Sequence-identification of *Candida* species isolated from candidemia

Naeimeh Fathi¹, Rasoul Mohammadi¹,², Mohammad Amin Tabatabaieefar³, Mohammad Ghahri⁴, Seyedeh Zahra Sadrossadati⁵

¹Department of Medical Parasitology and Mycology, School of Medicine, ²Infectious Diseases and Tropical Medicine Research Center, ³Department of Genetics and Molecular Biology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, ⁴Department of Biology, School of Applied Sciences, Imam Hossein University, Tehran, ⁵Department of Biology, Ashkezar Branch, Islamic Azad University, Ashkezar, Yazd, Iran

**Abstract**

**Background:** *Candida* species are the most prevalent cause of invasive fungal infections such as candidemia. Candidemia is a lethal fungal infection among immunocompromised patients worldwide. Main pathogen is *Candida albicans* but a global shift in epidemiology toward non-*albicans* species have reported. Species identification is imperative for good management of candidemia as a fatal infection. The aim of the study is to identify *Candida* spp. obtained from candidemia and determination of mortality rate among this population.

**Materials and Methods:** The study was performed during February 2014 to March 2015 in Tehran, Iran. Two-hundred and four blood cultures were evaluated for fungal bloodstream infection. Identification of isolates was carried out using phenotypic tests and polymerase chain reaction sequencing technique.

**Results:** Twenty-two out of 204 patients (10.8%) had candidemia. *Candida parapsilosis* was the most prevalent species (45.4%), followed by *C. albicans* (31.8%) and *Candida glabrata* (22.7%). Male to female sex ratio was 8/14.

**Conclusions:** The emergence of resistant strains of *Candida* species should be considered by physicians to decrease the mortality of this fatal fungal infection by appropriate treatment.

**Key Words:** *Candida parapsilosis*, candidemia, sequencing

**INTRODUCTION**

Candidemia is a significant public health problem among immunocompromised patients worldwide. During the last two decades, epidemiologic studies show that *Candida* species are the fourth most prevalent cause of nosocomial bloodstream infection (BSI) and are connected to high morbidity and mortality.¹⁻³ Risk factors contain exposure to broad-spectrum antibiotics, cytotoxic chemotherapy, corticosteroids, prolonged use of intravascular catheters, and dialysis.⁴⁻⁵ Invasive candidiasis (IC) involving BSI continues to increase worldwide and approach 50%.⁶⁻⁷ Main pathogen is *Candida albicans* but a global shift toward non-*albicans* species such as *Candida tropicalis*, *Candida krusei*, and *Candida glabrata* have detected.⁸⁻¹¹ This epidemiologic changing is of concern...
due to the varying susceptibility to antifungal agents with some of these emerging non-\textit{albicans} species.\cite{12,13} Due to the varying antifungal susceptibility of clinical isolates, \textit{Candida} spp. identification is necessary for good management of candidemia as a fatal infection. The goal of the present investigation is to identify \textit{Candida} spp. obtained from candidemia and determination of mortality rate among this population.

\section*{MATERIALS AND METHODS}

\subsection*{Strains}
Between February 2014 and March 2015, 204 blood cultures were evaluated for fungal infection. The samples were collected from Imam Khomeini Hospital Complex, Pediatrics Center, Baghiatallah, Hazrat Rasool, Imam Hossein, and Shariati Hospitals, Tehran, Iran. Direct microscopy, chlamydoconidia, and germ-tube production, and subcultured onto CHROMagar \textit{Candida} (Paris, France) were used for phenotypic identification.

\subsection*{Molecular identification}

\subsubsection*{Polymerase chain reaction}
DNA extraction was performed using of Whatman FTA filter matrix technology as delineated formerly.\cite{14,15} Briefly, a loopful of a single colony was suspended in 80–100 $\mu$l of distilled water and 5 $\mu$l of the suspension was transferred to a disc of FTA card (4 mm in diameter) and incubated at 25°C for at least 5 h. The dried papers were eluted in 400 $\mu$l sterile water for 10 s, then the paper was transferred to a new microtube containing 40 $\mu$l distilled water and incubated at 95°C for 15 min. The paper discs were removed, and the water including DNA was used for polymerase chain reaction (PCR) and stored at -20°C. PCR amplification and DNA sequencing of the ITS1-5.8S rDNA-ITS2 region was used for the identification of all \textit{Candida} strains. The universal fungal primers ITS1 (5'-TCC GTA GGT GAA CCT GCG G-3') and ITS4 (5'-TCC GCT TAT GGA TAT GC-3') were used to amplify the entire ITS rDNA region.\cite{16} PCR mixture contained 5 $\mu$l of 10X reaction buffer, 0.4 mM dNTPs, 1.5 mM MgCl$_2$, 2.5 U of Taq polymerase, 30 pmol of each ITS1 and ITS4 primers, and 2 $\mu$l of extracted DNA in a final volume of 50 $\mu$l.

\subsection*{Sequencing}
The amplicons were purified using the ethanol purification method, and cycle sequencing reactions in forward direction were performed (Bioneer, Korea). The sequencing products were analyzed with Chromas 2.3 (http://chromas.software.informer.com/2.4/). Resulting sequences of isolates were evaluated using NCBI BLAST searches against fungal sequences existing in DNA databases (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

\section*{RESULTS}

Twenty-two out of 204 patients (10.8\%) had candidemia. Age range of patients was between 16 days and 89 years (mean age; 28.8). Predisposing factors included catheter (31.8\%), cancer (22.7\%), pneumonia (9.1\%), diabetes (9.1\%), dialysis (4.5\%), hypoparathyroidism (4.5\%), cerebral infarction (4.5\%), candiduria (4.5\%), severe burn and inflammation of the esophagus due to ingestion of chemical materials (4.5\%), and congenital heart defect (4.5\%). Eight patients (36.4\%) were males and 14 patients (63.6\%) were females. Mortality rate was 4.5\% ($n=2$), \textit{Candida parapsilosis} was the most prevalent species (45.4\%), followed by \textit{C. albicans} (31.8\%) and \textit{C. glabrata} (22.7\%). Colony features on CHROMagar \textit{Candida} confirmed our findings. \textit{C. albicans}, \textit{C. parapsilosis}, and \textit{C. glabrata} caused green, white, and pink colonies, respectively. Seventeen patients (77.2\%) were taking antibiotics and nine patients (40.9\%) were taking Cortone (Cortisone Acetate). Nine patients (40.9\%) were hospitalized in Pediatric Intensive Care Unit (ICU), eight patients (36.3\%) in ICU, two patients (9.1\%) in Neonatal ICU, one patient (4.5\%) in heart-lung transplant ward, one patient (4.5\%) in liver transplantation unit, and one patient (4.5\%) in the bone marrow transplant unit. Table 1 summarizes the details of patients entered in this study.

\section*{DISCUSSION}

\textit{Candida} species are the most prevalent cause of invasive fungal infections. During 1995–2002, the frequency of \textit{Candida} species in blood cultures in the United States rose from 8\% to 12\%.\cite{14} Because of \textit{C. albicans} remains the most common \textit{Candida} spp. causing IC worldwide; however, the incidence of BSI due to \textit{C. albicans} was found to have decreased,\cite{15} rather than \textit{C. glabrata} has emerged as a prominent and potentially multidrug-resistant \textit{Candida} species.\cite{16} For example, the incidence of candidemia in consequence of \textit{C. glabrata} in Atlanta was shown to have increased from 1 case/100,000 people/year in 1992–1993 to 4.5 cases/100,000/year in 2008–2009.\cite{17} \textit{C. glabrata} was isolated from 22.7\% of patients in the present investigation, too. Pfaller \textit{et al.}\cite{15} showed that 5.7\% patients with candidemia were infected with 2 or more species of \textit{Candida}, whereas mixed fungal infection was not found in this study. They reported 51.2\% of patients had a concurrent bacterial infection as a result of immunocompromised nature of these patients. Bacterial coinfection was seen in 18.2\% of cases in this study [Table 1]. Cleveland \textit{et al.}\cite{18} revealed 61\% of patients were in an ICU within the 14 days before or after candidemia. Many studies
reported that men are infected to Candida BSI more frequently than women;^{[2,3,18]} however, 63.6% of all patients were female in this study. Many studies revealed C. albicans as the most common Candida species of candidemia; nevertheless, C. parapsilosis was predominant species in the present investigation. Matsumoto et al.^{[21]} reported 20% mortality rate among candidemia patients, but in this study, crude mortality was 4.5% (n = 2). The proportion of C. parapsilosis isolates varied considerably among the participating hospitals (the majority of isolates were obtained from pediatrics center). Burning of tissue is generally one of the first steps of systemic fungal infection. Candida infection in burn patients has been connected to prolonged hospitalization and high mortality. A 3-year-old female with candidemia had esophagus chemical burn as a predisposing factor in this study; however, she was healed due to the appropriate antifungal therapy. Lotfi et al.^{[22]} identified C. parapsilosis as the most prevalent species from candidemia (38%) in accordance with the present investigation (40.1%). Similar to the present survey, Gahari et al.^{[23]} by PCR-restriction fragment length polymorphism technique revealed C. parapsilosis as the most common yeast pathogen isolated from candidemia patients (34.4%). Mortality rate was 12.5% in their study. Limitations of this study include limited follow-up data, lack of antifungal susceptibility testing results, and restricted information on antifungal dosing practices.

**CONCLUSIONS**

The findings of this investigation confirm the high rate of candidemia in Tehran, Iran, and appear changes in epidemiological data such as increased proportion of C. parapsilosis and C. glabrata infections. Periodic surveillance studies are recommended to monitor alterations in the epidemiology of bloodstream Candida infections among high-risk population, management of serious conditions of disease, and early hospital discharge policy to control this fatal disease.

**Acknowledgments**

The authors greatly appreciate the cooperation of all staffs of Imam Khomeini, Pediatrics Center, Baghiatallah, Hazrat Rasool, Imam Hossein, and Shariati Hospitals, Tehran, Iran.

**Financial support and sponsorship**

Isfahan University of Medical Sciences, Isfahan, Iran (No. 394310).

**Conflicts of interest**

There are no conflicts of interest.

### Table 1: Characteristics of patients with candidemia in this study

| Gender | Age | Risk factors | Bacterial co-infection | Ward/unit | Candida spp. |
|--------|-----|--------------|------------------------|-----------|--------------|
| Male   | 16 days | Catheter | − | NICU | C. parapsilosis |
| Male   | 5 months | Pneumonia | + | PICU | C. albicans |
| Male   | 59 | Congenital heart defect | − | HLT | C. parapsilosis |
| Male   | 1 | Cancer | − | PICU | C. parapsilosis |
| Female | 70 | Cancer | − | ICU | C. albicans |
| Female | 37 | Diabetes | + | ICU | C. albicans |
| Female | 65 | Candiduria | − | ICU | C. albicans |
| Female | 22 | Catheter | − | ICU | C. parapsilosis |
| Female | 25 | Cancer | − | BMTU | C. parapsilosis |
| Female | 3 | Burn of esophagus | − | PICU | C. parapsilosis |
| Female | 89 | Cancer | − | ICU | C. parapsilosis |
| Female | 46 | Catheter | − | LTU | C. glabrata |
| Female | 28 days | Catheter | + | NICU | C. albicans |
| Female | 74 | Diabetes | − | ICU | C. parapsilosis |
| Female | 1 | Pneumonia | − | PICU | C. parapsilosis |
| Male   | 35 | Cancer | − | ICU | C. glabrata |
| Male   | 4 months | Catheter | + | PICU | C. albicans |
| Female | 3 months | Catheter | − | PICU | C. parapsilosis |
| Female | 11 | Hyoparathyroidism | − | PICU | C. albicans |
| Male   | 12 | Cancer | − | PICU | C. albicans |
| Female | 72 | Cerebral infection | − | ICU | C. parapsilosis |
| Female | 11 | Dialysis | − | PICU | C. parapsilosis |

PICU: Pediatric Intensive Care Unit, ICU: Intensive Care Unit, NICU: Neonatal Intensive Care Unit, HLT: Heart-lung transplant ward, BMTU: Bone marrow transplant unit, LTU: Liver transplantation unit, C. albicans: Candida albicans, C. parapsilosis: Candida parapsilosis
REFERENCES

1. Bar K, Wispelwingh H, Wenzel RP, Bearman GM, Edmond MB. Systemic inflammatory response syndrome in adult patients with nosocomial bloodstream infections due to enterococci. BMC Infect Dis 2006;6:145.
2. Pfaller MA, Diekema DJ. Epidemiology of invasive candidiasis: A persistent public health problem. Clin Microbiol Rev 2007;20:133-63.
3. Andes DR, Safdar N, Baddley JW, Playford G, Reboli AC, Rex JH, et al. Impact of treatment strategy on outcomes in patients with candidemia and other forms of invasive candidiasis: A patient-level quantitative review of randomized trials. Clin Infect Dis 2012;54:1110-22.
4. Arendrup MC, Bruun B, Christensen JJ, Fuursted K, Johansen HK, Kjaeldgaard P, et al. National surveillance of fungemia in Denmark (2004 to 2009). J Clin Microbiol 2011;49:325-34.
5. Pappas PG. Mycoses Study Group. Candidemia in the intensive care unit: Miles to go before we sleep. Crit Care Med 2011;39:884-5.
6. Pfaller MA, Diekema DJ. Epidemiology of invasive candidiasis: 3-year results from the Prospective Antifungal Therapy (PATH Alliance®) registry, 2004-2008. Diagn Microbiol Infect Dis 2012;74:323-31.
7. Diekema DJ, Messer SA, Brueggermann AB, Coffman SL, Doern GV, Herwaldt LA, et al. The changing epidemiology of healthcare-associated candidemia over three decades. Diagn Microbiol Infect Dis 2012;73:45-8.
8. Chow JK, Golan Y, Ruthazer R, Karchmer AW, Carmel Y, Lichtenberg D, et al. Factors associated with candidemia caused by non-albicans Candida species versus Candida albicans in the intensive care unit. Clin Infect Dis 2008;46:1206-13.
9. Samonis G, Kolterud DS, Saloustrinos E, Giannopoulos KP, Ntziora F, Christidou A, et al. Candida albicans versus non-albicans bloodstream infection in patients in a tertiary hospital: An analysis of microbiological data. Scand J Infect Dis 2008;40:414-9.
10. Chi HW, Yang YS, Shang ST, Chen KH, Yeh KM, Chang FY, et al. Candida albicans versus non-albicans bloodstream infections: The comparison of risk factors and outcome. J Microbiol Immunol Infect 2011;44:369-75.
11. Bassetti M, Trecarichi EM, Righi E, Sanguinetti M, Bisio F, Posteraro B, et al. Incidence, risk factors, and predictors of outcome of candidemia. Survey in 2 Italian university hospitals. Diagn Microbiol Infect Dis 2007;58:325-31.
12. Velasco E, Bigni R. A prospective cohort study evaluating the prognostic impact of clinical characteristics and comorbid conditions of hospitalized adult and pediatric cancer patients with candidemia. Eur J Clin Microbiol Infect Dis 2008;27:1071-8.
13. Wispelwingh H, Bischoff T, Tallent SM, Seifert H, Wenzel RP, Edmond MB. Nosocomial bloodstream infections in US hospitals: Analysis of 24,179 cases from a prospective nationwide surveillance study. Clin Infect Dis 2004;39:309-17.
14. Pfaller M, Neofytos D, Diekema D, Azie N, Meier-Kriesche HU, Quan SP, et al. Epidemiology and outcomes of candidemia in 3648 patients: Data from the Prospective Antifungal Therapy (PATH Alliance®) registry, 2004-2008. Diagn Microbiol Infect Dis 2012;74:323-31.
15. Zimbeck AJ, Iqbal N, Ahlquist AM, Farley MM, Harrison LH, Chiller T, et al. FKS mutations and elevated echinocandin MIC values among Candida glabrata isolates from U.S. population-based surveillance. Antimicrob Agents Chemother 2010;54:5042-7.
16. Loft TJ, Fraide JP, Lockhart SR. Multilocus sequence type analysis reveals both clonality and recombination in populations of Candida glabrata bloodstream isolates from U.S. surveillance studies. Eukaryot Cell 2010;9:619-25.
17. Cleveland AA, Farley MM, Harrison LH, Stein B, Hollick R, Lockhart SR, et al. Changes in incidence and antifungal drug resistance in candidemia: Results from population-based laboratory surveillance in Atlanta and Baltimore, 2008-2011. Clin Infect Dis 2012;55:1352-61.
18. Diekema D, Arbeyeville S, Boyken L, Kroeger J, Pfaller M. The changing epidemiology of healthcare-associated candidemia over three decades. Diagn Microbiol Infect Dis 2012;73:45-8.
19. Nuzzo M, Queiroz-Telles F, Alvarado-Matute T, Tiraboschi IN, Cortes J, Zurita J, et al. Epidemiology of candidemia in Latin America: A laboratory-based survey. PLoS One 2013;8:e59373.
20. Matsumoto E, Boyken L, Tendolkar S, McDaniel J, Castanheira M, Pfaller M, et al. Candidemia surveillance in Iowa: Emergence of echinocandin resistance. Diagn Microbiol Infect Dis 2014;79:205-8.
21. Lofti N, Shokohi T, Nourianbaladezaei SZ, Nasrolahi Omran A, Kondori N. High recovery rate of non-albicans Candida species isolated from burn patients with candidemia in Iran. Jundishapur J Microbiol 2015;8:e22929.
22. Ghahri M, Mirhendi H, Zomorodian K, Kondori N. Identification and antifungal susceptibility patterns of Candida strains isolated from blood specimens in Iran. Arch Clin Infect Dis 2013;8:e14529.