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A review on Multi Drug Resistant Candidiasis Species From Various Clinical Samples

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ABSTRACT

Background: Candida albicans are reportedly the most common human fungal pathogens. The objective was to analyse the distribution of C. albcans among isolates from various clinical specimens and to ascertain how their virulence factors were expressed. The Cand F and C and R oligonucleotides specifically identify eight of the most frequent Candida species that cause infection (C. albicans, C. glabrata, C. tropicalis, C. parapsilosis, C. krusei/P. kudriazevii, C. guilliermondii/M. guilliermondii, C. lusitaniae/C. lusitaniae, and C. dubliniensis) with a detection limit of 10 pg/µL of DNA or 103 yeasts/mL.

Keywords: Candida spp.; simplex PCR; diagnostic; molecular identification

INTRODUCTION

Candida is a common human commensal fungus that resembles yeast. They transform into pathogens and cause infections locally or broadly when the host's resistance to infection is compromised. [1] Candida species, which range in severity from non-life-threatening mucocutaneous candidiasis to life threatening invasive conditions such as bloodstream candidiasis, genitourinary candidiasis, vulvovaginal candidiasis, and oropharyngeal candidiasis, are responsible for the majority of fungal infections. Candida, a common commensal organism of the gastrointestinal tract, can grow on the skin. [2]

Invasive infections by Candida species are the most common cause of death in patients undergoing invasive procedures and immunocompromised people. [3] Aspergillus, Candida, and Cryptococcus are opportunistic microorganisms that can cause fungal infections in people with weaker immune systems, which can have detrimental effects. About 60–80% of infections resulting in candidiasis are caused by Candida albicans. Nonalbicans species have become more prevalent during the past few decades. [4] According to the literature, the oral cavity has been home to C. albicans, C. tropicalis, C. krusei, C. parapsilosis, C. guillermondii, C. sublimeness, and C. glabrata, are some examples of Candida species. Compared to other species of Candida, C. albicans possesses a pathogenic site factor that makes it more likely to cause disease. [5]

Candida-caused invasive infections have a high risk of death. An expanding population of individuals is vulnerable to and impacted by invasive fungal diseases due to better treatment options and survival rates for some critical illnesses, such as hematologic malignancies. Patients who are immunosuppressed or neutropenic and those in intensive care units are more likely to develop invasive candidiasis. Previously, it was believed that C. albicans was the species most usually in charge of candidiasis. However, some studies over the past few decades asserted that there has been a steady shift from C. albicans to non-albicans. NAC candida species include C. glabrata, C. tropicalis, and C. krusei . Due to the stability of genomic markers and better level of typing resolution, genotypic methods are preferred to phenotypic methods for identifying and characterising fungal isolates. Symptomatic and asymptomatic forms of candiduria exist. Most patients with Candida excretion in their urine don't have any symptoms. On the contrary, Patients with prostatitis, pyelonephritis, cystitis, epididymorchitis, and renal candidiasis, on the other hand, exhibit symptomatic candiduria, pregnancy, diabetes, and age. [6]

Compared to other Candida species of medical importance, C. tropicalis shares a closer genetic relationship with C. albicans. [7] In this study, we wanted to determine if the rDNA ITS region could be used as a molecular marker for assessing genetic diversity within and among therapeutically important and developing Candida species from a sizable Iraq yeast collection characterised by conventional plus molecular approaches.

Infections due to *Candida* species are major causes of morbidity and mortality and are associated with a wide variety of clinical manifestations ranging from superficial and mucosal infections to widely disseminated and bloodstream infections. [8] Global estimates suggest that invasive candidiasis occurs in more than a quarter of a million patients every year with incidence rates for candidemia of 2–14 per 100000 inhabitants in population-based studies. [9] *Candida albicans* is still a leading cause of candidemia, but other species (non-*albicans*) of *Candida* now comprise >50% of bloodstream infections in many parts of the world. [10]

Antifungal resistance is less common in *C. albicans* but has been reported with long-term antifungal use and with recurrent infections, such as those with chronic mucocutaneous candidiasis or recurrent oropharyngeal candidiasis in patients with uncontrolled human immunodeficiency virus infection. [1] Several of the non-*albicans Candida* species, such as *Candida krusei*, are intrinsically resistant or less susceptible to several classes of antifungals, whereas others, including *Candida glabrata*, develop acquired resistance following exposure to antifungal agents. [12] Resistance to >1 drug class (multidrug resistance) remains uncommon but has been increasingly reported, such as in *Candida auris.* Genetic and molecular mechanisms of resistance have been described for many strains, so that knowledge of these mechanisms may help guide selection of therapy. In these patients at risk for serious *Candida* infection, diagnosis remains difficult but is critical in allowing detection of resistance and determination of optimal treatment regimens. [13]

Yeast Identification

Clinical samples were cultured on blood agar, MacConkey agar, and chocolate agar. When yeasts were observed on Gram stain or wet smear, both in cultures or in clinical samples, the samples were plated on CHROMagar™ *Candida* Medium. Blood cultures were carried out in BACTEC flasks Peds Plus/F Culture Vials. [14] Blood cultures were monitored by the automated system BD BACTEC™ FX for five days. Cultures were incubated from 24 to 48 h at 37°C under aerobic conditions. Yeast species were preliminarily identified by CHROMagarTM Candida chromogenic medium, which identifies the main species of *Candida* by the color of the colonies. [15] CHROMagar™ cultures were incubated at 37°C for 48 h and scored for colony color according to the manufacturer's instructions. For the definitive identification of the yeasts, the BD Phoenix yeast

identification system BD PhoenixTM was used. This system is capable of identifying up to 64 species. [16-18]

With the isolated colonies, a suspension was made with a density equivalent to the McFarland standard Nº2. Suspensions were then inoculated onto yeast identification panels and loaded onto the BD Phoenix^{TM} M50 instrument. Results were registered after 4 to 8 h. The prevalence of *Candida* species was determined according to the hospital ward and gender of patients. [19]

Detection of Candida species from yeast pools

To assess the presence of three species of the *C. albicans* complex (*C. albicans* sensu stricto, *C. dubliniensis*, and *C. africana*), the approach proposed by Theill et al. was used with slight modifications. This method is based on the combination (pooling) of individual axenic cultures for the detection of a target species. This approach was also used to detect C. auris. [20]

To optimize the pooling method and demonstrate its sensitivity in detecting *C. auris*, 10 *Candida* species, including a reference strain of *C. auris*, were cultured individually in YPD liquid medium (1% yeast extract, 2% peptone, and 2% dextrose) at 30°C for 24 h under constant stirring at 200 rpm. Individual cultures were arranged in pools that included 300 µL of each culture. Six different pools were tested. Once the pools were prepared, DNA extraction was carried out using a previously described approach based on phenol chloroform. [20]

Molecular detection of C. auris

Briefly, pools were prepared using 10 yeasts previously identified as non-*albicans* species or as *C. tropicalis* by the BD Phoenix Yeast Identification System or CHROMagar^{TM} *Candida* Medium. This decision was based on previous reports of white to mauve colonies of *C. auris* on CHROMagar^{TM} , and because identification has not yet been optimized for any clade of *C. auris* in the BD Phoenix Yeast Identification System, this yeast could be misidentified as *C. catenulata*, *C. haemulonii*, or *C. parapsilosis*. [21]

The evaluation of suspected *Candida auris* infection starts with obtaining a clinical specimen from the site of infection. Specimens may be obtained from blood, urine, nose, pharynx, sputum, pleural cavity, heart, bile, peritoneal fluid, stool, vagina, bone, axilla, groin, wounds/surgical tissue, pus, ear, and cerebrospinal fluid and sent for culture, staining, and/or histopathologic evaluation. It is important to establish whether there is clinical evidence of a true infection, especially in the setting of cultures obtained from non-sterile sites, which may represent colonization. Growth of Candida species in blood culture systems typically takes 1 to 3 days and another 1 to 2 days for identification after subculture onto agar medium. [22]

The identification of *Candida auris* has presented challenges for laboratories, and it is commonly misidentified using clinical microbiology methods. While the aforementioned phenotypic characteristics of *Candida auris* colonies in culture may be useful in identifying it, they cannot be used for definitive diagnosis. Many biochemical methods and automated testing methods commonly misidentify *C. auris* for other *Candida* species, notably *C. haemulonii* and other yeast species. [23]

The most accurate identification is made with devices using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry with appropriate reference databases, which can differentiate *C. auris* from other *Candida* species. Accurate identification relies on the spectra for the sample organisms. This results in misidentifying *C. auris* as *C. albicans* and *C. haemulonii* by MALDI-TOF MS. Once spectra are added to the MALDI-TOF MS database, the labeling of *C. auris* to the species level becomes accurate, although the distinction between geographic strains depends on the number of spectra for each clade in the library. Other molecular methods have also been developed and are based on sequencing the D1-D2 region of the 28s rDNA or the internal transcribed region (ITS) of the rDNA. [24]

An automated molecular test using competitive DNA hybridization and electrochemical detection can rapidly distinguish 15 fungal pathogens, including *C. auris*. A multi-center study showed that this testing method has a 100% sensitivity and specificity for *C. auris*, *C. dubliniensis*, *C. famata*, and *C. krusei* and was able to distinguish between other *Candida* species, including *C. glabrata*, *C. lusitaniae*, *C. albicans*, *C. tropicalis*, and *C. parapsilosis*. The more accurate, reliable, and rapid forms of molecular tests are not always available in all facilities, which makes the diagnosis, management, and infection control efforts challenging. [25]

A range of molecular techniques, including amplified fragment length polymorphism (AFLP), have been utilized for the typing of *Candida auris* isolates. The role of AFLP analysis in the demarcation of the geographical clusters has been demonstrated. [26]

DISCUSSION

Candidiasis is one of the most common fungal infections in the immunosuppressed population. Its diagnosis must be accurate and rapid to allow the establishment of timely and specific treatment, especially in systemic or invasive infections, in which life can be compromised. [27,28] Cultures are considered the gold standard for diagnosing candidiasis; however, they present several limitations that hinder the proper management of patients. Among these limitations, it must be mentioned that the sensitivity is relatively low, particularly in blood samples where the negativity percentage is close to 50%.

Additionally, the isolation of yeasts through culture in bronchial lavages is not that simple. Furthermore, about 19% of false negatives have been reported in bronchial lavage. Another limitation of the gold standard is the time required to obtain results, requiring at least three days for isolation plus additional days for the species identification (48–96 h). [29] That is why molecular methods have gained importance. Even though diverse and novel procedures have been developed, with various degrees of sensitivity and specificity, their use in practice has been limited by the need for infrastructure and highly specialized personnel. Considering the prior, we have designed a pair of primers (CandF and CandR) that detect and identify the presence of eight of the most common Candida species causing disease by simplex end-point PCR, without the need to perform amplicon sequencing to define the species. [30]

CandF and CandR were designed based on the 18S-ITS1-5.8S-ITS2-28S region, taking advantage of the ITS regions intraspecific variability and the conserved sequencesofthe18S,5.8S, and28Sregions. In this way, eight of the most frequent pathogenic species were differentiated (C. albicans, C. glabrata, C. tropicalis, C. parapsilosis, C. krusei/P. kudriazevii, C. guilliermondii/M. guilliermondii, C. lusitaniae/C. lusitaniae, and C. dubliniensis) based on the amplicon size, avoiding the sequencing step that is used in other PCR assays that employ this genomic region as a basis. The oligonucleotides CandF and CandR proved to be species-specific. They only amplified the DNA of any of the eight Candida species for which they were designed and did not amplify

the genetic material of other fungal pathogens or human DNA. The detection limit of the designed oligonucleotides was 10 pg/µL, which is comparable to that of other trials reported in the literature. [31]

The parameters that determine the diagnostic usefulness of the PCR with the CandF and CandR oligonucleotides were adequate. The sensitivity found (73.9%) indicates that less than three out of ten patients with candidiasis will not be detected. On the other hand, 96.3% of patients who do not suffer from candidiasis will have a negative result when the PCR is used with CandF and CandR oligonucleotides. The PPV found (94.4%) indicates that a PCR test with the designed oligonucleotides has a high probability of association with candidiasis. At the same time, the NPV (81.2%) shows that 81.2% of patients with a negative PCR will have a negative culture. These results confirm that a PCR with the designed oligonucleotides can be used to diagnose patients with fever of unknown origin who do not respond to antibacterial treatment. Nevertheless, it is essential to mention that this PCR assay has both advantages and limitations, as with any other assay. Among the advantages, we can remark the rapid identification of eight of the most frequent pathogenic species, without the need to perform additional procedures to the amplification, such as sequencing. Another advantage is that the infrastructure needed to implement this simplex end-point PCR does not require much highly specialized equipment or infrastructure, so it can be easily implemented in any laboratory with the minimum tools to perform a PCR assay. [32]

Likewise, the cost of each assay is lower compared to other methods, such as real-timePCR. One additional advantage is the time reduction compared to the completion of a culture and the subsequent identification of the isolated yeast. In a single step, the PCR with CandF and CandR detects and identifies the yeast at the species level. It is worth noting that in this study, the species identification was consistent among PCR and Vitek 2, which is the automated biochemical system used to identify yeasts after their isolation in cultures. [33]

Among the disadvantages, we can mention that how amplicons are visualized (UV light) can lead to false-negative results, as low concentrations of template DNA can lead to little or no visible amplification products. Another limitation is that CandF and CandR oligonucleotides only identify

eight of the more than 20 species known to cause infection in humans, including C. auris, a yeast that has emerged globally as a multidrug-resistant pathogen associated with healthcare. [34]

Therefore, if the etiological agent is another species, it cannot be identified, leading to false-negative results. The latter occurred with sample, which did not amplify with the designed oligonucleotides but gave an isolated yeast biochemically identified as C. famata by conventional methods (culture and biochemical identification). Besides, another restraint is that the CandF and CandR oligonucleotides recognize C.glabrata, C.parapsilosis, and C.guilliermondii/ M.guilliermondii only at the complex level, which means that it is not possible to differentiate between C. glabrata sensu stricto, C. bracarensis, and C. nivariensis. Fortunately, the frequency of C. bracarensis and C. nivariensis, as well as the species that compose the C. parapsilosis and C. guilliermondii/M. guilliermondii complexes, is low. [35]

CONCLUSION

TheCandFandCandRoligonucleotidesspecificallyidentifyeightofthemostfrequent Candida species that cause infection (C. albicans, C. glabrata, C. tropicalis, C. parapsilosis, C. krusei/P. kudriazevii, C. guilliermondii/M. guilliermondii, C. lusitaniae/C. lusitaniae, and C. dubliniensis) with a detection limit of 10 pg/µL of DNA or 103 yeasts/mL. In addition, the PCR assay with the CandF and CandR primers showed a sensitivity, specificity, PPV, and NPV that justify their reliable use in diagnosing systemic candidiasis.

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