Article **ECORFAN Journal**
December 2015 Vol.1 No.1 20-26

# Molecular biology in the diagnosis of invasive candidiasis

FRÍAS-DE-LEÓN, María Guadalupe*†

*Juarez of Mexico Hospital, Division of Research. Av. 5160 National Polytechnic Institute, Col. Magdalena de las Salinas 07760, Mexico DF, Mexico*

Received July 17, 2015; Accepted October 27, 2015

## Abstract

Between invasive mycosis, invasive candidiasis (IC) ranks first worldwide for its morbidity and mortality associated with immunocompromised patients, representing an important public health problema. *Candida albicans* is the most common etiologic agent of CI; however, other non-albicans species also can cause it. Some of the non-albicans species differ from *C. albicans* in their susceptibility pattern to antifungal, even some are intrinsically resistant. For these reasons, the rapid and accurate identification of *Candida* species plays an important role in the selection of appropriate therapy. Conventional methods for laboratory diagnosis of IC have restrictions that have been tried to overcome with the development of molecular and proteomic methods; however, they also have disadvantages. So it is appropriate that the diagnosis of IC is based on the results of conventional tests and complemented with some molecular for greater accuracy in identifying the pathogen that involves proper management of patients.

**Molecular biology, candidiasis, diagnostic.**

**Citation:** FRÍAS-DE-LEÓN, María Guadalupe. Molecular biology in the diagnosis of invasive candidiasis. ECORFAN Journal-Republic of Guatemala 2015, 1-1: 20-26

* Correspondence to Author (email: magpefrias@gmail.com)
† Researcher contributing first author.

The incidence of invasive fungal infections has increased in immunocompromised patients and in critically ill patients [26]. Within invasive infections, invasive candidiasis (IC) prevails in first place worldwide, and thus represents an important public health problem [22]. The CI is caused by *Candida* yeasts, which includes more than 200 species, of which at least 17 have been reported to cause CI, being *Candida* albicans the most common, with more than 50% of cases, followed by *C. glabrata* (18%), *C. parapsilosis* (13%) and *C. tropicalis* (10%) and other rare species such as *C. guilliermondii*, *C. lusitaniae*, *C. norvegensis*, *C. inconspicuous*, *C. famata*, *C. intermedia*, C. zeylanoides, *C. pelliculosa*, *C. dubliniensis*, *C. rugosa*, *C. stellatoidea*, *C. kefyr*, *C. dubliniensis*, *C. famata*, *C. lusitaniae*, *C. norvegnesis*, *C. pelliculosa* [1,4,6,7,13,22,23]. Some of these species have low intrinsic resistance (*C. krusei*) or (*C. glabrata*) to azoles or echinocandins (*C. parapsilosis*) [19].

Importantly, the number of species associated with the CI may be higher, and that at least three species are part of complex: *C. glabrata* (*C. glabrata* strict sense, *C.* and *C. nivariensis bracariensis*), *C. parapsilosis* (*C. parapsilosis sensu stricto*, *C.* and *C. metapsilosis orthopsilosis*) and *C. guilliermondii* (sensu stricto C. guilliermondii, *C. fermentati*, *C. carpophila* and *C. xestobii*). Within these complexes, virulence and susceptibility to antifungal varies, so it is important to correctly identify [5]. C. parapsilosis complex, González et al. (14) report in CI prevalence of *C. parapsilosis sensu stricto* (95.3%), *C. orthopsilosis* (3.1%) and *C. metapsilosis* (1.6%).

Due to the etiological diversity of the CI, it is essential to identify, at the species level, the causal agent not only for diagnostic but also therapeutic and epidemiological.

For the diagnosis of IC, the clinical laboratory plays an important role in the isolation and identification of the pathogen by growing in different media and application of tests such as serological germ tube and assimilation of carbohydrates (VITEK 2 API and C AUX), among others [10]. However, these tests have several disadvantages, among which are: 1) low crop sensitivity to isolate the fungus and the need to require the fungal isolate to characterize, 2) the time required to get the result, 3) its low specificity, as they fail to differentiate between closely related species such as *C. albicans* and *C. dubliniensis*, and the species of complex *C. glabrata*, *C. parapsilosis* and *C. guilliermondii*, which are often mistakenly identified [2.3]. These disadvantages have limited the utility of phenotypic methods in clinical practice [3], so that several molecular methods have been developed to overcome the limitations of conventional tests. Molecular methods intended rapid and specific identification of the pathogen in clinical samples directly, without isolation of fungal [33].

In this paper, the advantages and disadvantages of recently developed molecular methods are reviewed, and that have shown promise in the diagnosis of IC: the polymerase chain reaction (PCR), fluorescent in situ hybridization probes peptides nucleic acids (PNA-FISH) and microarray.

***PCR***

The amplification of nucleic acids by PCR represents an alternative to improve early diagnosis of IC. This technique has been presented in different formats, including simplex PCR, multiplex, semi-nested, nested, coupled with enzyme immunoassay (PCR-EIA) in real time [9.29].

ISSN-On line: 2414-8849

White amplification (molecular markers) used to identify more *Candida* spp., In different formats PCR fragments corresponding to cytochrome P450 genes, topoisomerase II, heat shock, pH regulation, and rRNA (18S, 28S and 5.8S); however, rRNA are the most commonly used markers, because of its universal nature and number of copies [32] also contain regions of internal transcribed spacer 1 and 2 (ITS1 and ITS2) which show variability between species of the same gender [20]. Because of these characteristics, markers designed from rRNA genes have been specific and sensitive for detecting and identifying low concentrations of DNA of *C. albicans*, *C. tropicalis*, *C. parapsilosis*, *C. glabrata*, *C. dubliniensis*, *C. kefyr*, *C. krusei*, *C.* and *C. lusitaniae guililermondii* [16,17,20]. Of different formats PCR, real time PCR has the advantage of being able to quantify the fungal load, making it possible to monitor the persistence or resolution of infection, after treatment.

The PCR has proved a useful tool for the detection of *Candida* spp.,. Including commercial systems have been developed, as SeptiTest or SeptiFast for pathogen detection in clinical samples directly. The SeptiTest system employs a method of extracting DNA from blood and subsequently amplified by multiplex PCR and sequenced for identification of diverse *Candida* species. SeptiFast system in DNA extracted from blood samples and amplified by real-time PCR, wherein the amplicons are hybridized with specific fluorescent and 25 for identifying pathogens, including *Candida* probes. This procedure is completed in approximately six hours [1].

However, the PCR in different formats, has certain limitations that have prevented its applicability in the diagnosis of IC.

The main constraints are the lack of standardization of procedures for DNA extraction from clinical samples, and in some cases, the need for sequencing the amplicons, as this increases the cost of the test and time to get the results. Furthermore, in the case of real-time PCR, it has not been established between the limits of detection of DNA associated with *Candida* colonization or invasion, which may generate false positive results [8].

***PNA-FISH***

PNAs are synthetic peptides consisting pseudo base pairs having the same conformation of a nucleic acid. The PNA probes have neutral charge and very favorable characteristics for hybridization, as its high specificity, high affinity, fast kinetics and the lack of electrostatic repulsion. Additionally, the relative hydrophobicity of the PNA probes allows them to penetrate the hydrophobic cell wall of the microorganisms more easily [31]. Such probes are designed based on the region of the 26S rRNA of *C. albicans*, in order to identify the yeast in positive blood culture bottles. Upon hybridizing the probe with yeast, specific fluorescence is emitted for each species of *Candida*, which can be detected by fluorescence microscopy or flow cytometry. In addition, the PNA-FISH technique has had a positive impact on the selection of antifungal therapy, because when properly identify *C. albicans*, has managed to avoid excessive consumption of some antifungals, such as caspofungin [18,28,31].

Despite its advantages, the use of this technique has limited the clinical laboratories because of the cost of equipment (fluorescence microscopy) and reagents [30].

ISSN-On line: 2414-8849

**Microarrays**

The technology of microarray detection system represents a broad spectrum which can be very useful in view of the large number of pathogenic *Candida* species, it also allows the analysis of different types of biological samples (tissues, proteins, nucleic acids) [25]. Therefore, a microarray was developed for rapid diagnosis and simultaneous identification of 12 common fungal species of the genera *Candida* and Aspergillus. The probes were designed based on the analysis of changes in the ITS regions of the rRNA gene. Through the use of general primers (ITS1 and ITS4) directed towards the conserved regions of the 18S rRNA genes and 28S, respectively, the white region STI is simultaneously amplified and fluorescently labeled. This method has been validated with fungal isolates and clinical samples, showing satisfactory results in only four hours after DNA extraction, so it has a potential use in clinical laboratories mycology [21].

A great advantage of using microarrays is that can be analyzed simultaneously, multiple molecules in a single assay, making it possible not only to identify the pathogen, but also detect mutations that are associated with resistance to antifungal [25]. However, widespread use of microarrays is limited by the relatively small amounts of DNA pathogen found in biological samples. Trying to overcome these limitations,-Saltini Palka et al., [27] they proposed a large scale multiplex PCR (LSplex PCR) for amplification of several dozen genes nine pathogenic species, including *C. albicans*. This protocol employs a large number of pairs of oligonucleotides, 800 different to selectively amplify gene segments for the specific pathogen, and the amplicons are hybridized to a microarray probes.

This protocol increases 10 times LSplex detection sensitivity of the microarray. However, the high cost of this technology has prevented its use as a diagnostic tool.

As can be seen, the proposed molecular methods for the rapid and sensitive identification of clinical isolates of *Candida* spp., Also have limitations. Among the more important of the cost of equipment or reagents for implementation in routine clinical laboratories. Another disadvantage is that most of the methods have focused mainly on the unique identification of *C. albicans*, without taking into account the diversity of non-albicans species that can cause IQ.

Currently, it has also introduced the use of mass spectrometry (MALDI-TOF MS, for its acronym in English matrix-assisted laser desorption / ionization time-of-flight mass spectrometer) for the rapid identification of *Candida* spp., including closely related species [11,15,24]. However, although this novel method allows specific identification, isolation of the pathogen requires [12], which represents an important diagnostic use in IC disadvantage, as is common for the isolation of yeast is not achieved.

So due to the advantages and disadvantages of both the conventional methods such as molecular and proteomic, the most suitable is the diagnosis of IHD, like all infectious diseases, based on the result of conventional tests and is complemented by some of the molecular for greater accuracy in identifying the pathogen and therefore better management of patients.

**Acknowledgements**

To CONACYT for the support PDCP 2013-01 (216 112).

ISSN-On line: 2414-8849

**References**

[1] Ahmad S, Khan Z. Invasive candidiasis: A review of nonculture-based laboratory diagnostic methods. Indian J Med Microbiol. 2012;30(3):264-9.

[2] Asadzadeh M, Ahmad S, Al-Sweih N, Khan ZU. Rapid molecular differentiation and genotypic heterogeneity among *Candida parapsilosis* and *Candida orthopsilosis* strains isolated from clinical specimens in Kuwait. J Med Microbiol 2009;58:745-52.

[3] Avni T, Leibovici L, Paul M. PCR diagnosis of invasive candidiasis: Systematic review and meta-analysis. J Clin Microbiol. 2011;49:665-70.

[4] Bassetti M, Merelli M, Ansaldi F, de Florentiis D, Sartor A, Scarparo C, *et al*. Clinical and therapeutic aspects of candidemia: a five year single centre study. PLoS One. 2015;10:e0127534.

[5] Bertini A, De Bernardis F, Hensgens LA, Sandini S, Senesi S, Tavanti A. Comparison of *Candida parapsilosis*, *Candida orthopsilosis*, and *Candida metapsilosis* adhesive properties and pathogenicity. Int J Med Microbiol. 2013;303:98-103.

[6] Caggiano G, Coretti C, Bartolomeo N, Lovero G, De Giglio O, Montagna MT. *Candida* bloodstream infections in Italy: changing epidemiology during 16 years of surveillance. BioMed Res Int. 2015;2015:256580.

[7] Chen X, Shi W, Liu P, Xu D, Sun S. Development of molecular assays in the diagnosis of *Candida albicans* infections. Ann Microbiol. 2011(61):403-9.

[8] De Bedout C, Gómez BL. *Candida* y candidiasis invasora: un reto continuo para su diagnóstico temprano. Infectio. 2010;14(S2):S159-S171.

[9] Del Negro GM, Delgado AF, Manuli ER, Yamamoto L, Okay TS. Dual candidemia detected by nested polymerase chain reaction in two critically ill children. Med Mycol. 2010;48:1116-20.

[10] Deorukhkar SC, Saini S. Laboratory approach for diagnosis of candidiasis through ages. Int J Curr Microbiol App Sci. 2014;3(1):206-18.

[11] Ferroni A, Suarez S, Beretti JL, Dauphin B, Bille E, Meyer J, *et al*. Real-time identification of bacteria and *Candida* species in positive blood culture broths by matrix-assisted laser desorption ionization-time of flight mass spectrometry. J Clin Microbiol. 2010;48:1542-8.

[12] García P, Allende F, Legarraga P, Huilcaman M. Solari S. Identificación bacteriana basada en el espectro de masas de proteínas: Una nueva mirada a la microbiología del siglo XXI. Rev Chilena Infectol. 2012; 29(3):263-72.

[13] González GM, Elizondo M, Ayala J. Trends in species distribution and susceptibility of bloodstream isolates of Candida collected in Monterrey. Mexico, to seven antifungal agents: results of a 3-year (2004 to 2007) surveillance study. J Clin Microbiol. 2008;46:2902-5.

[14] González GM, Treviño-Rangel RJ, Palma-Nicolás JP, Martínez C, González JG, Ayala J, *et al*. Species distribution and antifungal susceptibility of bloodstream fungal isolates in paediatric patients in Mexico: a nationwide surveillance study. J Antimicrob Chemother. 2013; 68:2847-51.

ISSN-On line: 2414-8849

[15] Gu Z, Hall TA, Frinder M, Walsh TJ, Hayden RT. Evaluation of repetitive sequence PCR and PCR-mass spectrometry for the identification of clinically relevant *Candida* species. Med Mycol. 2011;23:259-65.

[16] Kamiya A, Kikichi A, Tomita Y, Kanbe T. Epidemiological study of *Candida* species in cutaneous candidiasis bases on PCR using a primer mix specific for the DNA topoisomerase II gene. J Dermatol Science. 2005;37:21-8.

[17] Katoa M, Ozekib M, Kikuchib A, Kanbe T. Phylogenetic relationship and mode of evolution of yeast DNA topoisomerase ii gene in the pathogenic *Candida* species. Gene. 2001;272:275-81.

[18] Kim HJ, Brehm-Stecher BF. Design and evaluation of peptide nucleic acid probes for specific identification of *Candida albicans*. J Clin Microbiol. 2015;53(2):511-21.

[19] Krcmery V, Barnes AJ. Non-*albicans Candida* spp. causing fungaemia: pathogenicity and antifungal resistance. J Hosp Infect. 2002;50:243-260.

[20] Leaw SN, Chang HC, Sun HF, Barton R, Bouchara JP, Chang TC. Identification of medically important yeast species by sequence analysis of the internal transcribed spacer regions. J Clin Microbiol. 2006;44:693-9.

[21] Leinberger DM, Schumacher U, Autenrieth IB, Bachmann TT. Development of a DNA microbarray for detection and identification of fungal pathogens involved in invasive mycoses. J Clin Microbiol. 2005;43:4943-53.

[22] Lim CSY, Rosli R, Seow HF, Chong PP. *Candida* and invasive candidiasis: back to basics. Eur J Clin Microbiol Infect Dis. 2012;31:21-31.

[23] Majid Zarrin, Ali Zarei Mahmoudabadi. Invasive candidiasis; a review article. Jundishapur J Microbiol. 2009;2(1):1-6.

[24] Martínez-Lamas L, Pérez del Molino ML, Pardo F, Varela E, Regueiro BJ. Matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry vs conventional methods in the identification of *Candida* non-albicans. Enferm Infecc Microbiol Clin. 2011;29:568-72.

[25] Miller MB, Tang YW. Basic oncepts of microarrays and potential applications in clinical microbiology. Clin Microbiol Rev. 2009;22(4):611-33.

[26] Ostrosky-Zeichner L. Invasive mycoses: diagnostic challenges. Am J Med. 2012;125(Suppl 1):S14-24.

[27] Palka-Santini M, Cleven BE, Eichinger L, Krönke M, Krut O. Large scale multiplex PCR improves pathogen detection by DNA microarrays. BMC Microbiology. 2009;9:1.

[28] Rigby S, Procop GW, Haase G, Wilson D, Hall G, Kurtzman C, *et al*. Fluorescence *in situ* hybridization with peptide nucleic acid probes for rapid identification of *Candida albicans* directly from blood culture bottles. J Clin Microbiol. 2002;40(6):2182-6.

[29] Shin JH, Nolte FS, Morrison CJ. Rapid identification of *Candida* species in blood cultures by a clinically useful PCR method. J Clin Microbiol. 1997;35:1454-9.

[30] Stone NR, Gorton RL, Barker K, Ramnarain P, Kibbler CC. Evaluation of PNA-FISH yeast traffic light for rapid identification of yeast directly from positive blood cultures and assessment of clinical impact. J Clin Microbiol. 2013;51:1301-2.

ISSN-On line: 2414-8849

[31] Trnovsky J, Merz W, Della-Latta P, Wu F, Arendrup MC, Stender H. Rapid and accurate identification of *Candida albicans* isolates by use of PNA FISHFlow. J Clin Microbiol. 2008;46(4):1537-40.

[32] White PL, Archer AE, Barnes RA. Comparison of non-culture-based methods for detection of systemic fungal infections, with an emphasis on invasive *Candida* infections. J Clin Microbiol. 2005;43:2181-7.

[33] White L, Perry MD, Barnes RA. An update on the molecular diagnosis of invasive fungal disease. FEMS Microbiol Lett. 2009;296:1-10.

ISSN-On line: 2414-8849