

Sequence-identification of *Candida* species isolated from candidemia

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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

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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

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due to the varying susceptibility to antifungal agents with some of these emerging non-*albicans* species.^[12,13]

Due to the varying antifungal susceptibility of clinical isolates, *Candida* spp. identification is necessary for good management of candidemia as a fatal infection. The goal of the present investigation is to identify *Candida* spp. obtained from candidemia and determination of mortality rate among this population.

MATERIALS AND METHODS

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 *Candida* (Paris, France) were used for phenotypic identification.

Molecular identification

Polymerase chain reaction

DNA extraction was performed using of Whatman FTA filter matrix technology as delineated formerly.^[14,15] Briefly, a loopful of a single colony was suspended in 80–100 µl of distilled water and 5 µl 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 µl sterile water for 10 s, then the paper was transferred to a new microtube containing 40 µl 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 ITS1-5.8SrDNA-ITS2 region was used for the identification of all *Candida* strains. The universal fungal primers ITS1 (5'-TCC GTA GGT GAA CCT GCG G-3') and ITS4 (5'-TCC TCC GCT TAT TGA TAT GC-3') were used to amplify the entire ITS rDNA region.^[16] PCR mixture contained 5 µl of 10X reaction buffer, 0.4 mM dNTPs, 1.5 mM MgCl₂, 2.5 U of Taq polymerase, 30 pmol of each ITS1 and ITS4 primers, and 2 µl of extracted DNA in a final volume of 50 µl.

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>).

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% ($n = 2$). *Candida parapsilosis* was the most prevalent species (45.4%), followed by *C. albicans* (31.8%) and *C. glabrata* (22.7%). Colony features on CHROMagar *Candida* confirmed our findings. *C. albicans*, *C. parapsilosis*, and *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.

DISCUSSION

Candida species are the most prevalent cause of invasive fungal infections. During 1995–2002, the frequency of *Candida* species in blood cultures in the United States rose from 8% to 12%.^[14] Because of *C. albicans* remains the most common *Candida* spp. causing IC worldwide; however, the incidence of BSIs due to *C. albicans* was found to have decreased,^[15] rather than *C. glabrata* has emerged as a prominent and potentially multidrug-resistant *Candida* species.^[16] For example, the incidence of candidemia in consequence of *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.^[17] *C. glabrata* was isolated from 22.7% of patients in the present investigation, too. Pfaller *et al.*^[15] showed that 5.7% patients with candidemia were infected with 2 or more species of *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 *et al.*^[18] revealed 61% of patients were in an ICU within the 14 days before or after candidemia. Many studies

Table 1: Characteristics of patients with candidemia in this study

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

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*

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^[3,19-21] 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, Ghahri *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.

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Conflicts of interest

There are no conflicts of interest.

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