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ARTICLE

Insights into *Candida tropicalis* nosocomial infections and virulence factors

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Abstract *Candida tropicalis* is considered the first or the second non-*Candida albicans Candida* (NCAC) species most frequently isolated from candidosis, mainly in patients admitted in intensive care units (ICUs), especially with cancer, requiring prolonged catheterization, or receiving broad-spectrum antibiotics. The proportion of candiduria and candidemia caused by *C. tropicalis* varies widely with geographical area and patient group. Actually, in certain countries, *C. tropicalis* is more prevalent, even compared with *C. albicans* or other NCAC species. Although prophylactic treatments with fluconazole cause a decrease in the frequency of candidosis caused by *C. tropicalis*, it is increasingly showing a moderate level of fluconazole resistance. The propensity of *C. tropicalis* for dissemination and the high mortality associated with its infections might be strongly related to the potential of virulence factors exhibited by this species, such as adhesion to different host surfaces, biofilm formation, infection and dissemination, and enzymes secretion. Therefore, the aim of this review is to outline the present knowledge on all the above-mentioned *C. tropicalis* virulence traits.

Keywords *Candida tropicalis* · Epidemiology · Risk factors · Virulence factors · Candiduria · Candidemia

Introduction

Nosocomial infections (NIs), or in other words hospital acquired infections, are now a serious public health problem, since these infections are among the leading causes of morbidity and mortality, causing an increase in hospitalization time and, consequently, high costs associated to patient's treatment [1, 2]. NIs have been particularly prominent in intensive care units (ICUs), where the incidence is two to five times higher than in the general population of hospitalized patients [3, 4]. The causes for the increased risk of NIs in ICUs have been associated with increased length of stay in ICU, invasive procedures, patients with compromised immune systems, and multiple exposure to antibiotics [5–7]. Beyond the hospital unit and the disease involving the patient, factors related to the infecting organism are of major importance to the progression of hospital acquired infections [8]. Most of the NIs are caused by microorganisms of the normal microbiota that attack the patient in special situations such as under immunosupression. In these patients, considered at risk, invasive fungal infections are often severe, with a rapid progression and difficultly to diagnose and/or treat [1, 7].

Fungal nosocomial infections (FNIs) incidence has increased significantly over recent decades. *Candida* species are the most frequently isolated fungi, corresponding to approximately 80% of FNIs, being the fourth responsible cause for blood stream infections and responsible for the overwhelming majority of urinary tract infections [7, 9, 10].

Until recently, *Candida albicans* was the *Candida* species that received major clinical attention. However, in parallel with the overall increase of fungal infections, it has been observed that infections caused by non-*Candida albicans Candida* (NCAC) species are emerging [7, 11, 12]. The reasons for this alteration in the pattern of *Candida* species distribution has not yet been completely understood, but could be attributed to the resistance of the NCAC species to antifungal agents, which are used for relatively long periods during hospitalisation [9, 12–14].

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Usually, *Candida tropicalis* is considered the first or the second NCAC species most frequently isolated from bloodstream (candidemia) [12, 13, 15, 16] and from urinary tract (candiduria) [17, 18] infections. Additionally, *C. tropicalis* is often found in patients admitted to ICUs, especially in patients with cancer, requiring prolonged catheterization, and/or receiving broad-spectrum antibiotics [8, 12]. This species appears to display higher potential for dissemination in the neutropenic host than *C. albicans* and other NCAC species. This propensity for dissemination in some way may explain the reported relatively high mortality associated with *C. tropicalis* [15, 19, 20].

Several virulence factors seem to be responsible for *C. tropicalis* infections, which present high potential for dissemination and mortality [21]. Adhesion to host surfaces (epithelial cells and medical devices), as well as biofilm formation [22, 23], secretion of enzymes (proteases and phospholipases) and haemolytic activity are considered important factors in *C. tropicalis* infection [22, 24, 25]. Therefore, this article aims to review and discuss *C. tropicalis* general characteristics, focusing on its microbiology, epidemiology, risk factors and mainly on its virulence factors.

Microbiology

Candida tropicalis, first known as *Oidium tropicale,* was differentiated among several *Candida* species in 1910 by Aldo Castellani. Meanwhile other names have been attributed to this species, such as *Monilia tropicalis*, *Candida vulgaris*, *Mycotorula dimorpha*, *Candida paratropicalis* and another 58 synonyms. Only in 1923, Berkhout introduced the present name [26, 27]. *Candida tropicalis* is a diploid ascomycete yeast and an opportunistic human pathogen, which colonizes several anatomically distinct sites, including the skin [28, 29], gastrointestinal [30] and genitourinary tracts [28], and may also be seen in the respiratory tract [29]. It can also be recovered from the environment, particularly from surfaces in medical settings [22, 29, 31]. Moreover, since 1960 *C. tropicalis* has been recognized as responsible for serious invasive candidosis [32, 33].

Infections caused by *C. tropicalis* can be acquired endogenously, when the individual is already colonized by the microorganism as part of the normal flora, but under altered conditions yeasts may be translocated and spread through the gastrointestinal tract to different anatomic sites, causing infection [8, 12, 15]. The exogenous infection can occur through contact of the hands of health professionals with patients or through catheters, implantable prostheses, as well as parenteral solutions, which were previously contaminated [15, 22, 34, 35].

The mechanism used by the commensal *C. tropicalis* to become a human pathogen is not yet clear. Moreover, *C. tropicalis* infections involve a broad spectrum of invasive diseases, affecting patients exposed to a wide variety of risk factors [8, 36, 37]. Among the invasive infections caused by *C. tropicalis*, the most common are candiduria and candidemia [13, 15, 17, 18, 38].

Identification

Colonies of *C. tropicalis* are cream-colored with a slightly mycelial border (Fig. 1a) on the routinely used Sabouraud dextrose agar (SDA) and appear dark blue (Fig. 1b) in *CHROMagar™ Candida* (CHROMagar, Paris, France) [26, 39]. Microscopically (Fig. 1c), on corn meal Tween 80 agar at 25°C (Dalmau method), *C. tropicalis* shows blastoconidia singly or in small groups all along graceful, long pseudohyphae and may also produce true hyphae.

In biochemical tests (fermentation and assimilation; Table 1), this yeast differs from the other important *Candida* species by being able to ferment and to assimilate glucose, sucrose, galactose, trehalose, and maltose, but not lactose or rafkose [26, 40, 41].

Genetically, *C. tropicalis* is more similar to *C. albicans*, since it contains the major repeat sequence (MRS)

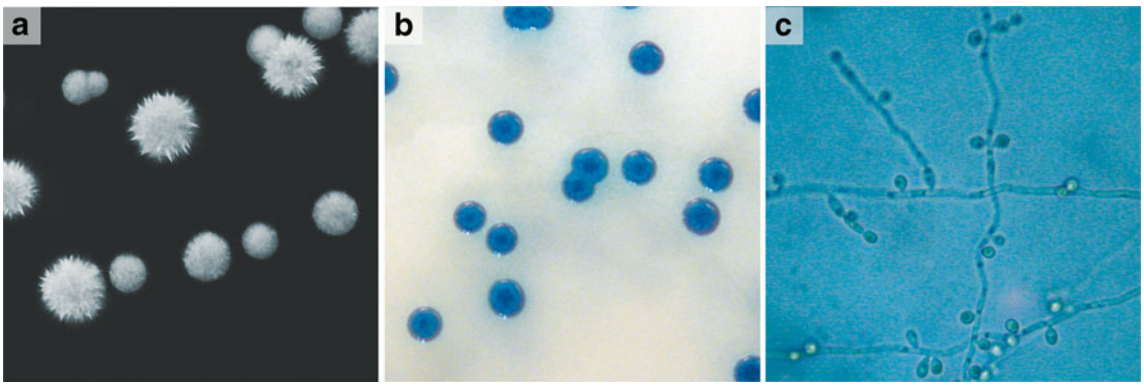

Fig. 1 *Candida tropicalis* morphology in routine culture media. **a** Colonies of *C. tropicalis* on Sabouraud dextrose agar. **b** On *CHROMagar™ Candida*. **c** On corn meal Tween 80 agar at 25°C (Dalmau method)

Table 1 Microbiological and biochemical characteristics of *C. tropicalis* compared with other important *Candida* species

Candida specie	Microbiology			Biochemical[a]											
	Hyphae	Pseudohyphae	Germinative tube	glu	gal	lac	mal	suc	meli	cel	tre	raf	mel	urease	KHO_3
C. tropicalis	+	+	–	FA	FA	–	FA	FA	–	A±	FA	–	A	–	–
C. albicans	+	+	+	FA	AF±	–	FA	A	–	–	AF±	–	A±	–	–
C. parapsilosis	–	+	–	AF±	A	–	A	AF±	–	–	AF±	–	A	–	–
C. glabrata	–	–	–	FA	–	–	–	–	–	–	A±	–	–	–	–

[a] Biochemical analyses: Fermentation and assimilation in the presence of a carbon source, such as glucose (glu), galactose (gal), lactose (lac), maltose (mal), sucrose (suc), melibiose (meli), celobiose (cel), trehalose (tre), raffinose (raf), melezitose (mel). Urea hydrolysis (urease) and assimilation of KHO_3

+ positive, − negative, *FA* fermentation and assimilation positive, *A* assimilation positive, *A*± assimilation variable, *AF*± assimilation positive with fermentation variable

elements, than *C. parapsilosis* and *C. glabrata* [42]. The discovery of MRS elements in *C. tropicalis* suggests that these repeats play a similar role in karyotypic variation in this species, although the contribution of these changes to pathogenesis is not known [43, 44].

For molecular identification, several procedures have been proposed to detect and differentiate *Candida* species *in vitro*, either by DNA extraction from cultured organisms [45, 46] or directly from clinical samples [47–49]. Methods such as polymerase chain reaction (PCR) assay [47, 49, 50] and real-time PCR assays [45, 51], described in Table 2, have been successfully used to identify *C. tropicalis* from clinical samples and even when this species is found in the presence of other fungi. Nevertheless, these methodologies are not yet standardized or readily available in most clinical laboratory settings nor have been validated in large clinical trials.

Risk factors

In general, the risk factors involved in the development of hospital-acquired *Candida* infections are associated with: extended periods in ICUs, administration of broad-spectrum antibiotics, patients with immunosupression, indwelling catheters, mechanical ventilation, candiduria, multiple sites of colonization, burns, and hemodialysis [5, 52–54]. However, the particularities of each *Candida* species may be influenced by specific risk factors. Studies have been shown, that in opposition to *C. parapsilosis*, *C. tropicalis* was less likely to occur among children of less than one year of age, but more likely to occur in patients with cancer or neutropenia [15, 55, 56], and is strongly associated with the presence of biofilms in urinary catheters. [18, 52, 57–59].

Candida colonization remains the most universally accepted predictive variable with regard to invasive candidiasis, being particularly true for high density colonization. In fact, colonization by *C. tropicalis,* especially from a specific body site can be highly predictive of the development of invasive disease with this organism [60]. Nevertheless, it has not yet been clarified whether colonization can be used alone to identify high-risk patients or if it should be combined with other variables indicating high risk [61]. According to Paul et al. [62], many risk factors traditionally linked to candiduria may be associated with urinary tract infections in general. Furthermore, Binelli et al. [63] found a significant association of candidemia with candiduria, although urine was not the main source of *C. tropicalis* bloodstream infection.

According to epidemiological data, when comparing patients with candidemia caused by *C. tropicalis* to those caused by other species of *Candida*, on average, the former are older patients (67 years vs. 56 years, P=0.01), present cancer (45.5% vs. 31.6%, P=0.04), and the portal of entry is the abdomen (32.2% vs. 11.9%, P=0.001). Additionally, these patients also have a high hospital mortality rate (61% vs. 44%, P=0.03) [64]. Further studies suggested that *C. tropicalis* is associated with higher dissemination potential and mortality in patients admitted in ICU, particularly in oncology patients, than *C. albicans* or any other NCAC species [13, 15, 65].

Epidemiology

The proportion of candidosis (candidemia and candiduria) caused by *C. tropicalis* varies widely with geographical area and patient group, with *C. tropicalis* being more prevalent, even compared with *C. albicans,* in certain countries [64, 66, 67]. Considering Table 3, it is possible to see that, among NCAC species, *C. tropicalis* has been considered the species most frequently isolated from candidosis in the Pacific-Asia region [13], Brazil [12, 15], and recently in Europe [7, 68]. Furthermore, important epidemiological studies revealed that 90% of invasive candidosis were due to NCAC species, with *C. tropicalis* accounting for about 4–6% in 1997–1998, 5.3% in 1999, and 7.3% in 2000–2003 [69]. Additionally, in general, *C.*

Table 2 Primers and probes used for polymerase chain reaction (PCR) and real-time PCR assay used for the identification of *C. tropicalis* from clinical samples and when this species is found in the presence of other fungi

Molecular method (reference)	Sequence (direction)	Description
PCR-based [47]	*C. tropicalis* I (F) 5′-GTTGTACAAGCAGACATGGACTG-3′ (R) 5′-CAAGGTGCCGTCTTCGGCTAAT-3′ (R) 5′-TCAAGGTACAGTTATGGCCAAGTT-3′ *C. tropicalis* II (F) 5′-CTGGGAAATTATATAAGCAAGTT-3′ (R) 5′-CTTGAGATACTCAATCTTTTATC-3′ (R) 5′-TCAATGTACAATTATGACCGAGTT-3′	Primer mixes specific to *Candida* DNA topoisomerase II genes. For the identification of *Candida tropicalis* to the species level, one species-specific forward primer and two species-specific reverse primers were designed within the region amplified by the degenerated primer pair
Multiplex PCR [50, 114]	ITS1 5′-TCC GTA GGT GAA CCT GCG G-3′ ITS2 5′-GCT GCG TTC TTC ATC GAT CG-3′	The method is based on the size variability of the ITS1 region in different species.The fungi-specific primers ITS1 and ITS2 are used to amplify a small conserved portion of the 18S rDNA region, the adjacent ITS1, and a small portion of the 28S rDNA region, generating different PCR products for *C. glabrata*, *C. guilliermondii*, *C. lusitaniae*, *C. parapsilosis*, *C. tropicalis* and *C. krusei*
PCR and pyrosequencing [49]	PCR: bio-fun (F) 5′-Biotin-ATTGGAGGGCAAGTCTGGTG-3′ fun (R) 5′-CCGATCCCTAGTCGGCAT-3′ Pyrosequencing: funS 5′-YTCAMAGTAAAAGTCCTGG-3′ or funS2 5′-TCAAAGTAAAAGTCCTGGTTC-3′ *C. tropicalis* pyrosequencing with primers funS or funS2: TTCGCCAAAAGGCTAGCCAGAAGGAAAGGCTCGGTTGGGTC	The tests are performed on amplicons derived from the 18S rRNA gene using PCR universal primers for amplification. The amplification products were subjected to pyrosequencing analysis—a method f DNA sequencing (determining the order of nucleotides in DNA) based on the "sequencing by synthesis" principle
LightCycler PCR [45, 92]	Primer CTR-PR (F) 5′-TCATACCAGTGATAGATGG-3′ CTR-PR (R) 5′-TTTTCTAGCTACTCCATGG-3′ Probes CTR-FL 5′-GTTGATTACCAATCCATGGTTACCTTAC-3′ CTR-RED 5′-ATTAGAACCTGCTGAAATTGTTTGG-3′	The LightCycler PCR combines rapid amplification of nucleic acids in glass capillary with melting curve analysis based on fluorescence resonance energy transfer for the sensitive detection of point mutations in various settings. Species-specific amplification (standard PCR) and hybridization (LightCycler PCR) of *Candida* DNA could be achieved using the species-specific primer pairs and the oligonucleotides, respectively
Real-time PCR assays [48]	*Candida*-specific primers Cand (F) CCTGTTTGAGCGTCRTTT ITS (R) TCCTCCGCTTATTGATAT *Candida*-specific probes C.trop-S Cy5-GGCCACCACAATTTATTTCA-biotin	The application of the biprobe technology facilitated a rapid screening for fungi (specific for the fungal ITS2 region) and simultaneous differentiation of 11 medically important *Aspergillus* and *Candida* species (with species-specific biprobes) in only two individual PCR mixtures and simultaneously in the same LightCycler run

(F) and (R) indicate forward and reverse primers, respectively

tropicalis appeared to be the first or second NCAC most frequently associated to candiduria. Nevertheless, regarding candidemia, *C. tropicalis* is less frequently isolated than *C. glabrata* [7, 17, 52, 65, 68].

Although the reasons for the increased detection of *C. tropicalis* in human infection are not completely clear, the advent of molecular genetics and the development of new methods of *Candida* identification and differentiation [45, 47, 70] may play an important role. Further, the changes of *C. tropicalis* incidence may also be attributed to the greater use of fluconazole nowadays [64, 67]. In the United States, due to fluconazole prophylaxis the frequency of candidosis caused by *C. tropicalis* has decreased. However, in other countries where it is not usual to use fluconazole prophylaxis, *C. tropicalis* appears more prevalent, e.g. second in Latin America, and even more common than *C.*

Table 3 Summary of incidence and antifungal resistance attributed to *Candida tropicalis* candidosis (candidemia and candiduria)

Candidosis	References	Region/Country (period)	Number of strains	*C. tropicalis* (%)	Other NCAC species[a] (%)	Resistance (%)
Candidemia	[115]	Kuwait (1996–2005)	607	12.4	36.2	Flu (0)
						Itra (0)
						Vor (0·5)
						5Flu (9·3)
	[116]	Europe (1997–1999)	2089	7	44	ND
	[75]	Italy (2000–2003)	94	16.0	35.1	Flu (0)
						Itra (0)
						Vor (0·5)
						5Flu (3)
	[15]	Brazil (2003–2006)	924	20	20	Flu (6·6)
						Itra (6·6)
						Vor (0·5)
						5Flu (20)
						Pos (6·6)
	[12]	Brazil (2003–2004)	712	20.9	25.4	Flu (0)
						Itra (0)
						Vor (0)
						5Flu (5)
	[13]	India (2007)	140	42.1	6.4	Flu (10·2)
						Itra (13·6)
						Vor (10·2)
						Cas (2)
	[117]	Europe/Asia/America (2008–2009)	1239	9.8	34.8	Flu (3·3)
						Vor (3·3)
						Pos (0·8)
						Cas (0)
Candiduria	[18]	USA (1991–1993)	530	7.9	19.7	ND
	[17]	Spain (1998–1999)	389	36	8.2	ND
	[63]	Brazil (1996–2000)	23	43.5	4.3	ND
	[118]	Slovakia	94	6.3	24.7	ND
	[52][b]	France (2001–2002)	233	6.5	30.5	Flu (0)
						Itra (0)
						Vor (0)
						5Flu (59)
						Cas (8·7)
	[68]	Portugal (2003–2006)	260	12.7	12.3	ND
	[73]	Brazil (2006–2007)	70	15.7	18.5	Flu (0)
						Itra (18·1)

Flu Fluconazole, *Itra* Itraconazole, *Vor* Voriconazole, *5Flu* 5-Flucytosine, *Pos* Posoconazole, *Cas* Caspofungin, *ND* not determined

[a] Percentage of *Candida glabrata* and/or *Candida parapsilosis*

[b] In vitro susceptibilities of 22 *C. tropicalis* bloodstream and urine isolates

glabrata in the Asia-Pacific region [69]. Nevertheless, the use of prophylactic fluconazole can become a risk factor since some cross-resistance between azoles [7, 11, 71] has been reported already. Several studies indicate that *C. tropicalis* has been showing a moderate level of fluconazole tolerance leading to the need of an increase in the drug concentrations and enabling a risk of azole resistance [65, 72, 73].

Curiously, according to Table 3, the epidemiological data related to antifungal resistance have been indicating an

increase of *C. tropicalis* resistance to 5-flucytosine [12, 15, 52, 74, 75]. Furthermore, it was observed 35% of resistance to 5-flucytosine by *C. tropicalis* isolates recovered from blood cultures in the active surveillance program on yeast-related fungemia implemented by the French National Reference Center for Mycoses and Antifungals (NRCMA) in the Paris area [74]. Additionally, Densos-Olliver et al. [74] studied the relationship between epidemiologic and genomic data of *C. tropicalis* 5-flucytosine resistance, and they observed that a clone of 5-flucytosine-resistant isolate, associated with malignancies, had lower mortality than the other *C. tropicalis* isolates. This suggests that geographic and temporal distribution of *C. topicalis* may be related with 5-flucytosine-resistant isolates in the Paris area.

In fact, the major problem with the development of invasive candidosis by *C. tropicalis* is that it is associated with higher mortality than other NCAC species and *C. albicans* [8, 20, 66, 76]. This propensity of *C. tropicalis* for dissemination and associated high mortality may be related to the virulence factors exhibited by this species such as biofilm formation, proteinases secretion and dissemination [11, 22].

Virulence factors

Mechanisms used by *Candida* species with the purpose of causing any type of injury to the host are related to virulence factors. Several mechanisms of pathogenicity have been associated with *C. tropicalis*, such as adhesion to different surfaces (Fig. 2), biofilm formation, capacity of dissemination (Fig. 3), and hyphae and enzymes production. These factors are concisely described in Table 4. Additionally, relevant findings indicate higher pathogenicity for *C. tropicalis* than other NCAC species. Unfortunately, the pathogenic mechanisms of *C. tropicalis* have not been yet fully elucidated [20, 21, 64, 65, 77].

Adhesion and biofilm formation

Candida tropicalis possesses a remarkable capacity to adhere to abiotic surfaces, human cells and tissues. It is known that *Candida* cells have several different adhesins (special cell wall proteins), which allows adhesion to specific substrates. *Candida* Als (agglutinin-like sequence) is considered an important protein family during the process of adhesion, mediating attachment to different epithelium cells, functioning as an adhesin. Furthermore, southern blot analysis with *ALS*-specific probes suggested the presence of *ALS* gene families in *C. tropicalis* [78, 79]. Furthermore, other factors, such as physicochemical interactions between yeast cells and materials surface, as well as environmental factors, can influence the initial adhesion of *C. tropicalis* [21, 80–82]. Several studies showed the ability of *C. tropicalis* to adhere, and consequently to form biofilms, in clinically relevant substrates like medical devices, and in different environmental situations, both *in vitro* and *in vivo*. Adherence of *Candida* cells to abiotic surfaces and to other cells is vital for biofilm formation [80, 82–85].

Candida biofilm formation is initiated when the yeast adheres to a surface, cells attach to each other and begin to proliferate—ultimately leading to the formation of a highly structured mature biofilm, comprised of complex intertwining layers of yeast, pseudohyphae and hyphae embedded in the extracellular matrix [86, 87]. The matrix is one of the most distinctive features of a microbial biofilm. This complex extracellular material might function to defend against phagocytic cells, to serve as a scaffold for maintaining biofilm integrity, and to limit diffusion of toxic substances into the biofilm, as antifungals [86, 88]. Further, studies indicate that *C. tropicalis* biofilms exhibit large amounts of matrix material completely resistant to antifungals [23, 65, 89–91]. Those data can explain why the

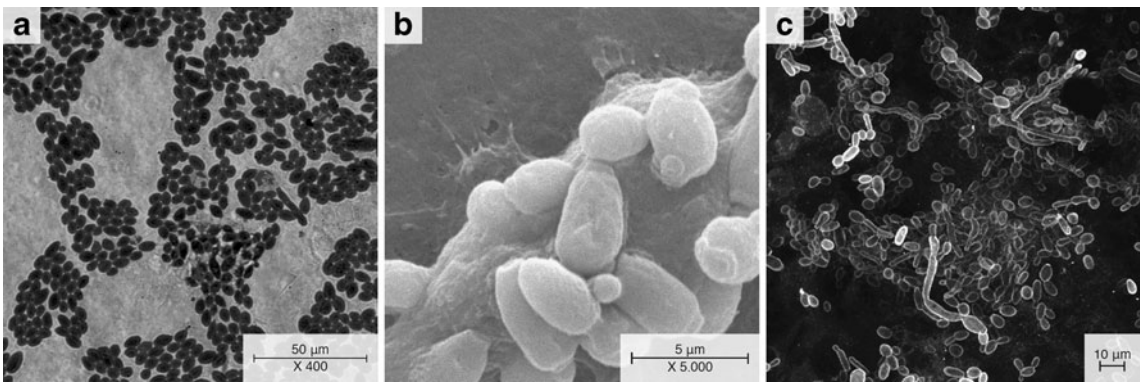

Fig. 2 *Candida tropicalis* adhered to different surfaces. **a** Optical micrograph of *C. tropicalis* on silicone coupons. **b** Scanning electron micrograph of *C. tropicalis* adhered to a human epithelial urinary bladder cell line. **c** Confocal laser scanning microscopy image of *C. tropicalis* adhered to a reconstituted human oral epithelium

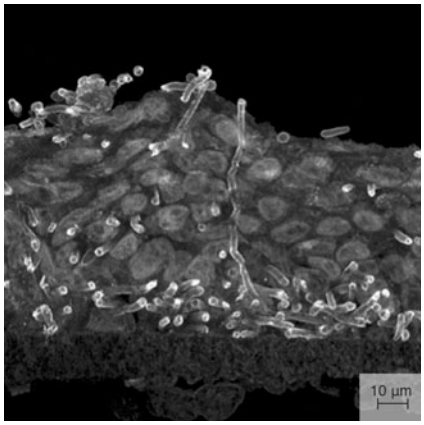

Fig. 3 Confocal laser scanning micrograph of *C. tropicalis* infecting reconstituted human oral epithelium

major risk factor of *C. tropicalis* in candidose development is related to the difficulty of treatment and, moreover, to the prolonged catheterization [64, 71, 92].

In a study done by Al-Fattani and Douglas [89], it was shown that the matrix of *C. tropicalis* contained carbohydrates, proteins, hexosamine, phosphorus and uronic acid. The major component in *C. tropicalis* matrix was hexosamine (27%), whereas in *C. albicans* matrix it was glucose (32%). It is important to emphasize that hexosamine is present in *Streptococcus epidermidis* as a polysaccharide, which is sometimes referred to as the intercellular polysaccharide adhesin (PIA) and is known to mediate cell–cell interaction within the biofilm [93]. Further, in the same study, biofilms of *C. albicans* were more easily detached from plastic surfaces by treatment with the enzyme lyticase than were those of *C. tropicalis*.

However, *C. tropicalis* biofilm and consequently matrix composition are extremely dependent on environmental conditions, such as medium composition, pH, oxygen and growth conditions (static or flow) [82, 84, 90]. According to a study by Jain et al. [59] comparing *Candida* biofilms grown in RPMI medium and artificial urine, biofilm formation is highly dependent on the growth medium. In particular, *C. albicans* strains produced more biofilm in artificial urine than in RPMI. Furthermore, other researches indicated that biofilms grown under conditions of continuous flow produced more matrix than those grown statically, and were significantly more resistant to amphotericin B [89].

Another important factor about the biofilm life cycle is related to dispersion/detachment or dissolution of cells, which release from the biofilm and seed new surfaces with the consequent establishment of disseminated candidiasis at distal organs. Additionally, there are indications that dispersed cells from biofilms are more virulent than planktonic cells [86]. Negri et al. [84] detected that *C. tropicalis* cells are able to detach from biofilms formed in catheters under a flow of artificial urine and move upflow. However, little is still known about *C. tropicalis* detachment cells from biofilms and more studies are necessary to better understand this process.

Infection and dissemination

Adherence of *C. tropicalis* to host cells, and consequently colonization, is seen as an essential early step in the establishment of disease, since high density colonization is indicative of high risk factor to the host [61, 64]. It is known that *C. tropicalis* is able to adhere, colonize and infect host tissues and further disseminate, both *in vivo* and *in vitro* [21, 22, 81, 83].

It is interesting to observe that, according to some researchers, *C. tropicalis* strains showed intermediate levels of adherence to buccal epithelial cells [30, 83] and to human epithelial cell monolayers [94], whereas *C. albicans* strains showed high *in vitro* adherence. However, in other studies, *C. tropicalis* showed similar or higher extent of adhesion than *C. albicans* when in contact with human epithelial cell monolayers [95] and endothelium from porcine vascular tissues [96]. Therefore, it is possible to verify that *Candida* species do not adhere in the same manner to the different mucosal types of cells, and also that there is distinct interaction between epithelium morphology and molecular events during *Candida* adhesion [97].

In a recent *in vivo* experimental study in mice, Okawa et al. [98] observed that the pathogenicity of *C. tropicalis* strains was not correlated with the adherence ability. Silva et al. [81] recently demonstrated that only filamentous forms of *C. tropicalis* were able to invade an oral epithelium reconstituted model. In fact, hyphae have an important role in tissue invasion, and *in vitro* research has shown that *C. albicans* lacking hyphal formation exhibited lower ability for tissue invasion compared with wild-type *C. albicans* strains [99]. The morphological forms exhibited by *C. tropicalis* are similar to those shown by *C. albicans*, but despite these few studies, there is no more evidence on the importance of *C. tropicalis* morphology in virulence. Furthermore, these studies indicate that after prolonged infection *C. tropicalis* increases its infectivity, causing more tissue damage and mice mortality [21, 81, 99]. Corroborating this fact, *C. tropicalis* was found to be highly invasive after 12 h of infection, with extensive tissue damage occurring after 24 h [81].

Thus, the pathogenic mechanisms of *C. tropicalis* seem to be different from those of *C. albicans* [21, 95]. A significant work [100] on pathogenicity of *Candida* species in an animal model showed that the most pathogenic group was *C. albicans* and *C. tropicalis*, followed by an intermediate group with *C. glabrata*, *C. lusitaniae* and *C. kefyr* and a least pathogenic group of *C. parapsilosis*, *C.*

Table 4 *Candida tropicalis* virulence factors analysed and major conclusions

Reference	Virulence factor	*Candida* sp. (*n*)	Comments
[96]	Adhesion to biotic surface and infection	*C. tropicalis* (1); *C. albicans* (1); *C. parapsilosis* (1); *C. glabrata* (1); *C. krusei* (1); *C. pseudotropicalis* (1)	*Candida* interaction with endothelium using porcine whole blood vessel. *C. albicans* and *C. tropicalis* adhered in a higher extent followed by *C. krusei*, *C. parapsilosis*, *C. pseudotropicalis*, and *C. glabrata*
[30]	Adhesion to a biotic surface	*C. glabrata* (12); *C. lusitaniae* (1); *C. kefyr* (1); *C. krusei* (2); *C. colliculosa* (1); *C. parapsilosis* (2); *C. tropicalis* (2); *C. albicans* (12);	This work studied the adherence of different *Candida* strains isolated from the human gastrointestinal tract. Adherence to buccal epithelial cells was maximal for *C. albicans, C. tropicalis* and *C. parapsilosis* and minimal for *C. krusei*
[21]	Adhesion to a biotic surface; dissemination *in vivo*; hydrophobicity; acidic conditions; sucrose assimilation	*C. tropicalis* (5); *C. albicans* (1)	*C. tropicalis* strains were tested for their lethality in mice, adherence to Hela cells, hydrophobicity, yeast growth under acidic conditions (pH 2.0–5.9) and sucrose assimilation. The pathogenicity in mice by all the tested *C. tropicalis* strains was not correlated with the adherence, the hydrophobicity, or yeast growth. The pathogenicity correlated well with the sucrose assimilation ability. Pathogenic mechanisms of *C. tropicalis* strains were different from those of the *C. albicans* assayed
[81]	Infection to epithelium; enzyme expression	*C. tropicalis* (6)	This study investigated the infectivity of *C. tropicalis* isolates using a reconstituted human oral epithelium (RHOE) and secreted aspartyl proteinase (*SAPT*) gene expression. *SAPT1-4* genes expression was strain-dependent, with *SAPT2-4* transcripts being frequently detected and *SAPT1* rarely detected. *C. tropicalis* was highly invasive with the ability to induce significant tissue damage. These features, however, do not appear to be related to specific *SAPT* gene expression
[80]	Adhesion and biofilm formation to abiotic surface; *Candida* surface properties	*C. tropicalis* (2); *C. parapsilosis* (2); *C. glabrata* (2)	This work compared both the adhesion and biofilm formation on silicone of urinary clinical isolates in the presence of urine. NCAC species were able to adhere to and survive on silicone in the presence of urine. Similar water contact angle values were obtained for all NCAC strains. *C. glabrata* strains presented higher colonization abilities than *C. tropicalis* and *C. parapsilosis* strains
[89]	Biofilm and drug resistance	*C. tropicalis* (1); *C. albicans* (1)	In this study, the chemical matrix composition of *C. albicans* and *C. tropicalis* biofilms and biofilm drug resistance were analysed. *C. tropicalis* biofilm matrix contained carbohydrates, proteins, hexosamine, phosphorus and uronic acid, but its major component was hexosamine, whereas in *C. albicans* matrix the major component was glucose. Biofilms of *C. tropicalis* synthesized large amounts of matrix material and such biofilms were completely resistant to both amphotericin B and fluconazole
[23]	Biofilm and drug resistance	*C. tropicalis* (2)	This study investigated the characteristics of *C. tropicalis* biofilm development regarding the different growth phases, morphology and antifungal susceptibility. Mature biofilms consisted of a dense network of yeast cells and filamentous forms of *C. tropicalis*. Increased resistance of sessile cells against fluconazole and amphotericin B was detected. Sessile cells overexpressed ERG11 and MDR1 by real-time PCR, indicating fluconazole resistance by *C. tropicalis* biofilm
[110]	Haemolyse activity	*C. tropicalis* (5); *C. albicans* (15); *C. dubliniensis* (2); *C. glabrata* (34); *C. parapsilosis* (5); *C. lusitaniae* (2); *C. famata* (3); *C. guilliermondii* (4); *C. rugosa* (1); *C. utilis* (1); *C. pelliculosa* (1); *C. kefyr* (2); *C. krusei* (4); *C. zeylanoides* (1)	This is the first study demonstrating the variable expression profiles of haemolysins by different *Candida* species. Total and partial hemolysis was detectable in *C. albicans, C. dubliniensis, C. kefyr, C. krusei, C. zeylanoides, C. glabrata, C. tropicalis,* and *C. lusitaniae*. Only partial-hemolysis was detectable in *C. famata, C. guilliermondii, C. rugosa, and C. utilis*. No hemolytic activity was observed in *C. parapsilosis* and *C. pelliculosa*
[108]	Ezymes production and biofilm; susceptibility testing	*C. tropicalis* (6); *C. albicans* (2); *C. glabrata* (5); *C. krusei* (3); *Candida* sp. (2)	The production of acid protease, phospholipase, haemolysin, and biofilm formation was evaluated. One isolate of *C. tropicalis* had a strong positive phospholipase activity and high resistance to fluconazole. All isolates showed hemolytic activity

Table 4 (continued)

Reference	Virulence factor	*Candida* sp. (*n*)	Comments
[104]	Enzymes production	*C. tropicalis* (9); *C. albicans* (60); *C. glabrata* (4); *C. parapsilosis* (2); *C. lusitaniae* (1); *C. famata* (3); *C. guilliermondii* (3); *C. rugosa* (2); *C. kefyr* (4); *C. krusei* (6); *C. lipolytica* (4)	Phospholipase and protease activities were tested in clinical *Candida* isolates with reference to the sources of strains. Protease producers in NCAC species were observed as *C. kefyr, C. lipolytica, C. parapsilosis* and *C. tropicalis*. *Candida albicans* isolates tested were phospholipase producers and only a few strains of *C. glabrata* and *C. kefyr* behaved in the same way. The quantity of phospholipase produced by *C. albicans* varied with the specific isolate and correlation with the site of infection
[107]	Enzymes production	*C. tropicalis* (19); *C. albicans* (77)	*Candida albicans* and *C. tropicalis* were obtained from whole saliva of patients presenting signs of oral candidosis. Proteinase activity was observed in both *C. albicans* and *C. tropicalis*, but phospholipase activity was only noted in *C. albicans*. In vitro resistance to antifungals was verified in both species, but *C. tropicalis* appears to be more resistant to the tested antifungals than *C. albicans*
[113]	Enzymes production	*C. tropicalis* (11); *C. albicans* (24); *C. glabrata* (22); *C. krusei* (5)	The aim was to determine the enzymatic activity and to investigate the distribution of phospholipase C gene of several *Candida* isolates from patients with pulmonary tuberculosis and non tuberculosis patients. Phospholipase activity from *C. albicans*, *C. tropicalis* and *C. krusei* exhibited some similarity in both groups. *Candida* isolates invariably showed high levels of phospholipase, proteinase, caseinase and lipase activities
[22]	Adhesion to abiotic surface and biofilm formation; adhesion to biotic surface; hyphae formation; enzymes production; susceptibility testing	*C. tropicalis* (7)	Characterization of *C. tropicalis* virulence and antifungal susceptibility. All clinical isolates presented one or more virulence factors. *Candida tropicalis* strains adhered significantly more to epithelium than to silicone. All strains were able to form biofilms and to express total haemolytic activity. However, protease was only produced by two isolates. Only one strain was phospholipase positive. Four strains were susceptible-dose dependent to itraconazole and one clinical isolate was found to be resistant. It was not possible to establish a relation among the virulence factors assayed

krusei and *C. guillerimondii*. It is important to emphasize the clinical relevance of those findings, since the major problems with the development of invasive *C. tropicalis* candidosis are associated with high dissemination and mortality.

Enzymes production

Once adhered to host cells, *C. tropicalis* requires other factors to penetrate into the deepest tissues, e.g., hyphal formation and production of enzymes. In fact, the pathogenic capabilities of this yeast have been related to the secretion of aspartyl proteinases, phospholipases and haemolytic activity [22, 24, 25, 65, 79, 101].

Candida produce a large variety of secreted hydrolases, and among the various potential virulence factors proposed, the secreted aspartyl proteinases (Sap) have been intensively investigated. It is now well established that the ability of *C. albicans* to adhere to mucosae, to invade in deep organs, and to resist to phagocytic cells, apparently requires the use of several different proteinases suitable to each particular condition during the infection. Like *C. albicans, C. tropicalis* presents *in vitro* Sap activity in a medium containing bovine serum albumin (BSA) as the sole source of nitrogen [22, 31, 79, 102].

Furthermore, Zaugg et al. [24] characterized a total of four *SAPT* gene families of *C. tropicalis*. According to this study, RT-PCR experiments revealed a strong *SAPT1* signal with RNA extracted from cells grown in BSA medium. The *SAPT2* and *SAPT3* gene products, Sapt2p and Sapt3p, which have not yet been detected in *C. tropicalis* cultures *in vitro*, were produced as active recombinant enzymes with the methylotrophic yeast *Pichia pastoris* as an expression system. However, a weak signal was obtained with all other *SAPT* genes under *in vitro* conditions tested, suggesting that the gene products Sapt2p, Sapt3p, and Sapt4p could be produced during infection.

This idea is highlighted by recent studies [81, 99] that investigated epithelial infection by *C. tropicalis* using a reconstituted human oral epithelium (RHOE) and *SAPT* gene expression. The results obtained by real-time PCR showed that *C. tropicalis* isolates were able to express

SAPT1-4 during the infection process. Moreover, expression was strain-dependent, with *SAPT2-4* transcripts being frequently detected and *SAPT1* rarely detected. Furthermore, *C. tropicalis* can be considered as highly invasive with the ability to induce significant tissue damage [81]. These features, however, do not appear to be related to specific *SAPT* gene expression. Therefore it is necessary to perform more investigations focusing on *SAPT* gene families of *C. tropicalis* for better understanding the specific role of these genes.

In addition to Saps, enzymes categorized as lipases (LIPs), i.e., enzymes that hydrolyze phospholipids into fatty acids, are often considered to be involved in *C. tropicalis* pathogenicity and are suggested to contribute to host cell membrane damage which could also expose receptors to facilitate adherence [101, 103, 104]. In *C. albicans*, ten genes encoding for LIPs (*LIP1-10*) have been identified and similar sequences were also detected in *C. tropicalis* [105]. Nevertheless, the most widely used diagnostic method for phospholipases (PLs) determination is based on yeast growth in an egg yolk agar medium [106]. According to recent studies, using this method, *C. tropicalis,* from different sources, appears to have a reduced ability to produce extracellular PLs *in vitro* when compared to *C. albicans* [29, 104, 107]. However, this production is highly species and strain dependent [22, 25, 108, 109].

Other important virulence factor recently described in literature is related with haemolytic activity which is tested on sheep blood agar supplemented with glucose [110]. It is known that enzymes as haemolysins are used by *Candida* species to degrade haemoglobin and facilitate recovery of the elemental iron from host cells, which is a contribution to pathogenicity in *Candida* species. Thus, haemolysins are considered key virulence factors enabling pathogen survival and persistence in the host [110–112]. The studies reported so far show that *C. tropicalis* are all able to produce haemolysins *in vitro*, inducing partial or total erythrocyte lyses, although the degree is strain dependent [110]. According to Luo et al. [110], total-haemolytic activities in *C. albicans* and *C. tropicalis* were significantly higher than in *C. glabrata*. However, Kumar et al. [113] observed the opposite, *C. glabrata* displayed the highest haemolytic activity when compared with *C. albicans* and *C. tropicalis*. Although significant studies showed the ability of *C. tropicalis* to produce haemolytic activity on sheep blood agar supplemented with glucose, it is important to assess whether the haemolytic activity observed is true or is a product of extracellular PLs of *Candida* species. Moreover, it is still necessary to have more advances in molecular studies to clarify the role of haemolytic activity in *C. tropicalis* pathogenesis.

Concluding remarks

In fact, the frequency of *Candida tropicalis* causing candidosis has been increasing in recent decades, probably due to several situations, e.g., new and efficient molecular methods of identification; antifungal resistance, mainly to fluconazole commonly used as prophylaxis agent; and factors related with host as well as invasiveness surgery, long periods in ICU, antibiotic administration and catheterization. Additionally, invasive disease developed by *C. tropicalis* is associated with colonization, high potential of dissemination and pathogenicity by this organism. This is mainly because *C. tropicalis* possesses a diversity of virulence factors that induce serious damage to patients and increases the mortality risk. However, much more research is necessary to get deeper insights into the strategies used by *C. tropicalis* to change from a harmless commensal microorganism to become a human pathogen of high clinical concern.

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