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Original paper

Characterization of non-albicans Candida species involved in human infections

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Abstract

The genus *Candida* includes about 200 different species, but only a few species are human opportunistic pathogens and cause infections, especially when the host becomes debilitated or immunocompromised. Here, we briefly review the current knowledge of pathogenic species of the non-*albicans Candida* species and then focus on current antifungal drugs and resistance mechanisms.

Better understanding of basic fungal biology and pharmacotherapy adaptation mechanisms, facilitated by progress in new technologies, including whole genome sequencing, has the potential to highlight the dynamic robust changes in fungal pathogens during the course of therapy.

Keywords *Candida non albicans*; antifungals resistance; epidemiology.

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Introduction

The incidence and prevalence of invasive fungal infections have increased since the 1980s, especially in immunocompromised patients and/or those hospitalized with serious underlying diseases (PFALLER [1]; ARENDRUP et al [2]; SARDI et al [3]). *Candida* species are part of the native vaginal and oro-gastrointestinal microbiota of humans and animals. *Candida albicans* was detected in at least 70% of the population (ESPINEL-INGROFF et al [4]). Commensal yeasts may cause systemic infection in immunocompromised individuals due to their great adaptability to different host niches. *Candida* comprises a heterogenous group of organisms, and more than 17 different *Candida* species are known to be the etiological agents of human infections; however, more than 90% of invasive infections are caused by *C. albicans*, *C. glabrata*, *C. parapsilosis*, *C. tropicalis* and *C. krusei* (CALDERONE [5]). In European countries, an analysis showed that more than half of the cases of candidaemia were caused by *C. albicans* isolates and the incidence rates for non-*albicans* candidaemia infections were 14 % for *C. glabrata*, 14 % for *C. parapsilosis*, 7 % for *C. tropicalis* and 2 % for *C. krusei* (TORTORANO et al [7]).

The wide variability in reported findings was due in part to differences in the underlying diseases affecting the patients described. For example, patients with leukemia were more likely to be infected by *C. albicans* or *C. tropicalis* but less likely to be infected by *C. glabrata* than patients with other types of cancer. The recent increase in the rate of bone marrow transplantation may also have contributed to discrepancies among reports. Bone marrow transplant recipients were more likely to be infected by *C. krusei* or *C. lusitaniae*. The other factors partially responsible for the variability among reports included common-source contamination and the pressures exerted by antimicrobial treatments ((FLÖRL [6]; TORTORANO et al [7]; JOHN, [8]).

In the last two decades, the number of infections due to non-*albicans Candida* (NAC) species has increased significantly (KAUFFMAN et al [9]; MANZANO-GAYOSSO et al [10], RUAN & HSUEH [11]). The apparent increased involvement of NAC species in human candidiasis may partly be related to improvements in diagnostic methods, such as the use of chromogenic media with the ability to differentiate *Candida* species, as well as the introduction of molecular techniques in the routine diagnosis of fungemia (LIGUORI et al [12]). However, the high prevalence of NAC species in disease could also be a reflection of their inherently higher level of resistance to certain antifungal drugs (GONZALEZ et al [13]) compared with *C. albicans*, as this would promote their persistence, possibly to the detriment of *C. albicans*, in mixed species infections treated with traditional antifungal agents. A number of factors have been involved in this increased occurrence of fungal disease, but it is generally accepted that the increased and widespread use of certain medical practices, such as immunosuppressive therapies, invasive surgical procedures and use of broad-spectrum antibiotics are among the most significant (SAMARANAYAKE et al [14], HAGERTY et al [15], KOJIC & DAROUICHE [16]).

General Description of NAC Species

The genus *Candida* is mprphologically characterized by yeasts of varied morphology which reproduce all by budding. These yeasts are uncapsulated, non-pigmented and some could produce a pseudomycelium or a mycelium. The yeasts of the genus *Candida* sp. can be found in all regions of the world and in all environments (RICHARD & CALDERONE [17]).

C. glabrata follows *C. albicans* as the second or third most prevalent cause of candidemia worldwide (TONI et al [18]). This species of yeast is non-dimorphic and no mating activity has been observed. Until recently, *C. glabrata* was thought to be a primarily nonpathogenic. However, with the ever increasing population of immunocompromised individuals, studies have shown that *C. glabrata* is a highly opportunistic pathogen of the urogenital tract and of the bloodstream. It is especially prevalent in HIV-positive people, and the elderly. *C. glabrata* exists exclusively as small blastoconidia under all environmental conditions, being the only species of *Candida* that does not form pseudohyphae at temperatures above 37°C, but only spores and is extremely resistant to diflucan. *C. glabrata* strains forms glistening, smooth, cream colored colonies which are relatively indistinguishable from those of other *Candida* species except for their relative size, which is quite small. A critical distinguishing characteristic of *C. glabrata* is represented by the haploid genome, in contrast to the diploid genome of *C. albicans* and of several other non-albicans *Candida* sp. (ASLANZADEH, [19]). *C. glabrata* strains ferments and assimilates only glucose and trehalose (WHELAN et al [20]).

C. tropicalis is recognized as a common medical yeast pathogen, existing as component of the normal human microbiota. *C. tropicalis* is similar to *C. albicans* in identification characteristics (PERLROTH et al [21]; MARTIN &WHITE [22]).

C. krusei is an emerging fungal nosocomial pathogen primarily found in the immunocompromised patients and in those with hematological malignancies. It was considered as a component of normal microbiota in female reproductive system, it can be isolated from adult stool and could cause pericardial inflammation. *C. krusei* strains can replace *C. albicans* in the oral cavities of HIV-infected patients, particularly after azole therapy (COLEMAN et al [23], CHAVANET et al [24], LISCHEWSKI et al [25], RUHNKE et al [26]). It is now generally accepted that *C. krusei* isolates is inherently resistant to fluconazole, and that fluconazole prophylaxis may promote the proliferation of this pathogen (MORAN et al [27]); REX et al [28]).

Another pathogenic species of *Candida* such as *C. parapsilosis* and *C. tropicalis* are generally recovered from blood cultures and the skin of immunocompromised patients, particularly in hospital environments, but these

species are rarely isolated from the oral cavity (HUBE et al [29], MORAN et al [30]). *C. lusitaniae* is a rare pathogen, mainly isolated from immunocompromised patients where it is often responsible for candidemia (LEVIN et al [31]). There have been revealed that *C. lusitaniae* strains easily develop resistance to amphotericin B (MORAN et al [30]). Like all pathogenic microorganisms, *Candida* species have developed different virulence mechanisms that confer the ability to colonize a host surface, to penetrate into deeper host tissue, and to evade host defenses (CHAKRABORTY et al [32], KWON-CHUNG et al [33]).

Structure and Reproduction

The cellular wall of fungi is composed of manno-proteins, β-glucan and chitin, which itself is composed of cellulose and hemicellulose. Chitin gives the cell wall its rigidity and resistance to the sunlight. β -glucans can be exposed on the fungal surface in specific areas. The composition of the cell wall also varies between different fungal species. Several CLRs have been identified that recognize these cell-wall structures, including transmembrane and soluble CLRs. The latter group, consisting of surfactant protein (SP)-A, SP-D and mannose-binding lectin (MBP), opsonize fungi and facilitate their recognition (VARSHA et al [35])**.**

The cellular wall is composed of thick filaments that are often called hyphae and are very similar to the roots of plants since fungi feed from these hyphae. These roots can puncture intestinal walls and organs in the human body creating leaky gut syndrome and other negative effects. Although it provides a rigid framework, which gives these pathogens their shape and protection from the environment, the cell wall is a dynamic structure that changes considerably, particularly during the morphological transitions that many fungi can undergo (yeast to hyphae) in *C. albicans*.

Inside of the cellular wall there is the fungal cell membrane composed of protein and fats, very similar to the structure of animal and human cells. Fungi have a typical nucleus, they reproduce asexually in many cases through the production of spores. They can also mate with other fungi when two mycelia, hyphae, or sporangia, meet, producing two multinucleate ball shaped cells that join together to form new nuclei. Asexual division is very much like that of direct division in bacteria (RICHARD [34]).

Candida sp. reproduces sexually and asexually by formation of spores or by budding.

Sexual reproduction is a pervasive attribute of eukaryotic species and is now recognized to occur in many clinically important human fungal pathogens. These fungi use sexual or parasexual strategies for various purposes that can have an impact on pathogenesis, such as the formation of drug-resistant isolates, the generation of strains with increased virulence or the modulation of interactions with host cells (VARSHA et al [35]).

Laboratory Diagnosis of *Candida* sp.

The colonies of *Candida* sp. isolates are cream to yellowish. They grow rapidly and mature in 3 days. The texture of the colony may be pasty, smooth, dry, wrinkled and dull, depending on the species. *Candida* sp. is unicellular yeast, though it can be a multicellular mold. Distinct features of yeasts can be identified by observing their morphology. Microscopes can be used for fast identification and detection of possible yeasts in a clinical sample. Specimens from exudates, sputum, urine and cerebrospinal fluid can be examined under reduced-light bright field microscope or by phase-contrast microscope (ASLANZADEH et al [39]). There have been demonstrated the presumptive identification of *C. albicans* using germ tube test. If *C. albicans* is present, short, slender, tube like structures (germ tube) can be observed under the microscope after 2 to 3 h at 30 to 37°C (MACKENZIE [40]). Reports have been revealed that *C. tropicalis* and *C. parapsilosis* are able to produce similar structures (CAMPBELL et al [41]).

There have been demonstrated that at low values of pH (< 6) *C. albicans* cells predominantly grow in the yeast form, while at a high pH (> 7) hypha growth is induced (LOIEZ et al [36]). Indeed, a number of conditions, including starvation, the presence of serum or N-acetyl-glucosamine, physiological temperature and CO_2 promote the formation of hyphae (SUDBERY [37]). Numerous *Candida* species can be detected by observing the changes in the indicator color when the yeast cultures utilize 1% carbohydrates, such as glucose, maltose, sucrose, trehalose and raffinose. These tests are now available as commercial kits such as API 20C or API 32C. Other than carbohydrates, hydrolysis of 1% fatty acid ester, 0.05% aryl-substituted glycosides, 0.3% urea and 0.01% acrylamide substrates can be detected with RapID Yeast Plus system. This method is currently the fastest commercial method for the identification of yeasts which requires a 4 h incubation period only. However, the identification of *C. dubliniensis* isolates was found better with API 32C than Vitek-2 YST system (CARDENES-PERERA et al [38]). API 32C was also useful in differentiating *C. albicans* from *C. dubliniensis* strains as this two species are phenotypically alike. API 32C is based on the assimilation of various carbohydrates and Vitek-2 YST system is based on the detection of enzymes in the yeast species. It was reported that Vitek-2 YST system is an automated new colorimetric card system which could correctly identify *Candida* species in 18 h which is faster than API 20C and API 32C (LOIEZ et al [36]).

Implications of NAC in Human Pathology

Candida species are commensal and colonize the skin and mucosal surfaces. The prevalence of infections caused by *Candida* species (candidiasis) has increased considerably over the past three decades, mainly due to the rise of the AIDS epidemic, where *Candida* infections constitute the most common fungal infection (ODDS, [42]; HASAN et al [43]). Also, critically ill or otherwise immunocompromised patients are more prone to develop both superficial and life-threatening *Candida* infections (ODDS, [42]).

An infection caused by *Candida* sp. is termed candidiasis or candidiasis. Mycoses caused by the species of this genus show a wide spectrum of clinical presentations and can be classified as superficial, as with cutaneous and mucosal infections, to deep widespread and of high severity, as is the case with invasive candidiasis. According to (FIDEL, [44]) the main transmission mechanism is through endogenous candidaemia, in which *Candida species* that constitute the microbiota of various anatomical sites under conditions of host weakness behave as opportunistic pathogens. Another mechanism for transmission is exogenous, and this occurs mainly through the hands of health professionals who care for patients.

NAC species cause 35-65% of all candidaemias in the general patient population (INGHAM et al. [46]). They occur more frequently in cancer patients, mainly in those with hematological malignancies and bone marrow transplant recipients (40-70%), but are less common among intensive care unit and surgical patients (35-55%), children (1-35%) or HIV-positive patients (0-33%). The proportion of NAC species among *Candida* species is increasing: over the two decades to 1990, NAC represented 10-40% of all candidaemias (KRCMERY & BARNES [47]). In contrast, in 1991-1998, they represented 35-65% of all candidaemias. The most common NAC species are *C. parapsilosis* (20-40% of all *Candida* species), *C. tropicalis* (10-30%), *C. krusei* (10-35%) and *C. glabrata* (5-40%). Although these four are the most common, at least two other species are emerging: *C. lusitaniae* causing 2-8% of infections, and *C. guilliermondii* causing 1-5% (INGHAM et al [46]). Other NAC species, such as *C. rugosa*, *C. kefyr*, *C. stellatoidea*, *C. norvegensis* and *C. famata* are rare, accounting for less than 1% of fungaemias in man. Regarding the virulence and pathogenicity, some NAC species appear to be of lower virulence in animal models, yet behave with equal or greater virulence in man, when comparison is made with *C. albicans*. Mortality due to NAC species is similar to *C. albicans* isolates, ranging from 15% to 35% (EUBANKS et al [48]). According to FIDEL, [44] the main transmission mechanism is through endogenous candidaemia, in which *Candida* species that constitute the microbiota of various anatomical sites under conditions of host weakness behave as opportunistic pathogens. Another mechanism for transmission is exogenous, and this occurs mainly through the hands of health professionals who care for patients. Also indicated in the spread of infection are health-care materials, such as contaminated catheters and intravenous solutions (COLOMBO et al [45]). The main risk factors for non-*albicans* candidaemia in immunocompetent patients are repeated abdominal surgeries, exposure to broad-spectrum antibiotics, diabetes, malignancies, renal failure.

Virulence and Resistance Factors in NAC

Candida pathogenicity is facilitated by a number of virulence factors, most importantly adherence to host surfaces including medical devices, biofilm formation and secretion of hydrolytic enzymes (e.g. proteases, phospholipases and haemolysins). Furthermore, despite extensive research to identify virulence factors in fungi, particularly in *C. albicans*, relatively little is known about NAC species (VOSS et al [49]). The virulence factors expressed or required by *C. albicans* are dependent on the type of infection, the stage and site of infection, and the nature of the host response. Thus, *C. albicans* must be highly adapted to an existence on and within the host, which indicates that this fungus possesses virulence attributes distinct from those of the closely related, but non-pathogenic yeast *Saccharomyces cerevisiae* (SÓNIA et al [50], SILVA et al [51]).

Currently, an increase in the number of yeasts that are resistant to antifungal drugs is recognized worldwide; therefore, the use of *in vitro* laboratory tests may be useful in choosing the appropriate therapy (COLOMBO et al [45]).

The largest family of antifungal drugs is represented by azole. Azoles disrupt the cell membrane by inhibiting the activity of the lanosterol 14-α-demethylase (HOF, [54]), enzyme involved in the biosynthesis of ergosterol. The azole family includes imidazoles (miconazole, econazole, clotrimazole, and ketoconazole) and triazoles (fluconazole, itraconazole, and the latest agent voriconazole (second-generation, synthetic triazole derivative of fluconazole) and posaconazole (hydroxylated analogue of itraconazole) (HOF, [54]).

Echinocandins (caspofungin, micafungin, and anidulafungin) are lipopeptidic antifungal agents that inhibit the synthesis of fungal wall by noncompetitive blockage of the (1,3)-β-D-glucan synthase (Fig. 1). This enzyme inhibition leads to the formation of fungal cell walls with impaired structural integrity, which finally results in cell vulnerability to osmotic lysis (GROVER [55]). All three agents (caspofungin, micafungin, and anidulafungin) exhibit concentration-dependent fungicidal activity against most species of *Candida* (CAPPELLETTY & EISELSTEIN-MCKITRICK [56], VAZQUEZ [57]) and have been approved by the regulatory agency FDA

for the treatment of esophageal and invasive candidiasis, including candidemia (OSTROSKY-ZEICHNER et al [58], DE WET et al [59]).

Polyenes such as nystatin and amphotericin B bind ergosterol and disrupt the major lipidic component of the fungal cell membrane resulting in the production of aqueous pores (Fig. 1). Flucytosine is a pyrimidine analogue transported into fungal cells by cytosine permeases. Then, it is deaminated to 5-fluorouracil and phosphorylated to 5-fluorodeoxyuridine monophosphate. This fluorinated nucleotide inhibits thymidylate synthase and thus interferes with DNA synthesis (Fig. 1, VERMES et al [60]). Allylamines and thiocarbamates also disrupt the cell membrane by inhibiting the squalene-epoxidase (SANGLARD et al [61]), enzyme involved in the biosynthesis of ergosterol. Griseofulvin (a tricyclic spiro-diketone, first isolated from *Penicillium griseofulvum*) acts by disrupting spindle and cytoplasmic microtubule production, thereby inhibiting fungal mitosis (Fig. 1, FRANÇOIS et al [62]).

Antifungal resistance is based on different mechanisms: i) reduced drug intracellular accumulation, ii) decreased target affinity/processivity for the drug, and iii) counteraction of the drug effect.

An intrinsically reduced susceptibility to fluconazole has been also reported for non-albicans species of *Candida* like *C. glabrata*, *C. krusei*, and *C. lusitaniae* (SAFDAR et al [63]). A mechanism responsible for decreasing the intracellular concentration of azole relies on an upregulation of two principal families of efflux pumps such us CgCDR1, CgCDR2 (named PDH1) and CgSNQ2 (another ABC transporter) (TORELLI et al [64]; SANGLARD et al [65]) in *C. glabrata*; CdCDR1 and CdCDR2 in *C. dubliniensis* (MORAN et al [66]); ABC1 and ABC2 in *C. krusei* (LAMPING et al [67]); and CDR1-homologue in *C. tropicalis* isolates (VANDEPUTTE et al [68]). Echinocandin drugs are recommended as the first line for invasive candidiasis. However, reports of echinocandin resistance in patients with infections due to *C. glabrata*, C. tropicalis, and *C. krusei* are rising (HAKKI et al [69]; PASQUALE et al [70]; ALEXANDER et al [71]; PFALLER et al [72]). There have been reported an increased level of resistance in *C. glabrata* between 2001 and 2010 (4.9% to 12.3%). Even more, emergence of co-resistance to both echinocandins and azoles in clinical isolates of *C. glabrata* has been reported (ALEXANDER et al [71]). In addition, intrinsic echinocandin resistance of *C. parapsilosis*, *orthopsilosis*, *C. metapsilosis*, and *C. guilliermondii* has been described (GARCIA-EFFRON et al [73]; CANTÓN et al [74]). A high number of isolates belonging to *C. glabrata* and *C. krusei* species resistant to amphotericin B has been reported (KONTOYIANNIS & LEWIS [75]). Additionally, some *Candida* spp. including *C. lusitaniae* and *C. guilliermondii*, besides *C. glabrata*, showed amphotericin B resistance (PAPPAS et al [76]).

Acquired resistance to flucytosine also results from point mutations in the FCY1 gene which encodes for the cytosine deaminase or FUR1 gene which encodes for the uracil phosphoribosyl transferase. These enzymes catalyze the conversion of 5-fluorocytosine to 5-fluorouracil and 5-fluorouracil to 5-fluorouridine monophosphate, respectively. The most frequently acquired resistance to flucytosine is based on point mutations in the FUR1 gene. Several point mutations have been described in *C. glabrata*, and *C. lusitaniae* (PEMÁN et al [77]; CHAPELAND-LECLERC et al [78]; VANDEPUTTE et al [79]).

The ability of *Candida* sp. to form drug-resistant biofilms is an important factor in their contribution to human disease. As in the vast majority of microbial biofilms (RAJENDRAN et al [52]), sessile cells within *C. albicans* biofilms are less susceptible to antimicrobial agents than are planktonic cells (KUHN & GHANNOUM [53]). The progression of drug resistance within *Candida* biofilms has been associated with a parallel increase in the biofilm maturation process.

Conclusion

This review provides information on the current state of knowledge on the biology, epidemiology, pathogenicity and antifungal resistance of the NAC species, the most frequently involved in candidiasis after *C. albicans*. The rapid development of antifungal resistance, the toxicity of some agents, and the increase in the frequency of non-albicans *Candida* spp. infections support the need for more effective and less toxic treatment strategies.

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