-733,560 against fluconazole-resistant and -susceptible *Candida albicans* isolates. Antimicrob. Agents Chemother. **40:** 1277–1279.
11. **Meyer, W., T. G. Mitchell, E. Z. Freedman, and R. Vilgalys.** 1993. Hybridization probes for conventional DNA fingerprinting used as single primers in the polymerase chain reaction to distinguish strains of *Cryptococcus neoformans*. J. Clin. Microbiol. **31:**2274–2280.
12. **Mitchell, T. G., R. L. Sandin, B. H. Bowman, W. Meyer, and W. G. Merz.** 1994. Molecular mycology: DNA probes and applications of PCR technology. J. Med. Vet. Mycol. **32:**351–366.
13. **National Committee for Clinical Laboratory Standards.** 1992. Reference method for broth dilution antifungal susceptibility testing of yeasts. Proposed standard M27-P. National Committee for Clinical Laboratory Standards, Villanova, Pa.
14. **Pfaller, M. A.** 1995. Epidemiology of fungal infections: the promise of molecular typing. Clin. Infect. Dis. **20:**1535–1539.
15. **Phillips, G., and C. Colledge.** 1991. Fungal infections in neonates. J. Antimicrob. Chemother. **28:**159–161.
16. **Reagan, D. R., M. A. Pfaller, R. J. Hollis, and R. P. Wenzel.** 1995. Evidence of nosocomial spread of *Candida albicans* causing bloodstream infection in a neonatal intensive care unit. Diagn. Microbiol. Infect. Dis. **21:**191–194.
17. **Rodríguez-Tudela, J. L., and J. V. Martínez-Suárez.** 1994. Improved medium for fluconazole susceptibility testing of *Candida albicans*. Antimicrob. Agents Chemother. **38:**45–48.
18. **Ruiz-Díez, B., V. Martínez, M. Alvarez, J. L. Rodr–iguez-Tudela, and J. V. Martínez-Suárez.** 1996. Molecular tracking of *Candida albicans* in a neonatal intensive care unit (NICU): long-term colonizations *vs* catheter-related infections, abstr. J23, p. 222. *In* Programs and abstracts of the 36th Interscience Conference on Antimicrobial Agents and Chemotherapy. American Society for Microbiology, Washington, D.C.
19. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
20. **Scherer, S., and D. A. Stevens.** 1988. A *Candida albicans* dispersed, repeated gene family and its epidemiologic applications. Proc. Natl. Acad. Sci. USA **85:**1452–1456.
21. **Schönian, G., O. Meusel, H.-J. Tietz, W. Meyer, Y. Gräser, I. Tausch, W. Presber, and T. G. Mitchell.** 1993. Identification of clinical strains of *Candida albicans* by DNA fingerprinting with the polymerase chain reaction. Mycoses **36:**171–179.
22. **Sherertz, R. J., K. S. Gledhill, K. D. Hampton, M. A. Pfaller, L. B. Givner, J. S. Abramson, and R. G. Dillard.** 1992. Outbreak of *Candida* bloodstream infections associated with retrograde medication administration in a neonatal intensive care unit. J. Pediatr. **120:**455–461.
23. **Solomon, S. L., H. Alexander, and J. W. Eley.** 1996. Nosocomial fungemia in neonates associated with intravascular pressure-monitoring devices. Pediatr. Infect. Dis. J. **5:**680–685.
24. **Strausbaugh, L. J., D. L. Sewell, T. T. Ward, M. A. Pfaller, T. Heintzman, and R. Tjoelker.** 1994. High frequency of yeast carriage on hands of hospital personnel. J. Clin. Microbiol. **32:**2299–2300.
25. **Vassart, G., M. Georges, R. Monsieur, H. Brocas, A. S. Lequarre, and D. Christophe.** 1987. A sequence in M13 phage detects hypervariable minisatellites in human and animal DNA. Science **235:**683–684.
26. **Waggoner-Fountain, L. A., M. W. Walker, R. J. Hollis, M. A. Pfaller, J. E. Ferguson II, R. P. Wenzel, and L. G. Donowitz.** 1996. Vertical and horizontal transmission of unique *Candida* species to premature newborns. Clin. Infect. Dis. **22:**803–808.
27. **Wey, S. B., M. Mori, M. A. Pfaller, R. F. Woolson, and R. P. Wenzel.** 1989. Risk factors for hospital-acquired candidemia. A matched case-control study. Arch. Intern. Med. **149:**2349–2353.
28. **Yoon, C. S., and D. A. Glawe.** 1993. Pretreatment with RNase to improve PCR amplification of DNA using 10-mer primers. BioTechniques **14:**908–910.