

## Review

# Candiduria by *Candida tropicalis* evolves to fatal candidemia

Vidigal, Pedrina Gonçalves<sup>1</sup>, Santos, Simone Aparecida<sup>2</sup>, Maria Aparecida Fernandez<sup>2</sup>, Patrícia de Souza Bonfim<sup>1</sup>, Hilton Vizi Martinez<sup>3</sup> and Terezinha Inez Estivalet Svidzinski<sup>1\*</sup>

<sup>1</sup>Department of Clinical Analysis, Universidade Estadual de Maringá, Brazil.

<sup>2</sup>Department of Cell Biology, Universidade Estadual de Maringá, Brazil.

<sup>3</sup>Hospital Universitário de Maringá, Universidade Estadual de Maringá, Brazil.

Accepted 16 March, 2011

***Candida* species are opportunistic pathogens that belong to the normal human microbiota. In the past decades, the incidence of nosocomial fungal diseases has increased, and *Candida* spp. is still one of the main causal agents. Urinary tract is usually involved with disseminated infections, but interpretation of yeast findings, from representative specimens of this anatomical site, remains controversial. We report a candiduria case in a 64-year-old male patient from intensive care unit (ICU) who developed candiduria due to *Candida tropicalis*, which complicated to fatal candidemia despite antifungal treatment.**

**Key words:** *Candida tropicalis*, candiduria, candidemia.

## INTRODUCTION

In recent years, *Candida* spp. has emerged as an important nosocomial pathogen (Colombo and Guimarães, 2007; Klotz et al., 2007; Pfaller and Diekema, 2007; Galvan and Mariscal, 2006). Candiduria is a frequently documented condition in ICU, but it remains a common dilemma, faced by clinicians, whether determining if a patient is suffering from a fungal infection or if the fungal presence is only due to normal colonization. In hospitalized patients, the urinary tract (UT) is one of the most propitious anatomical sites for the development of infections, once it is normally colonized by such microorganisms (Schaberg et al., 1991; Guler et al., 2006). However, the lack of a proper and safer protocol to characterize candiduria as a UT infection is usually a serious problem (Kauffman, 2005).

On the other hand, candiduria has been considered an early marker of disseminated fungal infection in critically ill patients. According to Ang et al. (1993), ascending infections may uncommonly result in *Candida*

pyelonephritis, which is responsible for up to 3 to 10% of candidaemia secondary episodes. Nonetheless, the criteria for yeast quantification continue to be controversial, once they are not properly standardized and specimens from this site could be easily contaminated. In this report, we describe a case of candiduria caused by *Candida tropicalis*, which resulted in a fatal candidaemia.

## Case summary

A 64 year-old male patient was admitted in February 2008, in the Emergency room of the Maringá University Hospital, Maringá, Brazil, due to acute obstructive abdomen and enterorrhagia. This patient was submitted to an abdomen surgery due to obstruction of the small intestine with a necrotic area of 1 cm. After the surgery, the patient was transferred to intensive care unit (ICU). Prior the hospitalization, his current medical history included diabetes mellitus and hypertension. At admission, empirical treatment with antibiotics (metronidazole, gentamicin and ceftriaxone) was adopted in the first day of hospitalization due to patient's febrile condition.

\*Corresponding author. E-mail: [terezinha@email.com](mailto:terezinha@email.com). Tel: +55 44 3011-4809. Fax: +55 44 3011-4860.

**Table 1.** Oligonucleotides used in nested-PCR, RAPD and DNA sequencing.

Identification	Sequence (5' 3')	Reference
ITS1/4 (Nested-PCR)	F: TCCGTAGGTGAACCTGCGG R: TCCTCCGCTTATTGATATGC	Nakajima-Iijima et al. (1985)
CTR1/2 (Nested-PCR)	F: CAATCCTACCGCCAGAGGTTAT R: TGGCCACTAGCAAATAAGCGT	Luo and Mitchell (2002)
M2 (RAPD)	CTTGATTGCC	Melo et al. (1998)
P4 (RAPD)	AAGAGCCCGT	kit Ready-To-Go/RAPD Analysis Beads
OPA (RAPD)	AGCTGACCGT	Lockhart et al. (1997)
OPE (RAPD)	GGACTGCAGA	Lockhart et al. (1997)
CTF* (Sequencing)	TCAACTTGTACACCAGATT	
CTR* (Sequencing)	TTTTTGGTTAGACCTAAGC	

F= forward; R= reverse. \* Primers were designed by the authors using data from GenBank.

### Microbiological evaluation

Hemoculture was analyzed through Bactec® system, while urine was cultured in Cystine-Lactose-Electrolyte Deficient agar. Hemoculture and uroculture have not exhibited growth of bacteria. We also carried out cultures from faeces, orotracheal secretion, tip of central venous catheter (CVC) and surgical site samples. None of these cultures revealed the presence of yeasts. Quantitative uroculture, obtained in the 30<sup>th</sup> day of hospitalization under aseptic conditions, demonstrated the presence of yeast with counting superior to 10<sup>5</sup> CFU/ml. Seven days later, repeated blood samples were consecutively collected and revealed restricted development of yeasts. All yeasts were grown on CHROMagar™ *Candida* (CHROMagar, Paris, France), showing the presence of colonies of steel blue color. Morphological, physiological and biochemical characteristics from blood and urine samples were examined by standardized methods frequently used in yeast taxonomy (Ahearn and Simmon, 1998). Both strains (from urine and blood) were identified as *C. tropicalis* by all tests carried out. To confirm these results obtained from classical yeast identification techniques, molecular studies were conducted.

### Molecular study methods

Genomic yeast DNA extraction from both samples was carried out according to Amberg et al. (2006) with modifications. Firstly, we carried out Nested-PCR using primers ITS1/2 (DNA of *Candida*) and CTR1/2 (Table 1). These primers amplified bands of 500 and 357bp, respectively, characterize *Candida tropicalis* species. Genotyping study was conducted through Random

Amplification of Polymorphic DNA (RAPD) and sequencing techniques. RAPD analyses was performed with Ready-To-Go RAPD Analysis Beads kit (Amersham Biosciences Corp., Piscataway, NJ, USA) (Table 1). No polymorphic bands were obtained (Supplemental Digital Content 1), which strongly suggested that *C. tropicalis* isolates from blood and urine samples appear as identical clones. DNA profile obtained by RAPD detected a homogenous profile with high similarity between the two isolates. Further investigation of these findings was carried out analyzing both isolates, and performing DNA sequencing with specific primers (Table 1). Sequencing reactions were carried out with amplicons (Supplemental Digital Content 2) through DYEnamic ET Dye Termination kit (GE®). The sequences obtained were submitted to NCBI bank through BLASTn platform. Alignment was performed using ClustalW2 (EMBL-EBI) software. The alignment showed that the analyzed sequences were considered homologous (Figure 1). Accurate identification of the clinical isolates were achieved, and these sequences were 99% identical to *C. tropicalis* gene ITS1 (GenBank accession no. AB467295.1) strain NT1106 (Chen, 2008).

### Reflection upon laboratory results and outcomes

In this case, urine yeast finding was considered infectious due to repetitive quantification of *C. tropicalis* superior to 10<sup>5</sup> CFU/ml. RAPD and sequencing analysis allowed to verified high similarity between strains isolated in both blood and urine samples, suggesting its migration by haematogenous dissemination. Antifungal susceptibility test proposed by CSLI was conducted, and these samples were both (blood and urine) considered

Urine	-----TTGCGCCCTTTGGTATT	17
Blood	-----AACGCACATTGCGCCCTTTGGTATT	25
DNAr	TGAATTGCAGATATTCGTGAATCATCGAATCTTTGAACGCACATTGCGCCCTTTGGTATT	60
	*****	
Urine	CCAAGGGCATGCCTGTTTGAGCGTCATTTCTCCCTCAAACCCCCGGGTTTGGTGTGAGC	77
Blood	CCAAGGGCATGCCTGTTTGAGCGTCATTTCTCCCTCAAACCCCCGGGTTTGGTGTGAGC	85
DNAr	CCAAGGGCATGCCTGTTTGAGCGTCATTTCTCCCTCAAACCCCCGGGTTTGGTGTGAGC	120
	*****	
Urine	AATACGCTAGGTTTGTGTTGAAAGAATTTAACGTGGAAACTT-----	118
Blood	AATACGCTAGGTTTGTGTTGAAAGAATTTAACGTGGAAACTTATTTTAAGCGACTTAGGTT	145
DNAr	AATACGCTAGGTTTGTGTTGAAAGAATTTAACGTGGAAACTTATTTTAAGCGACTTAGGTT	180
	*****	
Urine	-----	
Blood	TATCC-----	150
DNAr	TATCCAAAACGCTTATTTTG	201

**Figure 1.** Alignment of blood and urine isolates. Alignment of sequences obtained from urine and blood samples with the ribosomal DNA region of *C. tropicalis*, using the ClustalW2 program (EMBL-EBI). "\*" indicates that nucleotides are identical for all aligned sequences.

susceptible to the tested drugs (fluconazole, itraconazole, voriconazole and amphotericin B). Antimicrobial treatment with vancomycin and fluconazole was administered during 8 days, after obtaining the first clinical laboratory results. Subsequently, the combination was substituted by vancomycin and amphotericin B for 33 days because of febrile condition. Later, the administration of vancomycin was replaced by piperacillin/tazobactam, which was then changed to meropenem. No corticosteroids were prescribed to the patient. The patient did not show health improvement. Approximately 60 days later, in a context of multiple organ failure, he died. The clinical evolution and laboratorial findings from this patient have given evidences for the physicians to consider candidaemia as cause of death.

## DISCUSSION

UT is one of the most propitious anatomical sites for the development of infections in hospitalized patients, even though it is still a problem with suspicious significance (Schaberg et al., 1991; Guler et al., 2006). Fungemia and UT infections incidence is rising gradually and consists an important public healthcare problem. It is estimated that 10 to 15% of UT nosocomial infections are due to *Candida* spp., and its prevalence is still increasing (Alvarez-Lerma et al., 2003). In the last two decades, this increase has been notable, probably due to several pre-disponent factors, which are related to the occurrence of candiduria (Sobel, 2002). Besides that, some data verified that *Candida* has been isolated in 22% of urine samples from patients admitted at ICU (Alvarez-Lerma et

al., 2003).

The recovery of *Candida* spp. in urine samples exposes clinicians to a great challenge due to the magnitude of clinical possibilities, such as; pyelonephritis; cystitis; occurrence of a haematogenous dissemination from renal cortex; or yet colonization of anatomical sites as bladder and perineum (Lundstrom and Sobel, 2001; Kaufmman, 2005). The distinction between an authentic infection and a contamination is complicated and still searches for standardized criteria (Alvarez-Lerma et al., 2003; Sellami et al., 2006; Blot et al., 2008). Therefore, a frequent problem faced by clinicians consists of confirming whether a patient is suffering from real infection or if the candiduria is due to normal colonization or urine contamination.

The evidences to determine a proper correlation between candiduria and invasive candidiasis is not yet clear or well established, due to publication of conflicting results and the lack of consensus (Hollenbach, 2008). For that reason, trying to identify the source of the infection, consist of an important measure for prevention and control strategies. It was not possible to rule out the presence of disseminated candidiasis by other tests, such as, transesophageal echo (TEE) or renal ultrasounds in our patient. But recently, Revankar et al. (2011) conducted a long term follow up of patients with candiduria. The authors observed one death case, which was attributed to candidaemia and also confirmed by hemoculture.

However, the used molecular tests allowed us to conclude that the yeast isolated from urine and blood samples were identical. Actually, our data are not in agreement with previous ones published by Binelli et al. (2005). According to the authors, molecular typing did not

allow them to state that the UT was the source of disseminated fungal disease. On the other hand, they recognize that microbiological findings suggested a significant correlation between candiduria and candidemia. Ruan et al. (2008) reported a candiduria case by *C. parapsilosis*. Molecular typing of the urinary samples collected from this patient during a period of 7 years, exhibited similar pattern, suggesting that all isolates belonged to the same strain. Miranda et al. (2009) have also evaluated diverse *Candida* colonization anatomical sites as potential sources for candidaemia. The authors used a combination of restriction endonuclease analysis (REAG) and randomly amplified polymorphic DNA (RAPD) methodologies.

The microbiological findings and molecular studies of this patient suggest that candiduria caused by *C. tropicalis* evolved to a fatal case of candidemia, even though antifungal treatment was administered. This fact could be explained by evidences of identical genotype pattern present in urine and blood samples and also due to absence of this species in other sites evaluated. Our hypothesis is supported by Viale (2009), who stated that, candiduria can be reliably considered as a marker of high density of colonization. Thereby, it potentially represents a more practical, less resource-intensive screening marker of heavy colonization and high risk of infection, than is currently possible using parameters such as the multiple-site colonization index.

*C. tropicalis* is well known for its potential to cause infections, which easily results in ample haematogenous dissemination. Therefore, colonization of UT should be well monitored, considering its frequency in hospitalized patients. Hence, further investigations of yeast findings are very important and indispensable on routine diagnostics to promote efficient and fast decisions concerning the management of critically ill patients.

## ACKNOWLEDGEMENTS

This work was supported by Fundação Araucária and T.I.E.S. and also, grants from CNPq. The authors thank Cristiane S. S. Mesquita for her lab assistance.

## REFERENCES

- Ahearn DG, Simmon RB (1998). Yeast pathogenic for humans. In: The yeast - a taxonomic study. 4th edition. Elsevier, Amsterdam, pp. 454-463.
- Alvarez-Lerma F, Nolla-Salas J, León C, Palomar M, Jordá R, Carrasco N, Bobillo F (2003). Candiduria in critically ill patients admitted to intensive care medical units. *Intensive Care Med.*, 29: 1069-1076.
- Ang BSP, Telenti A, King B, Steckelberg JM, Wilson WR (1993). Candidemia from a urinary tract source: microbiological aspects and clinical significance. *J. Infect. Dis.*, 17: 662-666.
- Amberg DC, Burke DJ, Strathern JN (2006). Preparation of genomic DNA from yeast using glass beads. [<http://cshprotocols.cshlp.org/cgi/content/extract/2006/1/pdb.prot4151>].
- Binelli CA, Moretti ML, Assis RS, Sauaia N, Menezes PR, Ribeiro E, Geiger DCP, Mikami Y, Miyaji M, Oliveira MS, Barone AA, Levin AS (2006). Investigation of the possible association between nosocomial candiduria and candidaemia. *Clin. Microbiol. Infect.* 12: 538-543.
- Blot S, Dimopoulos G, Rello J, Vogelaers D (2008). Is *Candida* really a threat in the ICU? *Curr. Opin. Crit. Care*, 14: 601-604.
- Chen Y (2008). Isolation and characterization of marine yeast at eastern coast of Taiwan. Yisheng Chen Ming Chuan University, Department of Biotechnology.
- Colombo AL, Guimarães T (2007). Candidúria: uma abordagem clínica e terapêutica. *Rev. Soc. Bras. Med. Trop.*, 40: 332-337.
- Galvan B, Mariscal F (2006). Epidemiologia de la candidemia en UCI. *Rev. Iberoam. Micol.*, 23: 12-15.
- Guler S, Ural O, Findik D, Arslan U (2006). Risk factors for nosocomial candiduria. *Saudi Med. J.*, 27: 1706-1710.
- Hollenbach E (2008). To treat or not to treat – critically ill patients with candiduria. *Mycoses*, 51(Suppl 2): 12-24.
- Kauffman CA (2005). Candiduria. *Clin. Infect. Dis.*, 41: 371-376.
- Klotz SA, Chasin BS, Powell B, Gaur NK, Lipke PN (2007). Polymicrobial bloodstream infections involving *Candida* species: analysis of patients and review of the literature. *Diagn. Microbiol. Infect. Dis.*, 59: 401-406.
- Lockhart SR, Joly S, Pujol C, Sobel JD, Pfaller MA, Soll DR (1997). Development and verification of fingerprinting probes for *Candida glabrata*. *Microbiol.*, 143: 3733-3746.
- Lundstrom T, Sobel J (2001). Nosocomial Candiduria: a review. *Clin. Infect. Dis.*, 32: 1602-1607.
- Luo T, Mitchell TG (2000). Rapid identification of pathogenic fungi directly from cultures by using Multiplex PCR. *J. Clin. Microbiol.*, 40: 2860-2865.
- Melo AS, Almeida LP, Colombo AL, Briones MR (1998). Evolutionary distances and identification of *Candida* species in clinical isolates by Randomly Amplified Polymorphic DNA (RAPD). *Mycopathologia*, 142: 57-66.
- Miranda LN, van der Heijden IM, Costa SF, Sousa API, Sienna RA, Gobara S, Santos CR, Lobo RD, Pessoa Jr VP, Levin AS (2009). *Candida* colonisation as a source for candidaemia. *J. Hosp. Infect.* 72:9-16.
- Levin AS (2009). *Candida* colonisation as a source for candidaemia. *J. Hosp. Infect.*, 72: 9-16.
- Nakajima-Iijima S, Hamada H, Reddy P, Kakunaga T (1985). Molecular structure of the human cytoplasmic beta-actin gene: interspecies homology of sequences in the introns. *Proc. Natl. Acad. Sci.*, 82: 6133-6137.
- Pfaller MA, Diekema DJ (2007). Epidemiology of invasive candidiasis: a persistent public health problem. *Clin. Microbiol. Rev.*, 20: 133-163.
- Revankar SG, Hasan MS, Revankar VS, Sobel JD (2011). Long-term follow-up of patients with candiduria. *Eur. J. Clin. Microbiol. Infect. Dis.*, 30: 137-140.
- Ruan SY, Chien JY, Hsueh PR (2008). Persistent *Candida parapsilosis* funguria associated with an indwelling urinary tract stent for more than 7 years. *J. Med. Microbiol.*, 57: 1585-1587.
- Sellami A, Sellami H, Makni F, Bahloul M, Cheikh-Rouhou F, Bouaziz M, Ayadi A (2006). La candidurie en milieu de réanimation: signification et intérêt de la numération des levures dans les urines. *Ann. Fr. Anesth. Reanim.*, 25: 584-588.
- Schaberg DR, Culver DH, Gaynes RP (1991). Major trends in the microbial etiology of nosocomial infection. *Am. J. Med.*, 91: 72-75.
- Sobel JD (2002). Controversies in the diagnosis of candiduria: What is the critical colony count? *Infect. Dis.*, 4: 81-83.
- Viale P (2009). *Candida* colonization and candiduria in critically ill patients in the intensive care unit. *Drugs*, 69: 51-57.