Molecular Investigation of a Fungemia Outbreak Due to *Candida parapsilosis* in an Intensive Care Unit

Murat Dizbay[1], Ayse Kalkanci[2], Busra Ergut Sezer[1], Firdevs Aktas[1], Sibel Aydogan[2], Isil Fidan[2], Semra Kustimur[2] and Takashi Sugita[3]
[1]Gazi University Faculty of Medicine, Department of Clinical Microbiology and Infectious Diseases and Infection Control Committee; [2]Department of Microbiology; Ankara, Turkey; [3]Meiji Pharmaceutical University, Department of Microbiology Kiyose; Tokyo, Japan

We investigated a nosocomial cluster of four *Candida parapsilosis* fungemia episodes that occurred in a neurological intensive care unit over a two-week period. The four infected patients had received parenteral nutrition through central lines, and all four had catheter-related candidemia. All of the isolates were susceptible to all of the antifungals tested, including amphotericin B, fluconazole, voriconazole, and caspofungin. They had strictly related fingerprints, based on randomly amplified polymorphic DNA analysis. Additional DNA sequencing data revealed that they were same strain. Although no isolate of *Candida parapsilosis* was recovered from other clinical, surveillance, or environmental samples, nosocomial spread of this yeast ceased, following the reinforcement of infection-control measures. *Candida parapsilosis* may require an intravascular foreign body to cause fungemia, but this outbreak shows that it can be transmitted nosocomially and can cause epidemics.
Key-Words: Candidemia, *Candida parapsilosis*, nosocomial outbreak, intensive care unit, RAPD, DNA sequencing.

Candida species are the third most common cause of nosocomial bloodstream infections (BSI) in intensive care units, with a 47.1 % crude mortality rate in the United States [1]. Candidemias have been found to be associated with increasing mortality, length of hospital stay and hospital costs [2,3]. Among the *Candida* species identified to date, *Candida albicans* still ranks first, but recent studies show a shift towards increasing prevalence of non-*albicans Candida* spp. [4-6]. This change has been attributed partly to the widespread use of antifungal agents and to an increasing number of immunocompromised hosts; however, it may also reflect an increasing awareness that non-*albicans Candida* isolates are important opportunistic pathogens. In a neurological intensive care unit (NICU) of our hospital, we observed a nosocomial cluster of fungemia due to *Candida parapsilosis* that affected four patients. We retrospectively examined the characteristics of the candidemia episodes in these patients and employed molecular techniques to determine whether the fungal isolates from these patients were of clonal origin.

Material and Methods

Patients

Four cases of *C. parapsilosis* fungemia were identified within a two-week period in an NICU at the University Hospital of Gazi, a 1,000-bed tertiary care hospital, in Ankara, Turkey. The NICU has one patient room (a total of seven beds), mainly occupied by elderly patients affected by stroke, cerebrovascular disease, or intracranial hemorrhage. At the time the candidemia cases were detected, the four affected patients were in same room. Their periods of hospitalization overlapped, and they were all cared for by NICU staff members. The clinical characteristics of the four patients are summarized in Table 1.

Active surveillance program is routinely performed for infection control in the NICU. After the second case of *C. parapsilosis* fungemia was diagnosed, molecular epidemiological investigations were initiated. Physicians and nursing staff of the intensive care unit were screened for oral and hand carriage of *Candida* spp. Extensive sampling was undertaken from fomites and other environmental sources of the ward (floors, disinfectant solutions, multi-dose vials, infusion pumps, commercially prepared parenteral nutrition bags, and other medical equipments), and cultures were made. Finally, compliance with standard infection control measures, including rigorous hand-washing, was emphasized.

Strains

All of the *C. parapsilosis* strains were isolated from the cultures of blood specimens or central venous catheter (CVC) tips from the four patients. The yeastlike organisms were identified according to their morphological characteristics, biochemical profiles obtained with the ID 32C kit (bioMérieux, France), assimilation of six additional sugars, and growth morphology on corn meal agar plates.

Antifungal Susceptibility Tests

Antifungal susceptibility testing for amphotericin B, fluconazole, voriconazole and caspofungin was performed for each of the strains by the microdilution method, according to the Clinical Laboratory Standards Institution (CLSI, formerly NCCLS) reference microdilution method documented in M27-A2 [7].

Randomly Amplified Polymorphic DNA and DNA Sequencing

All of the *C. parapsilosis* isolates were typed by randomly amplified polymorphic DNA (RAPD). Two oligonucleotide primers, forward 5'-ATG ACT CCA GCT GGT TC-3' and reverse 5'- TAG ATC AAG AAT GCA, were used for RAPD analysis. Briefly, approximately 10 ng *Candida* DNA was added to a 0.5 mL microfuge tube containing 20 pmol

Received on 12 April 2008; revised 20 September 2008.
Address for correspondence: Dr. Isil Fidan. Gazi University, Faculty of Medicine, Department of Medical Microbiology. Dekanlik Binasi 2.Kat Besevler/ANKARA 06500, TURKEY. Phone: +90 312 2024626. Fax: +90 312 4358632. E-mail: isilfidan@yahoo.com.

oligonucleotide primer, 250 µM each of dATP, dTTP, dCTP and dGTP, 3 mM $MgCl_2$, 2.5 U Taq DNA polymerase and 10X buffer in a final volume of 25 µL (all of the chemicals were obtained from Metis Biyoteknoloji, Turkey). The amplification procedure was performed with an initial denaturation step at 94°C for 1 min, followed by 45 cycles of, 30 s at 94°C, 60s at 42°C, and 90s at 72°C, with a final extension at 72°C for 7 min in a thermal cycler (Hybaid, UK). The products were separated in 2% (w/v) agarose gels containing 0.5 µg ethidium bromide mL^{-1} and viewed on a UV transluminator.

DNA sequencing was additionally performed for identifying *C. parapsilosis*. The D1/D2 regions of 26S rDNA in the rRNA gene were sequenced directly from PCR products using the primer pair NL1 (GCA TAT CAA TAA GCG GAG GAA AAG) and NL4 (GGT CCG TGT TTC AAG ACG G). The PCR products were sequenced using an ABI 310 DNA sequencer and a BigDye Terminator Cycle Sequencing Ready Reaction kit (Perkin-Elmer), according to the manufacturer's instructions. The sequence data were analyzed using the National Center for Biotechnology Information (Bethesda, Md., USA) BLAST system (available at http://www.ncbi.nlm.nih.gov/BLAST/).

Results

Strains

Ten samples obtained from blood specimens or catheter tips removed from the four patients were culture-positive for yeast organisms. All isolates showed identical phenotypic characteristics and were identified as *C. parapsilosis*. The quality of identification of *C. parapsilosis* with ID 32C was acceptable in all strains. Yeasts were identified morphologically as *C. parapsilosis* on corn meal agar plates.

Antifungal Susceptibility Tests

All isolates were susceptible to amphotericin B (MIC, 0.125 µg/mL), fluconazole (MIC, 0.125 µg/mL), voriconazole (MIC, 0.250 µg/mL) and caspofungin (MIC, 0.062 µg/mL).

RAPD Analysis

RAPD analysis was performed for further characterization of *C. parapsilosis* isolates. The overall RAPD profiles of the clinical isolates were very similar to each other, but very different from the profiles of the *C. parapsilosis* ATCC 22019 reference strain (Figure 1). These results suggest that the isolates from the four patients were derived from the same strain. *Candida parapsilosis* was not isolated from the surveillance cultures of the healthcare workers or from environmental sources. The review of infection control procedures used in the NICU and in the pharmacy did not show any relapse.

DNA Sequences

The DNA sequences of all the isolates completely matched to that of *C. parapsilosis* from the GenBank DNA database. Therefore, all the isolates were identified as a single strain of *C. parapsilosis*. Clonal spread was confirmed by DNA sequencing results (Figure 2).

Discussion

Numerous outbreaks of systemic candidiasis in hospitalized patients have been reported [2,4,8]. *Candida* spp. was found to be responsible for outbreaks associated with parenteral nutrition, invasive devices, and intraoperative contamination, as well as cross-infection via the hands of hospital personnel or to extrinsic contamination of parenteral nutrition solutions that occurred during preparation or administration [3,5,6,8]. Since the hands of healthcare workers have been documented to be a reservoir of *Candida* spp., exogenous infection or cross-infection of the patients can be common [9,10]. Although an investigation was retrospectively carried out in each of these outbreaks, no definitive source of the fungal strains could be found.

Candida parapsilosis particularly affects critically ill neonates and ICU patients, likely because of its association with parenteral nutrition and central catheter lines [11,12]. The affinity of *C. parapsilosis* for foreign material is shown by infections [13]. *Candida parapsilosis* is increasingly responsible for hospital outbreaks, and the hands of healthcare workers can be the predominant environmental source. Total parenteral nutrition (TPN) solutions may promote *C. parapsilosis* adhesion and growth. Recently, biofilm-forming potential was cited as a reason that patients with *C. parapsilosis*-infected catheters should have the device removed [14].

The prevalence of *C. parapsilosis* fungemia has increased over the years, and now, in some areas, *C. parapsilosis* is the second most common species found in patients with candidemia [15]. Risk factors for systemic infections with *C. parapsilosis* are prolonged neutropenia, aggressive chemotherapy, or use of broad spectrum antibacterial agents, and the alteration of local defenses by the obstruction or breakdown of the skin and mucosa. Epidemiological data obtained from fungemia patients revealed that non-*albicans* strains are gaining importance because of low-susceptibility rates to licensed antifungal drugs [16]. The reasons for the rising incidence of *C. parapsilosis* candidemia are not completely known, although indwelling venous catheters and parenteral nutrition have been recognized as specific risk factors [4,17,18]. Most experimental studies have indicated that the adherence of *C. parapsilosis* to acrylic surfaces is greater than that of *C. albicans* [19].

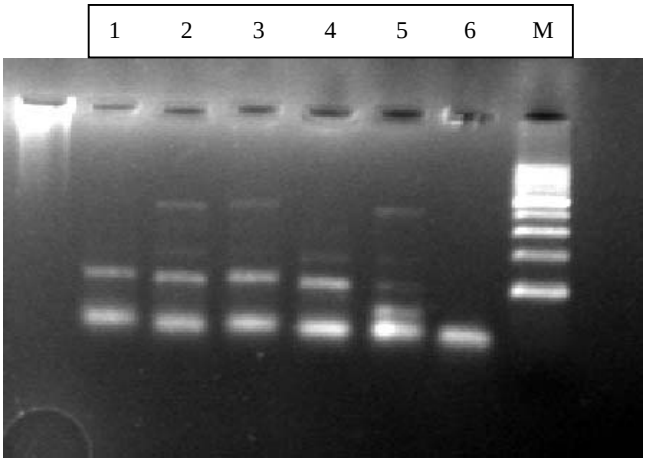
We identified four episodes of *C. parapsilosis* fungemia in four patients (mean age, 75.8 years) admitted to a NICU of a tertiary care hospital over a two-week period. All of the patients had neurological disease, had received parenteral nutrition solutions through a CVC, and had been cared for by personnel of the ICU during overlapping periods. All of the four patients had persistent CVC-related candidemia. All of the patients underwent CVC removal and received antifungal therapy following the diagnosis of candidemia. Fungemia

Table 1. Clinical characteristics of four patients with nosocomially-transmitted *Candida parapsilosis* fungemia.

Characteristic	Patient 1	Patient 2	Patient 3	Patient 4
Age (years)/sex	73/male	79/female	78/male	73/male
Underlying disease	Intracranial infarction	Cerebrovascular disease	Syncope	Generalized tonic-clonic seizure
Potential risk factors for candidemia[a]				
CVC	Yes	Yes	Yes	Yes
Mechanic ventilation	Yes	Yes	Yes	Yes
Bladder catheter	Yes	Yes	Yes	Yes
Parenteral nutrition	Yes	Yes	Yes	Yes
BSA	Yes	Yes	Yes	Yes
VAP	Yes	No	Yes	Yes
Diabetes mellitus	No	No	No	No
Malignancy	No	No	No	No
No. of positive blood cultures (culture site)	3 (peripheral veins)	2 (peripheral veins)	4 (peripheral veins)	1 (peripheral vein)
Therapy				
Antifungal therapy	AMB, L-AMB, Caspofungin	AMB	Fluconazole,	AMB
Catheter removal	Yes	Yes	Yes	Yes
Catheter-related candidemia	Yes	Yes	Yes	Yes
Outcome				
Candidemia	Cleared	Cleared	Failed	Cleared
Clinical	Excitus	Recovery	Excitus	Recovery

CVC, central venous catheter; ND, not determined; AMB, amphotericin B; L-AMB, liposomal amphotericin B; VAP, ventilation associated pneumonia; BSA, broad spectrum antibiotics. [a]Risk factors occurring within one week prior to the onset of candidemia.

Figure 1. Random amplified polymorphic DNA fingerprints of *Candida parapsilosis* clinical isolates. Lanes 1 to 4, isolates from the blood of patients, lane 5, *C. parapsilosis* clinical isolate, lane 6, *C. parapsilosis* ATCC 22019 reference strain. lane M, lambda HaeIII molecular size marker.

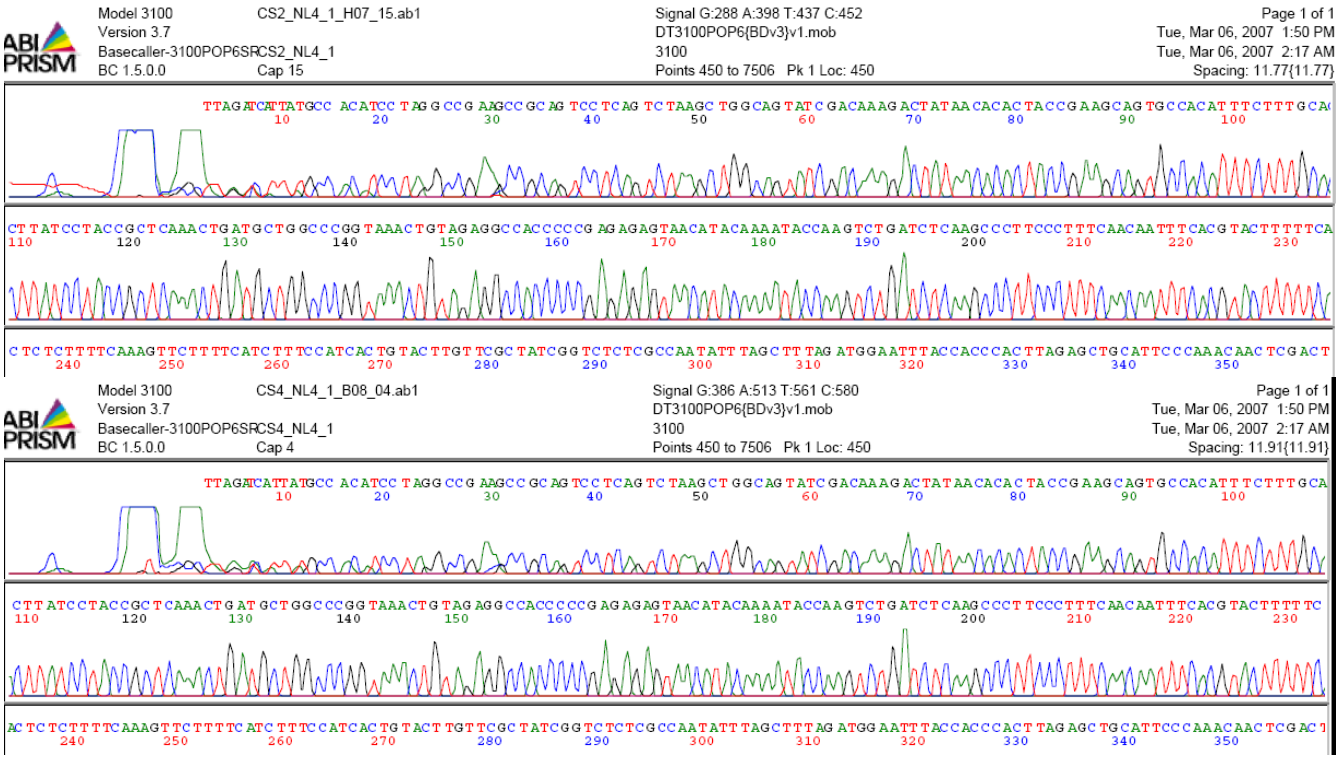


cleared in two of the patients, but two of them died later as a consequence of their underlying disease.

All of the *C. parapsilosis* strains isolated from blood samples were identified in our laboratory by morphological and biochemical methods that are used by many clinical laboratories. Recent studies have shown that fungal 26S rDNA and ITS region sequence data facilitate earlier and more reliable identification than phenotypic methods. Oligonucleotide primers based on the sequences of 18S or 26S rDNA have been designed for PCR and used in the detection of fungi. Unfortunately, these sophisticated molecular methods are available only in reference laboratories.

Molecular epidemiology methods are required for the demonstration of clonal relationships among isolates. The RAPD or arbitrarily primed polymerase chain reaction (AP-PCR) analysis is technically simple and often detects variations among *Candida* spp. isolates that are not discriminated with other typing methods. Though it has good discriminatory capacity, low cost and is easy to use, morphotyping has low reproducibility [20]. We used RAPD analysis to investigate the molecular epidemiology of blood isolates recovered from the patients hospitalized in the NICU of a tertiary care hospital. The clinical value of a single procedure, such as RAPD analysis, for determining both species and biotype is most clear in the area of molecular epidemiology. According to some authors, given the increase in nosocomial infections caused by *Candida* species, there is an urgent need for a rapid and simple procedure that would allow for the analysis of both the outbreaks and the incidence of person-to-person transmission of these organisms [21]. As such, more in-depth subsequent epidemiological analyses are required to more accurately clarify whether there are simply genetic similarities in a characteristic cloned population among these isolates or whether hospital

Figure 2. Nucleotide sequence of the D1/D2 region of the large (26S) subunit rRNA genes.



procedures help disseminate the agents among these patients through cross-infections. DNA sequencing is such method that can be used for both in the identification of species and the demonstration of clonal spread [22]. In our study, *C. parapsilosis* isolates obtained from the patients were identified as the same strain by direct DNA sequence analysis. The DNA examination results therefore suggest that *C. parapsilsosis* was transmitted to the patients in the hospital environment.

At the time of our investigation, we were unable to identify *C. parapsilosis* in any of the other clinical or environmental samples. Since patient screening was not carried out at hospital admission, and the cluster was only investigated retrospectively, it is difficult to determine whether these patients became infected from a common source within the hospital environment or if the yeast was transmitted from the first patient to the others. Although the outbreak strain was not isolated from the hands of the ward personnel during our retrospective investigation, the transmission of *C. parapsilosis* between patients and staff would seem likely. Indeed, the nosocomial spread of fungal infections ceased just after the standard infection control measures were reinforced in the ICU.

In conclusion, this outbreak shows the potential for nosocomial epidemic transmission of *C. parapsilosis*, an organism that may require an intravascular foreign body to cause fungemia. Investigation of an outbreak by molecular methods has diagnostic value when the primary source can not be found. This study proves the importance of a molecular approach for investigating suspected outbreaks in hospital survey.

References

1. Wisplinghoff H., Bischoff T., Tallent S.M., et al. Nosocomial bloodstream infections in US hospitals: analysis of 24,179 cases from a prospective nationwide surveillance study. Clin Infect Dis **2004**;39:309-17.
2. Zaoutis T.E., Argon J., Chu J., Berlin J.A., Walsh T.J., Feudtner C. The epidemiology and attributable outcomes of candidemia in adults and children hospitalized in the United States: a propensity analysis. Clin Infect Dis **2005**;41:1232-9.
3. Falagas M.E., Apostolou K.E., Pappas V.D. Attributable mortality of candidemia: a systematic review of matched cohort and case-control studies. Eur J Clin Microbiol Infect Dis **2006**;25:419-25.
4. Barberino M.G., Silva N., Rebounças C., et al Evaluation of blood stream infections by *Candida* in three tertiary hospitals in Salvador, Brazil: A case-control study. Brazil J Infect Dis **2006**;10:36-40.
5. Bakir M., Cerikcioglu, N., Barton R., Yagci A. Epidemiology of candidemia in a Turkish tertiary care hospital. APMIS **2006**;111:601-10.
6. Warren D.K., Quadir W.W., Hollenbeak C.S., et al. Attributable cost of catheter-associated bloodstream infections among intensive care patients in a nonteaching hospital. Crit Care Med **2006**;34:2243-4.
7. National Committee for Clinical Laboratory Standards (2002) Reference method for broth dilution antifungal susceptibility testing of yeasts; approved Standard. Second edition, document M27-A2. National Committee for Clinical Laboratory Standards, Wayne, Pa., **2002**.
8. Shin J.H., Og Y.G., Cho D., et al. Molecular epidemiological analysis of bloodstream isolates of *Candida albicans* from a university hospital over a five-year period. J Microbiol **2005**;43:546-54.

9. Huang Y.C., Lin T.Y., Leu H.S., Wu J.L., Wu J.H. Yeast carriage on hands of hospital personnel working in intensive care units. J Hosp Infect **1998**;39:47-51.
10. Bonassoli L.A., Bertoli M., Svidzinski T.I.E. High frequency of *Candida parapsilosis* on the hands of healthy hosts. J Hosp Infect **2005**;59:159-62.
11. Levin A.S., Costa S.F., Mussi N.S., et al. *Candida parapsilosis* fungemia associated with implantable and semi-implantable central venous catheters and hands of health care workers. Diagn Microbiol Infect Dis **1998**;30:243-9.
12. Sadfar A., Fine J.P., Maki D.G. Meta-analysis: methods for diagnosing intravascular device-related bloodstream infection. Ann Intern Med **2005**;142:451-66.
13. Lupetti A., Tavanti A., Davini P., et al. Horizontal transmission of *Candida parapsilosis* candidemia in a neonatal intensive care unit. J Clin Microbiol **2002**;40:2363-9.
14. Barchiesi F., Caggiano G., Di Francesco L.F., et al. Outbreak of fungaemia due to *Candida parapsilosis* in a pediatric oncology unit. Diagn Microbiol Infect Dis **2004**;49:269-71.
15. Clark T.A., Slavinski S.A., Morgan J., et al. Epidemiologic and molecular characterization of an outbreak of *Candida parapsilosis* bloodstream infections in a community hospital. J Clin Microbiol **2004**;42:4468-72.
16. Almirante B., Rodrý´guez D., Cuenca-Estrella M., et al. Epidemiology, risk ractors, and prognosis of C*andida parapsilosis* bloodstream ýnfections: case-control population-based surveillance study of patients in Barcelona, Spain, from 2002 to 2003. J Clin Microbiol **2006**;44:1681-5.
17. Bassetti M., Righi E., Costa A., et al. Epidemiological trends in nosocomial candidemia in intensive care. BMC Infect Dis **2006**; 6:21 (http://www.biomedcentral.com/1471-2334/6/21).
18. Kuhn D.M., Chandra J., Mukherjee P.K., Ghannoum M.A. Comparison of biofilms formed by *Candida albicans* and *Candida parapsilosis* on bioprosthetic surfaces. Infect Immun **2002**;70:878-88.
19. Ergon, M.C., Yucesoy M. Molecular epidemiology of *Candida* species isolated from urine at an intensive care unit. Mycoses **2005**;48:126-31.
20. Humhreys H. Does molecular typing make any contribution to the care of patients with infection? Clin Microbiol Infect **2004**;10:269-71.
21. McGinnis M.R., Nunn G.B. Sequence based fungal identification and classification. In: Molecular Microbiology Diagnostic Principles and Practice. ASM Press Washington DC, p: 561-8, **2004**.
22. Yamamoto T., Ueta E., Kamatani T., Osaki T. DNA identification of the pathogen of Candidal aspiration pneumonia induced in the course of oral cancer therapy. J Med Microbiol **2005**;54:493-6.