Molecular biology in the diagnosis of invasive candidiasis

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Abstract

Between invasive mycosis, invasive candidiasis (IC) ranks first worldwide for its morbidity and mortality associated with immunocompromised patients, representing an important public health problem. 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.

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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. norvegensis, 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].
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].
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.

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References


