



An Update on *Candida tropicalis* Based on Basic and Clinical Approaches

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Candida tropicalis has emerged as one of the most important *Candida* species. It has been widely considered the second most virulent *Candida* species, only preceded by *C. albicans*. Besides, this species has been recognized as a very strong biofilm producer, surpassing *C. albicans* in most of the studies. In addition, it produces a wide range of other virulence factors, including: adhesion to buccal epithelial and endothelial cells; the secretion of lytic enzymes, such as proteinases, phospholipases, and hemolysins, bud-to-hyphae transition (also called morphogenesis) and the phenomenon called phenotypic switching. This is a species very closely related to *C. albicans* and has been easily identified with both phenotypic and molecular methods. In addition, no cryptic sibling species were yet described in the literature, what is contradictory to some other medically important *Candida* species. *C. tropicalis* is a clinically relevant species and may be the second or third etiological agent of candidemia, specifically in Latin American countries and Asia. Antifungal resistance to the azoles, polyenes, and echinocandins has already been described. Apart from all these characteristics, *C. tropicalis* has been considered an osmotolerant microorganism and this ability to survive to high salt concentration may be important for fungal persistence in saline environments. This physiological characteristic makes this species suitable for use in biotechnology processes. Here we describe an update of *C. tropicalis*, focusing on all these previously mentioned subjects.

Keywords: *Candida tropicalis*, virulence factors, antifungal resistance, phenotypic and molecular identification, update

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INTRODUCTION

In the last decades, medicine advances related to the discovery of several medical devices which seek for a longer survival of patients with several infirmities, such as AIDS, hematological malignancies, cancer, and other immunosuppressive diseases promoted a longer lifespan. On the other hand, the number of opportunistic fungal infections increased, mainly the ones caused by the *Candida* genus (Pincus et al., 2007; Araújo et al., 2017). In this context, *Candida tropicalis* emerges as one of the most important *Candida* species in terms of epidemiology and virulence. It is able to produce true hyphae, an exclusive property of *Candida albicans* and its sibling species *Candida dubliniensis*. *C. tropicalis* has also been considered a strong biofilm producer species and is highly adherent to epithelial and endothelial cells (Marcos-Zambrano et al., 2014). In addition, several recent investigations have reported the recovery of *C. tropicalis* resistant to the antifungal drugs

currently available, such as the azoles derivatives, amphotericin B, and echinocandins (Choi et al., 2016; Seneviratne et al., 2016). In addition, *C. tropicalis* has been considered an osmotolerant microorganism and this ability to survive to high salt concentration may be important for fungal persistence in saline environments, contributing to the expression of virulence factors *in vitro* and resistance to antifungal drugs (Zuza-Alves et al., 2016). This property explains *C. tropicalis* potential use in biotechnological processes such as the production of xylitol from corn fiber and the ethanol from marine algae (Rao et al., 2006; Ra et al., 2015).

BIOLOGY AND TAXONOMY

C. tropicalis was originally isolated from a patient with fungal bronchitis in 1910 and named *Oidium tropicale* (Castellani, 1912). It is a yeast belonging to the filo Ascomycota, from the Hemiascomycetes class (Blandin et al., 2000), which has a single Order created in 1960 by Kudrjavzev, called Saccharomycetales (Kirk et al., 2001). This monophyletic lineage comprises about 1,000 known species, including several yeasts of medical importance such as *C. tropicalis* (Diezmann et al., 2004).

According to Kurtzman et al. (2011) *C. tropicalis* colonies on Sabouraud Dextrose Agar (SDA) are white to cream, with a creamy texture and smooth appearance and may have slightly wrinkled edges. Therefore, it is indistinguishable from other *Candida* species. After 7 days of microculture on cornmeal agar containing Tween 80, incubated at 25°C, spherical or ovoid blastoconidia, which may be grouped in pairs or alone, measuring $\sim 4\text{--}8 \times 5\text{--}11 \mu\text{m}$, pseudohyphae in branched chains, and even true hyphae may be observed (Silva et al., 2012; **Figure 1**). With respect to the biochemical characteristics, it is known that *C. tropicalis* is capable of fermenting galactose, sucrose, maltose, and trehalose, besides assimilating these and others carbohydrates through the oxidative pathway (Kurtzman et al., 2011).

GENETIC CHARACTERISTIC

C. tropicalis is a diploid yeast, whose genome was sequenced in 2009 (strain MYA-3404) in a study conducted by Butler et al. (2009). It has a genomic size of 14.5 Mb, containing 6,258 genes encoding proteins and a guanine-cytosine content of 33.1%. The number of chromosomes is not known with precision, but Doi et al. reported 12 chromosomes per cell for *C. tropicalis* (Doi et al., 1992).

It has been widely believed that *C. tropicalis* is an asexual yeast. However, some studies performed recently have reported that mating between diploid cells α and a , generating a/α tetraploid cells may occur (Porman et al., 2011; Xie et al., 2012; Seervai et al., 2013). Such mating is regulated by colony phenotypic switching, where cells change from a white to an opaque state. Seervai et al.

(2013) demonstrated that tetraploid strains of *C. tropicalis* can be induced to undergo parasexual cycle without meiotic reduction. This process results in a or α diploid cells competent for mating, being able to form tetraploid cells, which show chromosomal instability after incubation and return to the diploid state after ~ 240 generations (Seervai et al., 2013). Genetic recombination has also been demonstrated, besides ploidy changes (aneuploidies and polyploidy), affecting cells gene expression and protein production (Morrow and Fraser, 2013). This reduction in ploidy is considered a mechanism of adaptation and may be associated with cell stress (Berman and Hadany, 2012). This adaptive mechanism may also generate karyotype variation within the host, and may be induced by various stressors, such as thermal shock, exposure to UV light, and growth in l-sorbose or d-arabinose as the only carbon source (Legrand et al., 2008; Arbour et al., 2009; Bouchonville et al., 2009; Morrow and Fraser, 2013). It is important to emphasize again that meiosis occurrence has never been described in *C. tropicalis*.

C. tropicalis has greater genetic similarity with *C. albicans* than the other *Candida* species of medical interest (Butler et al., 2009), as may be observed in **Figure 2**. This intimate evolutionary relationship is also evident in phenotypic and biochemical characteristics of both species. Phylogenetically, this pattern of evolution can be explained due to predominant clonal reproduction. However, with recombination events frequent enough to generate a population with similar characteristics (Wu et al., 2014).

IDENTIFICATION

Conventional Methods for *Candida tropicalis* Identification

C. tropicalis has been quite reasonably well-identified with phenotypic methods until the present moment (**Table 1**). This is contradictory to some other *Candida* spp., where molecular identification is mandatory due to the existence of cryptic species.

Although the classical methodology is of easy execution, it is very laborious and time-consuming making it difficult to be used in microbiology routine laboratories (**Table 1**; Pincus et al., 2007; Sariguzel et al., 2015).

The use of chromogenic media, with different substrates that react with specific enzymes of the main *Candida* species induce the formation of colonies with different colors and has been used for the presumptive identification of *C. tropicalis*. They have all been used for the screening of distinct species, besides being used to check the purity of *Candida* colonies and may be helpful to detect mixed infections. Quite a few number of different chromogenic culture media are currently commercially available for yeasts identification, and they have been successfully used for the initial screening of *C. tropicalis* colonies (**Table 1**).

Several commercially available kits used for yeasts identification based on carbohydrates used by oxidative pathways have been in the market in order to facilitate the process used for yeasts identification (**Table 1**). *C. tropicalis* identification with commercial methods have been performed since 1975; since then, several papers have been published in

Abbreviations: *C. albicans*, *Candida albicans*; *C. tropicalis*, *Candida tropicalis*; CLSI, Clinical and Laboratory Standards Institute; MALDI-TOF/MS, Matrix-assisted laser desorption time-of-flight mass; ELISA, Enzyme-linked immunosorbent assay.

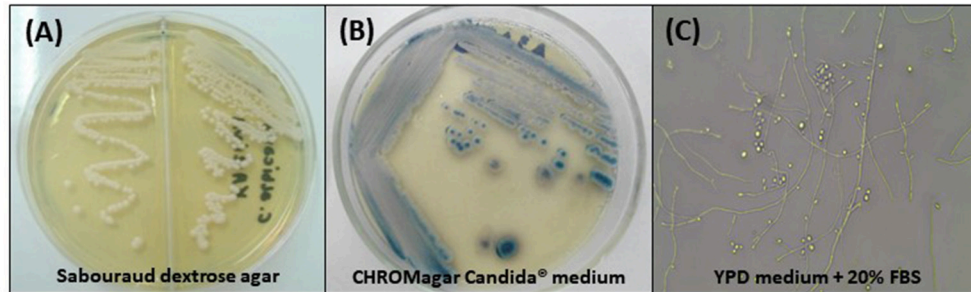


FIGURE 1 | Phenotypic characteristics of *Candida tropicalis*. **(A)** Cream-colored, dull, smooth colonies, after 48 h of incubation at 30°C on Sabouraud dextrose agar; **(B)** Colonies with typical dark blue color on CHROMagar Candida® medium after 96 h of incubation at 35°C; **(C)** Micromorphological aspects after incubation in YPD medium containing 20% fetal bovine serum (FBS) for 7 days at 30°C, 400x: blastoconidia in single or branched chains, true hyphae and abundant pseudohyphae.

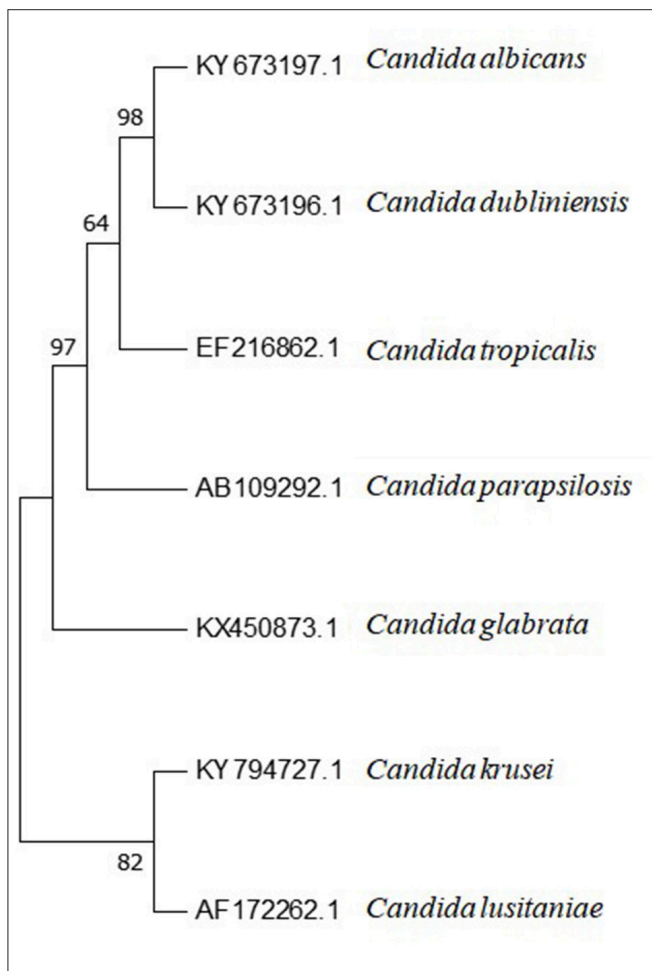


FIGURE 2 | Phylogenetic tree of *Candida* spp. internal transcribed spacer 1 (ITS1)-5.8S ribosomal RNA gene and internal transcribed spacer 2 (ITS2) complete sequences and their accession numbers, obtained from Genbank database at <https://www.ncbi.nlm.nih.gov>. Sequences were aligned using BioEdit software (v7.2.61). Aligned sequences were used for phylogenetic analysis conducted with Mega 7.0.26 Software. The method used for tree constructions was maximum parsimony. Phylogram stability was assessed by bootstrapping with 1,000 pseudoreplications.

the literature evaluating the efficiency of this method. In a recent study by Stefaniuk and collaborators), the API ID32C system (bioMérieux) was used for the identification of 124 *Candida* clinical isolates, where 21 *C. tropicalis* isolates (100% of cases) were accurately identified (Stefaniuk et al., 2016). In a study performed by Alfonso et al., with 240 isolates of different *Candida* species, the authors found the accurate identification of 34 isolates of *C. tropicalis* with the API ID 32C system (Alfonso et al., 2010). Gundes et al. compared the efficiency of different commercial methods used in the identification of 116 yeasts of medical interest, demonstrating the accuracy of 87% (101 out of 116) for API 20C® against 82.7% (96 out of 116) with Candifast® system. However, *C. tropicalis* was accurately identified in 100% of cases with both methods (Günder et al., 2001). The AuxaColor™ Kit (Bio-Rad) identification has been shown to be accurate in 63.8–95.2% of cases (Pincus et al., 2007). Recently, in a meta-analysis performed by Posteraro et al. (2015), including a total of 26 studies that evaluated yeasts identification methods, they observed that *C. tropicalis* was accurately identified in 168 out of 184 cases tested with AuxaColor™ and 55 out of 66 cases by using API ID32C® (Posteraro et al., 2015).

Besides semi-automated methods currently available, there are other methods completely automated for yeasts identification (Table 1). Won and collaborators performed a study that compared the efficiency of several medically important yeast species identification with the automated systems Vitek2® and BD Phoenix™. This study included a total of 341 isolates, from 49 species and *C. tropicalis* (36 isolates) was accurately identified in 34 cases with BD Phoenix™ System and in 32 occasions with Vitek2® (Won et al., 2014).

The conventional methods of identification including the classical methods, semi-automated and automated systems may not be completely accurate on some cases and may lead to an incomplete identification, needing supplementary tests or even give a wrong identification for some species (Marcos and Pincus, 2013; Chao et al., 2014). Therefore, molecular biology advances are of extreme importance for microorganism's identification because of the fact they are more accurate, and may reduce costs

TABLE 1 | Conventional methods used for *Candida tropicalis* laboratorial identification.

	Method	Principle	Advantages	Disadvantages	References
Classical methodology	Auxanogram and zymogram	Assimilation and fermentation of several different carbon and nitrogen sources	Easy execution and low cost	Laborious and time-consuming, subjectivity of interpretation	Pincus et al., 2007; Sariguzel et al., 2015
	Microculture on cornmeal agar containing Tween 80	Yeasts incubation on culture medium with Tween 80 and low oxygen tension esporulation and filamentation			
	Urease test	Urea hydrolysis alkalizes the medium, causing the pH indicator to change. The medium goes from yellow to pink, indicating positivity			
Chromogenic media	Chromagar <i>Candida</i> [®] , <i>Candida</i> ID2 [®] , CandiSelect4 [®] , <i>Candida</i> Brilliance [®]	Different substrates react with specific enzymes of the main <i>Candida</i> species and induce the formation of colonies with different colors for presumptive identification	Rapid screening of different species and checking the purity of <i>Candida</i> colonies, detects mixed infections; high sensitivity and specificity	Presumptive identification for only five species of the <i>Candida</i> genus	Alfonso et al., 2010; Sariguzel et al., 2015; Zhao et al., 2016
Semi-automated methods	API 20C AUX, API ID 32C system	Galleries with different carbon sources, where growth and assimilation is observed by turbidity in the respective well	Good reproducibility and easy execution	May not be completely accurate on some cases and may lead to an incomplete identification, needing supplementary tests or even give a wrong identification for some species; higher cost. Not all the rare <i>Candida</i> species are included in the galleries	Bowman and Ahearn, 1976; Stefaniuk et al., 2016
Automated methods	CandFast [®] system	The identification well contains cycloheximide, besides seven carbohydrates, where fermentation is analyzed after acidification and alteration of media colors due to the presence of a pH indicator	Used for identification and antifungal susceptibility testing		Gündeş et al., 2001
	AuxaColor [™] Kit	Assimilation of 13 sugars, besides the enzymatic detection of N-acetyl-galactosaminidase, phenoloxidase and L-proline arilamidase	Good reproducibility and easy execution		Pincus et al., 2007
	Vitek2 [®] System BD Phoenix [™]	Fluorometric and colorimetric methods for microorganism's identification and analysis in a software which contains a database with 52 yeast species Polystyrene strips contain three fluorescent control wells (a negative and two positives) with 47 wells containing lyophilized substrates	Rapid results, requires minimal preparation of reagents		Posteraro et al., 2015 Grant et al., 2016

involving identification during the whole process, resulting in a decreased time for the release of results (Chao et al., 2014; Posteraro et al., 2015).

Molecular Methods and Proteomics for the Identification of *Candida tropicalis*

Recently, the evaluation of the protein profile of each species has been used as the basis for yeasts identification and has been proven as more efficient than the conventional methods (Santos et al., 2011; Chao et al., 2014; Stefaniuk et al., 2016). The protein profile by mass spectrophotometry is a simple methodology of easy sample preparation and short time for analysis (Table 2; Keceli et al., 2016).

The accurate identification of *C. tropicalis* by proteomics analysis has been demonstrated in several studies which compared identification methods (Chao et al., 2014; Angeletti et al., 2015; Panda et al., 2015; Sariguzel et al., 2015; Keceli et al., 2016; Stefaniuk et al., 2016). *C. tropicalis* was accurately identified in 22/22 (100%) (Sow et al., 2015), in 21/21 (100%) (Stefaniuk et al., 2016), in 18/18 (100%) (Angeletti et al., 2015), in 17/17 (100%) (Chao et al., 2014), in 13/13 (100%) (Keceli et al., 2016), and in 2/2 (100%) by VITEK-MS (Sariguzel et al., 2015). The system performance of the MALDI Biotyper system also showed satisfactory results for the identification of *C. tropicalis*, where the accurate identification was found for 21/21 (100%) (Stefaniuk et al., 2016), 17/17 (100%), and in 18/18 (100%) (Angeletti et al., 2015) of cases.

Several studies have also been performed to evaluate PNA-FISH performance for different *Candida* species isolated from different anatomic sites, where conclusive results for *C. tropicalis* ranged from 96 to 100% of cases (Table 2; Hall et al., 2012; Stone et al., 2013; Calderaro et al., 2014; Gorton et al., 2014).

Although the methods used for microorganism's identification by using PNA-FISH and protein profile analysis using mass spectrophotometry techniques are accurate and have high sensitivity and specificity, molecular sequencing has been considered the gold standard technique for microorganisms identification recently (Keceli et al., 2016). rDNA ITS region sequencing has been quite satisfactorily used for *C. tropicalis* identification elsewhere. The main target for yeasts DNA molecular sequencing is the ribosomal (rDNA) region (Pincus et al., 2007). This region contains conserved domains separated by variable regions (the small sub unities 18S and 5.8S, besides the large subunit 26S, while these sub unities are separated by the interspacer regions ITS1 and ITS2) which contain species-specific sequences used as the preferential target for universal primers used of identification (Table 2; Merseguel et al., 2015; Shi et al., 2015; Benedetti et al., 2016).

Candida tropicalis Genotyping

Genotyping methods have largely been used recently to investigate a genetic correlation of different strains of the same species or even among different species (Table 2). These methods may be applied to the investigation of infections caused by similar or identical strains, besides the observation of possible

micro-evolution or strains substitution during colonization and infection (da Costa et al., 2012; Almeida et al., 2015).

Recently, Almeida and collaborators employed RAPD technique with three different random primers (OPA-18, OPE-18, and P4) to evaluate the genetic variability of 15 clinical isolates of *C. tropicalis* obtained from patients with candiduria (Almeida et al., 2015). The analyses of the dendrogram constructed with DNA bands with the best discriminatory power primer (OPA-18) showed four well-defined clusters (I, II, III, and IV), where cluster I and II showed above 90% similarity among them, while clusters III and IV had 70% similarity.

Da Costa et al. (2012) genotyped by RAPD 15 strains of *C. tropicalis* oral isolates with primers OPA-01, OPA-09, OPB-11, OPE-18, and SEQ-06 (da Costa et al., 2012). OPA-01 showed the best discriminatory power, presenting ten distinct patterns for *C. tropicalis* isolates, with 80% similarity (da Costa et al., 2012). Another study using primers OPE-03, RP4-2, OPE-18, and AP50-with 12 catheter tip and urine isolates, obtained 9 different clusters with similarities coefficients (SABs) ranging from 0.8 to 1.0, where different strains were considered unrelated (if SAB was below 0.8), moderately related (SAB 0.8–0.89), highly related (SAB 0.90–0.99), and identical (SAB 1.0) (Marol and Yücesoy, 2008).

Almeida et al. (2015) typed 15 isolates of *C. tropicalis* with microsatellites and obtained the presence of five different alleles with the marker *URA3* and eight different allelic combinations with the *CT14* locus, being this marker considered to have a better discriminatory power than the *URA3* locus (Almeida et al., 2015).

By evaluating 65 clinical isolates of *C. tropicalis* obtained from different anatomic sites, Wu et al. used different markers of sequence tandem repeats, as follows: Ctrm1, Ctrm7, Ctrm10, Ctrm12, Ctrm15N, Ctrm21, Ctrm24, and Ctrm28 and selected six loci for population genetic analyses (Ctrm1, Ctrm10, Ctrm12, Ctrm21, Ctrm24 and Ctrm28), obtaining a total of 7 (Ctrm24 e Ctrm28) to 27 (Ctrm1) distinct genotypes (Wu et al., 2014).

The methodology known as MLST (Multilocus Sequence Typing) was originally described by Maiden et al. (1998). Therefore, by using MLST, strains from different geographic regions and various anatomic sources may be analyzed and compared. Strains maintenance, substitution, and multiple colonization may be investigated (Maiden et al., 1998; Chen et al., 2009; Wu et al., 2012, 2014).

The first MLST studies on *C. tropicalis* were performed in 2005, by Tavanti et al. with DNA sequencing of 6 housekeeping genes (*ICL1*, *MDR1*, *SAPT2*, *SAPT4*, *XYR1*, and *ZWF1 α*). In this study, 106 isolates of *C. tropicalis* (104 human clinical isolates and 2 from animal origin) were evaluated, where 87 DSTs were obtained, grouped within three different highly related clades (Tavanti et al., 2005). In the study performed by Wu et al., with 58 strains of *C. tropicalis* from different anatomic sites by MLST, 52 different DSTs grouped within 6 different clades were obtained (Wu et al., 2012). Therefore, MLST is considered a very robust molecular technique used for typing with high discriminatory power, being widely used to evaluate intra-specific variability for different microorganisms including *C. tropicalis* (Tavanti et al., 2005; Odds and Jacobsen, 2008; Chen et al., 2009; Wu et al., 2012).

TABLE 2 | Molecular methods and proteomics for the identification and genotyping of *Candida tropicalis*.

	Method	Principle	Advantages	Disadvantages	References
Proteomics	Protein profile by mass spectrophotometry	Uses an ionizing matrix and has been assembled to automated methods of microorganisms identification such as MALDI Biotyper and VITEK-MS and several other mass spectrometer	Simple methodology of easy sample preparation and short time for analysis, more efficient than the conventional methods, accurate identification	Higher cost of equipment, necessity for specialized training; possible lack of a robust database	Chao et al., 2014; Angeletti et al., 2015; Panda et al., 2015; Sariguzel et al., 2015; Kececi et al., 2016; Stefanjuk et al., 2016
Molecular identification	Molecular rDNA sequencing	Based on the ability of DNA polymerase to copy a DNA strand from the template in the presence of a primer. The inclusion of fluorescent markers with different colors allows the differentiation of the chains truncated by the respective fluorescence	Robust technique, automated, Higher accuracy, gold standard identification	Requirement for specialized equipment, expensive reagents, and highly trained personnel	Pincus et al., 2007
	PNA-FISH	Based on the use peptide nucleic acid probes directed to specific rRNA species of the main <i>Candida</i> species tagged with fluorescent dyes	High sensitivity and specificity	There may be some problem in discriminating closely related microorganisms	Stender, 2003; Hall et al., 2012; Stone et al., 2013; Calderaro et al., 2014; Gorton et al., 2014; Aydemir et al., 2016
Genotyping	Randomly Amplified Polymorphic DNA (RAPD)	Based on the amplification of DNA fragments by polymerase chain reaction (PCR) by using shortprimers containing random sequences	Fast, simple and low-cost method for detecting polymorphisms; Does not require radioactively labeled probes; use of arbitrary primers, no need of initial genetic or genomic information, and the requirement of only tiny quantities of target DNA	Dominant technique; low reproducibility and low discriminatory power; difficult standardization, possible problems of interpretation	Wu et al., 2014; Almeida et al., 2015
	Microsatellites analysis	Based on the amplification by PCR of small tandem sequence repeats from 2 to 6 highly polymorphic nucleotides, present on chromosomal telomeric regions	Easy execution, reproducible, appropriate for large-scale epidemiological studies, good discriminatory power,	Technical challenges during the construction of enriched libraries and species-specific primers	
	Multilocus Sequence Typing (MLST)	Based on the amplification of 6–10 housekeeping genes by PCR, with further PCR products purification and gene sequencing. Gene sequencing generates the sequence type (ST) for haploid organisms and diploid sequence types (DST) for the diploids microorganisms, which also may be compared to a database	Robust technique with high discriminatory power, excellent reproducibility, easy standardization; data that can be shared and compared between different laboratories easily through the Internet	Requirement for specialized equipment, expensive reagents, and highly trained personnel; phylogenetic relationships and resolution of clones can be masked by the use of slowly evolving housekeeping genes	Maiden et al., 1998; Tavanti et al., 2005; Odds and Jacobsen, 2008; Chen et al., 2009; Wu et al., 2012

VIRULENCE FACTORS

The ability of yeasts to adhere, infect, and cause diseases altogether is defined as a potential of virulence or pathogenicity. According to Cauchie et al. (in press), it was previously believed that species of the *Candida* genus were passively involved in the process of establishment of infection. However, it is now established that these yeasts play an active role in the infectious process through the action of several virulence factors (Cauchie et al., in press).

Adhesion to Epithelial and Endothelial Cells

Adhesion of blastoconidia to host cells is considered the first step for both colonization and the establishment of *Candida* infections and involves interactions between fungal cells and host surfaces (Cannon and Chaffin, 2001). It is a complex and multiphase process, including different factors, such as the microorganism involved, the composition of adhesion surfaces and several environmental factors (Silva-Dias et al., 2012).

Galan-Ladero et al. (2013) performed a study with 29 *C. tropicalis* isolates with hydrophobicity potential (Galan-Ladero et al., 2013). The cell wall structure is composed by hydrophobic proteins embedded in a cellular matrix which may favor the initial interaction, because hydrophobic particles tend to attach to a high variety of plastic materials and host proteins such as laminin, fibrinogen, and fibronectin (Tronchin et al., 2008).

Genes which codify proteins related to adhesion processes are differentially expressed, accordingly to a variety of hosts and environmental conditions (Sohn et al., 2006; Verstrepen and Klis, 2006). Despite the fact that *ALS* genes (Table 3) are highly involved with adhesion in *C. albicans*, it has been reported that several *Candida* species also have the ability to adhere to human buccal and vaginal epithelial cells, besides to the gastrointestinal epithelia of mice and several different plastic materials, motivating studies on adhesion in Non-*C. albicans Candida* (NCAC) species (Klotz et al., 1983).

Punithavathy and Menon (2012) evaluated the presence of *ALS* genes in 48 isolates of *C. tropicalis* obtained from HIV-negative and positive patients. The authors found that 12 isolates (25%) expressed the *ALS1* gene, 24 isolates (50%) expressed *ALS2*, and 23 of them (48%) showed *ALS3* expression (Punithavathy and Menon, 2012).

HWPI ("Hyphal wall protein") gene codify another important adhesin present on the hyphal cell wall (Table 3). *In vitro* studies demonstrated the presence of high amounts of Hwp1p at hyphal cell walls, while low amounts are present in blastoconidia (Naglik et al., 2006) and pseudo-hyphae (Snide and Sundstrom, 2006). The *HWPI* gene is involved in adhesion to human buccal epithelial cells (HBEC), codifying the first protein needed for biofilm formation (Sundstrom et al., 2002; Nobile et al., 2008).

The expression of this adhesin was recently reported for *C. tropicalis* in a study performed in Malaysia (Wan Harun et al., 2013) which investigated the presence of *HWPI* in NCAC species by using mRNA expression. *HWPI* mRNA transcription was positively regulated in *C. tropicalis*, indicating the ability of this species to express this adhesin. This study suggests that *HWPI*

in *C. tropicalis* shares an identical sequence with *C. albicans*. Therefore, this is contradictory with the description of the presence of *HWPI* only in *C. albicans* (Ten Cate et al., 2009).

In fact, most of the studies report *C. albicans* as more adherent than other NCAC species, but *C. tropicalis* is considered the second most adherent species of the *Candida* genus (Calderone and Gow, 2002; Lyon and de Resende, 2006; Biasoli et al., 2010). For instance, Costa et al. evaluated the ability of adherence of *Candida* isolates obtained from the oral cavity of HIV individuals, patients with candidemia and catheter tips and found *C. albicans* as the most adherent species (average of 227.5 cells/100 HBEC) while *C. tropicalis* showed in average 123.5 cells/100 HBEC (Costa et al., 2010). Conversely, another study investigating adhesion by oral isolates of *C. albicans* and *C. tropicalis* to laminin and fibronectin detected by ELISA, reported *C. tropicalis* adhesion significantly higher than what was found for *C. albicans* (da Costa et al., 2012).

More recently, Menezes et al. (2013) evaluated the ability *Candida* spp. clinical isolates to adhere to glass cover slips (Menezes et al., 2013). They found higher adherence of *C. tropicalis* than *C. albicans* and yeasts belonging to the *C. parapsilosis* complex. A recent study performed in Brazil, with isolates from the oral cavity of kidney transplant recipients also demonstrated high ability of adherence to HBEC by *C. tropicalis* (Chaves et al., 2013). In this study, while *C. albicans* isolates showed about 237 cells/150 HBECs in average, an isolate of *C. tropicalis* had 335 cells/150 HBECs, reinforcing the remarkable role of adhesion as an important virulence factor in *C. tropicalis*.

Morphogenesis and Phenotypic Switching

Subsequently to the adhesion step to host cells, bud-to-hyphae transition (also called morphogenesis) is highly relevant to some pathogenic yeasts, including *Candida* spp. (Calderone and Gow, 2002). It is one of the most important steps for the establishment of candidiasis and is considered a necessary step for several virulence processes, including invasion of host epithelial layers, endothelial rupture, survival to phagocytic cells attack, biofilm formation, and thigmotropism (Lackey et al., 2013).

Studies concerning morphogenesis are very well-established for *C. albicans*, with very well-established environmental signals, transcription regulators, and target genes involved in filamentation (Gustin et al., 1998; Kumamoto and Vences, 2005; Wapinski et al., 2007; Lackey et al., 2013). However, there are considerably less studies concerning morphogenesis in other NCAC species. Several *Candida* species may develop pseudo-hyphae, but quite a few are able to form true hyphae, including *C. albicans*, *C. dubliniensis*, and *C. tropicalis*. The latter do not show the same degree of filamentation than *C. albicans*; however, because of the fact they are frequently associated with infectious processes, they certainly have mechanisms of adaptation that may favor filamentation in specific environmental conditions (Lackey et al., 2013).

Galan-Ladero et al. (2013) evaluated the filamentation among *C. tropicalis* isolates obtained from different anatomic sites of patients admitted in a Spanish tertiary hospital (Galan-Ladero

TABLE 3 | Genes recognized as virulence factors in *Candida tropicalis*.

	Gene	Gene product in <i>C. tropicalis</i>	Biological function	References
Adhesion to epithelial cells	<i>ALS</i>	Als1 Als2 Als3	Adhesin	Hoyer et al., 2001; Punithavathy and Menon, 2012
	<i>HWP1</i>	Hwp1p	Hyphal cell wall adhesin	Wan Harun et al., 2013
Morphogenesis	<i>UME6</i>	Ume6p	Positive transcription regulator responsible for hyphae morphology and extension; induces <i>HGC1</i> transcription	Lackey et al., 2013
	<i>NRG1</i>	Nrg1p	Negative transcription regulator; inhibiting filamentation	
	<i>HGC1</i>	Hcg1p	Forms a complex between cyclin/Cdk and CDC28 kinase, to inhibit cell separation and activation of Cdc42 regulator (involved in vesicular transport in hyphae and actin polymerization)	Zheng et al., 2007; Gonzalez-Novo et al., 2008; Lackey et al., 2013
	<i>PHR1</i>	Phr1p	Remodeling of the cell wall, necessary for maintenance of hyphae shape and growth, adhesion to abiotic surfaces and invasion of the epithelium	Calderon et al., 2010
	<i>CDC12</i>	cdc12p septin	Formation of the cytoskeleton during cell growth in filamentation; Binding to cdc3p actin ligand	Chang et al., 1997; Li et al., 2012
	<i>WOR1</i>	Wor1p	Transcription factor that induces filamentation	Slutsky et al., 1987; Porman et al., 2013
Phenotypic switching	<i>EFG1</i>	Efg1	Activator or a repressor of hypha formation	Mancera et al., 2015
	<i>WOR1</i>	Wor1p	Master regulator of the white-opaque switching	Slutsky et al., 1987; Porman et al., 2013
Biofilm formation	<i>ALS</i>	Als1 Als2 Als3	Adhesin	Hoyer et al., 2001; Punithavathy and Menon, 2012; Wan Harun et al., 2013
	<i>HWP1</i>	Hwp1p	Hyphal cell wall adhesin	
	<i>BCR1</i>	Bcr1p	Transcription factor for regulation of adhesin production	
	<i>RBT5</i>	Rbt5p	Filamentation of cells in the biofilm	Nobile and Mitchell, 2006; Fitzpatrick et al., 2010
	<i>UME6</i>	Ume6p	Negative dispersion regulator of biofilm cells	
	<i>WOR1</i>	Wor1p	Negative regulator of mature biofilm cell release	Uppuluri et al., 2010
	<i>NRG1</i>	Nrg1p	Positive regulator of cells dispersion in biofilm	
	<i>ERG11</i>	Erg11p	Mechanisms of resistance	Lupetti et al., 2002
	<i>MDR1</i>	Mdr1p	Active drug efflux pump	Marie and White, 2009; Morschhäuser, 2010
Proteinase activity	<i>SAPT1</i> <i>SAPT2</i> <i>SAPT3</i> <i>SAPT4</i>	Sapt1p	Protein hydrolysis	Togni et al., 1996; Zaugg et al., 2001; Silva et al., 2011
Phospholipases activity	<i>PLB1</i> <i>PLC1</i>	Plb1, Plc1	Hydrolysis of ester bonds in glycerol phospholipids	Bennett et al., 1998; Hoover et al., 1998
Hemolytic activity	<i>RBT5</i>	Rbt5	GPI-anchored cell-wall protein involved in hemoglobin utilization	Nobile and Mitchell, 2006; Fitzpatrick et al., 2010

et al., 2013). The authors described high levels of filamentation for 76.6% of the isolates at the specific environmental conditions. Wapinski et al. (2007) reported that at least 55 out of the 105 genes involved in *C. albicans* filamentation are conserved in *C. tropicalis* (Wapinski et al., 2007).

Lackey et al. (2013) induced *C. tropicalis* cells filamentation and analyzed gene expression at the conditions provided (Lackey et al., 2013). They found significant filamentation in serum and glucose medium at 37°C. Optical microscopy showed the

presence of elongated yeast-like cells, pseudohyphae, and true hyphae that were shorter than the ones found in *C. albicans*. They also verified that the negatively regulated gene *NRG1* has an important role in inhibiting filamentation in other NCAC species, suggesting that this gene may be related with poorer filamentation found among these species (Table 3). The *UME6* gene is transcriptionally induced during filamentation in *C. tropicalis*, similarly to what happens in *C. albicans* (Table 3; Banerjee et al., 2013).

Porman et al. (2013) reported the elevated expression of the transcriptional regulator *WOR1* (Table 3) in *C. tropicalis* cells cultivated on Spider medium (Porman et al., 2013). The micromorphological analysis of isolates with wrinkled phenotype showed that the most filamentous strains had *WOR1* overexpression. *Wor1p* homologs were also found in *Saccharomyces cerevisiae* (Cain et al., 2012) and *Histoplasma capsulatum* (Nguyen and Sil, 2008), controlling morphological transition within these species. This finding may suggest the existence of a common ancestor gene found in the *C. tropicalis* genome (Porman et al., 2013).

In addition, *Wor1* which is the master regulator of the white-opaque switching, a phenomenon which is related to the reversible transition of cells from a white phase to an opaque phase, where cells are larger and elongated, while colonies have wrinkled appearance (Slutsky et al., 1987). Besides morphology, these two different cell types exhibit dramatic differences regarding to the preferred anatomic sites they colonize and infect, in addition to specific responses to environmental and nutritional signals and mating behavior (Mancera et al., 2015). In *C. tropicalis*, *WOR1* overexpression direct cells to the opaque phase which is involved in biofilm formation and morphogenesis (Porman et al., 2013).

It was described in the *C. tropicalis* genome an ortholog of the transcription factor *Efg1* (enhanced filamentous growth), commonly found in *C. albicans* (Table 3; Mancera et al., 2015). The deletion of both alleles of the *EFG1* gene revealed that *Efg1p* is essential for filamentation, biofilm formation, and white-opaque switching in *C. tropicalis*, similarly to *C. albicans*, indicating conservation in the function of this ortholog gene.

Zhang Y. et al. (2016) reported a gray phenotype in *C. tropicalis* recently, whose cells are small and elongated, show intermediate mating competence and virulence in rats' animal models (Zhang Y. et al., 2016).

Biofilm Formation

The ability of yeast cells to form biofilms is an important determinant of virulence in *Candida* spp. and has been considered the main form of microbial growth recently (Donlan and Costerton, 2002; Fanning and Mitchell, 2012). Biofilms are complex structures formed by a community of microorganisms adhered to solid surfaces of either biotic or abiotic nature. Therefore, *in vitro* biofilm formation may be organized by three important steps, as follows: adhesion and colonization of yeast cells on a surface; cellular growth and proliferation, forming a basal layer; and pseudohyphal and/or true hyphal formation (for the species that are able to form filaments), with the subsequent secretion of an exopolymeric extracellular matrix which embeds microorganisms with low growth rates and altered phenotypes (Hawser and Douglas, 1995; Baillie and Douglas, 1999; Chandra et al., 2001; Ramage et al., 2001; Douglas, 2003). The exopolymeric matrix (EPS) may be secreted by different populations of either unique or multiple microbial species (Adam et al., 2002). Some advantages of biofilm formation include: the protection of microorganisms against environmental damage, nutrients availability, metabolic cooperation, and the acquisition of genetic modification (Douglas, 2002).

The formation of the microbial community involves a cascade of molecular mechanisms and fine alterations in gene expression (Nobile and Mitchell, 2006; Araújo et al., 2017). Signaling molecules which naturally occur in fungal cells as a response to environmental stimuli are part of this process present in the *Candida* genus (Ramage et al., 2006). This regulation is called "quorum sensing" (QS) mechanism and is the main communication form among several microorganisms correlated to population density (Albuquerque and Casadevall, 2012).

Farnesol is kind of self-regulator, a sesquiterpene with the ability to inhibit biofilm formation and altering the expression of 274 genes in *C. albicans*, specifically involved in filamentation. Weber et al. (2010) investigated the role of farnesol in biofilm formation of *C. tropicalis*. They found that besides inhibiting cellular aggregates, cells of the *C. tropicalis* mature biofilm were also influenced by farnesol, which may be related to their dispersion to other body sites (Nickerson et al., 2006; Ramage et al., 2006).

The initial step for biofilm formation is dependent of cellular adhesion cells to substrates and further formation of a basal layer (Nobile and Mitchell, 2006). *C. tropicalis* adhesins are also involved in biofilm formation (Table 3; Punithavathy and Menon, 2012; Wan Harun et al., 2013), and are regulated by the *BCR1* gene (also considered a cell wall regulator). In addition, the *RBT5* gene was also found in the *C. tropicalis* genome (Fitzpatrick et al., 2010).

Other genes involved in *C. tropicalis* biofilm formation are *WOR1*, *UME6*, *NRG1*, *ERG11*, and *MDR1* (Table 3). Besides being involved with morphogenesis and phenotypic switching, *WOR1* is one of the main transcriptional factors involved in biofilm formation (Xie et al., 2012; Porman et al., 2013).

UME6 and *NRG1* are key transcription regulators directly involved in morphogenesis in *C. tropicalis* (Finkel and Mitchell, 2011). The overexpression of *UME6* reduces the liberation of mature sessile cells, while the decreased expression of *NRG1* promotes cells dispersion (Uppuluri et al., 2010).

With respect to the expression of resistance genes to antifungal drugs, *ERG11* (ergosterol biosynthesis), and *MDR1* (multidrug resistance) genes (Table 3) are related with resistance to fluconazole. Bizerra et al. (2008) reported the increased expression of these genes in sessile cells of *C. tropicalis* isolated from vulvovaginal candidiasis (VVC) and uroculture resistant to both fluconazole and amphotericin B. Punithavathy and Menon (2012) also demonstrated higher resistance to fluconazole of sessile cells liberated from mature biofilms of *C. tropicalis*.

There are evidences that biofilm cells formed on medical devices constantly released in the bloodstream guarantee the successful establishment of disseminated candidiasis (Fanning and Mitchell, 2012). Marcos-Zambrano et al. (2014) investigated biofilm formation in different *Candida* species obtained from episodes of fungemia and found *C. tropicalis* isolates were the strongest biofilm producers. In fact, another study reported that the high thickness of the EPS matrix of *C. tropicalis* biofilm cells may impair oxygen and nutrients diffusion to cells, and may be responsible for the lower metabolic activity (Alnuaimi et al., 2013).

Pannanusorn et al. (2013) also described *C. tropicalis* as the most efficient biofilm producers among bloodstream isolates as compared to other NCAC species. Paiva et al. (2012) evaluated the *in vitro* biofilm formation by *C. tropicalis* isolates obtained from VVC. This species was also considered the strongest biofilm producer compared to *C. albicans*, yeasts belonging to the *C. parapsilosis* complex, *C. glabrata*, and *C. guilliermondii*. A similar trend was also described by Udayalaxmi et al. (2014) with strains isolated from the urogenital tract (samples from vaginal fluid and urine) of patients from a tertiary hospital in the South of India. Therefore, *C. tropicalis* has been considered an important biofilm producer species of the *Candida* genus.

Lytic Enzymes

In order to facilitate host tissues invasions, several pathogenic microbes secrete lytic enzymes such as proteinases, phospholipases, and hemolysins to destroy, alter, or damage the integrity of host membranes, leading to the dysfunction, or rupture of host cells (Sanita et al., 2014).

Pathogenic *Candida* species produce a great variety of hydrolases, including secreted aspartic proteinases (Saps). These proteins have been intensely investigated, and possess a wide range of substrates, including collagen, queratin, and mucin. They have the ability to degrade epithelial barriers, antibodies, complement, and cytokines (Hube and Naglik, 2001), and are encoded by a great gene family. The *SAP* gene family is composed by 10 genes and was initially described in *C. albicans* (Ruchel et al., 1983). These genes are differentially regulated and expressed under several laboratory conditions and are activated during different stages of infections *in vivo*. In addition, some of the *SAP* genes are more important to superficial rather than systemic infections, and are also involved in other pathogenic process in *C. albicans*, such as adhesion, host tissue invasion, and immunological system cells evasion (Hube and Naglik, 2001).

It is well-known since 1983 that *C. tropicalis* is able to secrete proteinases as one of the most important determinants of virulence of this species (Macdonald and Odds, 1983; Ruchel et al., 1983). In 1991, Togni et al. reported the nucleotide sequence of a gene involved with the extracellular secretion of proteinases by this yeast, while in 1996 the same authors reported the secretion of Sapt1p by *C. tropicalis* (Togni et al., 1991, 1996). Subsequently, the crystallographic structure of this protein was published, and was considered very similar to the Sap2p of *C. albicans* (Symersky et al., 1997).

A study performed by Zaugg et al. (2001) suggested the existence of a *SAPT* gene family in the *C. tropicalis* genome, leading to four genes cloning: *SAPT* (1–4; **Table 3**). However, only Sapt1p was purified from culture supernatant and biochemically characterized.

Silva et al. (2011) investigated epithelial invasion by *C. tropicalis* using a reconstituted human buccal epithelia model. All the isolates tested were able to colonize this tissue and cause a great damage after 24 h. Real time PCR showed that *SAPT2-4* transcripts were detected, while *SAPT1* expression was rarely observed. In addition, the authors showed that there was no increase in *SAPT1* expression, suggesting that the high invasive capacity of *C. tropicalis* may not be related with the specific

expression of this gene. Following the same trend, Togni et al. (1996) reported that *SAPT1* gene disruption in *C. tropicalis* seemed to have low effect in attenuation of virulence in mice, in a model of systemic infection.

Costa et al. (2010) evaluated proteinase activity of 15 isolates of *C. albicans* and 15 of *C. tropicalis* obtained from the saliva of dental patients in Brazil. All *C. tropicalis* isolates showed higher enzymatic production than *C. albicans*. These results are contradictory to most of the studies which suggest higher proteinase activity in *C. albicans* than in *C. tropicalis* (Zaugg et al., 2001; Sachin et al., 2012).

In addition to the secretion of proteinases, the secretion of phospholipases constitutes important determinants of virulence in *Candida* spp. This heterogeneous group of enzymes catalyzes the hydrolysis of ester bonds in glycerol phospholipids, with each enzyme participating in a specific reaction (Ghannoum, 2000). Secretion of phospholipases is therefore considered a key attribute for invasion of host epithelia, since phospholipids are major components of all cell membranes. In addition, the breakdown of these molecules promotes great instability in host cells, resulting in cellular lysis (Schaller et al., 2005).

One of the first studies that analyzed the production of phospholipases in *Candida* spp. was published in 1984 by Samaranyake et al. which demonstrated the secretion of these enzymes only in *C. albicans* isolates, without any detection in *C. tropicalis* (Samaranyake et al., 1984). However, other authors later reported phospholipase activity in isolates of this species. A recent study conducted by Jiang et al. (2016) with 52 strains of *C. tropicalis* found phospholipase activity in 31 isolates from different clinical sources. However, strains showed low enzyme production. Another study with 29 strains of several anatomic sites obtained from hospitalized patients, described low or no phospholipase activity in *C. tropicalis* (Galan-Ladero et al., 2010).

Conversely, a study conducted by Deorukhkar et al. (2014) investigating the expression of several virulence factors in 125 clinical isolates of this species concluded that the secretion of phospholipases was the main determinant of virulence expressed by these strains. The authors suggest that the variability of results between different authors may be a result of biological differences among the isolates tested.

Related to the expression of these enzymes in the presence of antifungal drugs, Anil and Samaranyake (2003) analyzed the effect of previous exposure of *C. albicans* and *C. tropicalis* to antifungal drugs on extracellular phospholipase activity. They concluded that the enzymatic activity of both species reduced significantly after previous exposure to nystatin and amphotericin B. In fact, they showed that *C. albicans* had greater phospholipase expression than *C. tropicalis*.

Phospholipases are classified into four major groups, named from A to D, all already well-described for *C. albicans* (Schaller et al., 2005). However, a few studies address this gene regulation in *C. tropicalis*. Phospholipase B (PLB; **Table 3**) is known to catalyze the hydrolytic cleavage of sn-1 and acyclic glycerophospholipid sn-2 esters (Ghannoum, 2000) and is primarily responsible for phospholipase activity in *C. albicans* (Schaller et al., 2005).

In 1998, Hoover et al. published an investigation with degenerate oligonucleotides (derived from conserved regions of the *PLB1* gene of *S. cerevisiae* and other fungi) to amplify homologous fragments of *PLB1* in *C. albicans* and *C. tropicalis* by PCR. The main PCR product obtained was a 540 bp fragment with a high probability of *PLB1*-correspondence of other fungi, and significant homology was found between the deduced amino acid sequence of the PCR product of *C. albicans* and *C. tropicalis* and the corresponding regions of *PLB1* sequence of *S. cerevisiae*, *Torulaspora delbrueckii*, and *Penicillium notatum* (~70–75% resemblance, ~55–65% identity). In that same year, Bennett et al. (1998) evaluated the presence of homologous sequences to *C. albicans* PLC in NCAC species, including five isolates of *C. tropicalis*. A DNA sequence homologous to *CAPLC1* was detected in only three of these isolates. Thus, the need for further studies addressing the molecular mechanisms related to phospholipase activity in *C. tropicalis* is evident.

The hemolysins are another group of proteins that significantly contribute for the dissemination of *Candida* infections, specifically in facilitating hyphal penetration in host tissues (Luo et al., 2004; Tsang et al., 2007). Hemolytic factors secreted by fungi cause hemoglobin liberation from red blood cells for further utilization by yeasts as an iron source (Giolo and Svidzinski, 2010). This chemical element is an essential cofactor to a great number of metabolic processes, such as oxygen transport, gene expression regulation, and DNA synthesis. Therefore, the ability of iron acquisition is of fundamental importance for microorganisms survival and establishment of infectious processes (Giolo and Svidzinski, 2010).

Manns et al. (1994) reported iron acquisition from erythrocytes by *C. albicans* as a consequence of a protein factor that promoted host cells lysis. In 1997, Tanaka et al. reported that this factor is liberated from the culture medium supernatant, and concluded that it was a cell wall manoprotein. The same phenomenon was observed in *C. tropicalis* (Favero et al., 2014), and although this factor is known as directly involved with yeasts pathogenicity, it is still poorly understood (Favero et al., 2011).

The study conducted by Luo et al. (2001) was the first one to show differences in hemolysin production by different *Candida* species on SDA plates containing sheep blood. The authors also observed that the hemolysis induced by this method could be divided into categories according to the standard microbiological nomenclature, including: total hemolysis (beta), partial hemolysis (alpha), or hemolysis absence (gamma). In this study with 80 isolates of 14 different *Candida* species, all the five isolates of *C. tropicalis* showed a large clear halo around colonies, proving the ability of *C. tropicalis* in producing beta hemolysis. Similarly, Favero et al. (2011) detected hemolysin production in *C. tropicalis* strains after incubation in both solid and liquid SDA containing either human or sheep blood.

A study produced by Rossoni et al. (2013) evaluated the hemolytic activity in different *Candida* species obtained from the oral cavity of HIV positive patients. Strong hemolytic activity was observed in 75% of *C. tropicalis* isolates evaluated, only after *C. albicans*. Similar results were found for *Candida* isolates obtained from different anatomical sites (blood, synovial,

and peritoneal liquid) where, again, *C. albicans* proceeded *C. tropicalis* in hemolysins production (de Melo Riceto et al., 2015).

Contradictory to these results, Favero et al. (2014) analyzing clinical *Candida* spp. isolates from bloodstream infection, reported low hemolytic activity in *C. albicans*, while *C. tropicalis* was the species tested with greater hemolysins production.

The genetic regulation of hemolysins production in the *Candida* genus was not still largely investigated (Anil et al., 2014). It is known that in *C. glabrata*, the *HLP* gene (hemolysin-like protein) encodes a protein associated with hemolytic activity (Luo et al., 2004). In *C. albicans* the *Csap* is involved with iron acquisition from host erythrocytes during hyphal development (Okamoto-Shibayama et al., 2014). This enzyme is a member of the Rbt5 protein (Table 3), also described in *C. tropicalis*, as previously mentioned. However, there are currently no studies in the literature concerning the genetic elucidation of hemolytic activity in *C. tropicalis*.

IN VIVO MODELS OF INFECTION BY *CANDIDA TROPICALIS*

The characterization of the expression of most variable virulence factors by *Candida* spp. and other fungi are necessary for the understanding each particular pathway involved in microorganisms pathogenicity (Takakura et al., 2003; Solis and Filler, 2012; de Campos Rasteiro et al., 2014). However, experiments performed *in vivo* involve different variables which cannot be controlled like what happens in experimental conditions *in vitro*, including the presence of body fluids, pH variation, commensal microorganisms, and their metabolites and host response during infection. Therefore, *in vivo* experimental models are needed for the global understanding of infectious disease pathogenicity, interactions with host cells and immune response as well as it is a more appropriate approach to evaluate new therapeutic strategies (Takakura et al., 2003; Solis and Filler, 2012; de Campos Rasteiro et al., 2014).

Several studies have been described in the literature with animal models of infections by *C. tropicalis* using mice (Bayegan et al., 2010; Mariné et al., 2010; Koga-Ito et al., 2011; Chen et al., 2014; Nash et al., 2016a; Wang et al., 2016; Zhang Y. et al., 2016). Nash et al. (2016a) evaluated the co-infection of six different *Candida* species (*C. albicans*, *C. tropicalis*, *C. parapsilosis*, *C. krusei*, *C. dubliniensis*, and *C. glabrata*) with *Staphylococcus aureus*, intraperitoneally inoculated. They evaluated mortality rates and attributed a score of 1–4 to evaluate characteristics of morbidity (creepy hair, absence of mobility, arched posture, and ocular secretion). *C. tropicalis* associated with *S. aureus* showed the second highest mortality rate (behind *C. albicans*) and a mortality index of 3 (Nash et al., 2016a).

Animal models of systemic infections may be induced by the inoculation of *C. tropicalis* via the lateral tail vein (Table 4; Zhang Y. et al., 2016). This via of infection was established by Zhang Y. et al. (2016) to evaluate virulence of a new phenotype described by *C. tropicalis*, the “Grey phenotype,” besides the other phenotypes already described (White–Opaque). After systemic

TABLE 4 | *In vivo* models of *Candida tropicalis* infection.

Organism	Site of infection	References
Mice	Lateral tail vein	Zhang Q. et al., 2016
<i>Bombyx mori</i> larvae	Larval hemolymph	Hamamoto et al., 2004; Nwibo et al., 2015; Uchida et al., 2016
<i>Drosophila melanogaster</i> larvae	Injected in the thorax	Zanette and Kontoyiannis, 2013
<i>Galleria mellonella</i> larvae	Last left proleg	Forastiero et al., 2013

infection through the tail vein with strains of each phenotype (Gray, White, and Opaque), animal organs have been removed and macerated and fungal load was evaluated. The authors found that cells of the Gray phenotype showed intermediate distribution, but greater than cells with the White phenotype for all the organs evaluated (kidney, lungs, spleen, liver, and brain), 24 h and 7 days after infection.

Other mice models of *Candida* infections have been described in the literature such as the VVC model described by Fidel et al. (1997), where doses of estradiol valerate are subcutaneously administered (0.1 mg/100 μ l of sesame oil) in the vagina of animals infected with 5×10^4 cells/20 μ l PBS in order to successfully establish the vaginal infection (Fidel et al., 1996, 1997; Garvey et al., 2015; Nash et al., 2016b). Nevertheless, to the best of our knowledge, they were still not employed for the experimental investigation of VVC caused by *C. tropicalis*.

Alternative models of experimental infections have been broadly used for virulence and interactions with the host studies (Table 4; Hamamoto et al., 2004; Forastiero et al., 2013; Mesa-Arango et al., 2013; Zanette and Kontoyiannis, 2013; de Souza et al., 2015; Ishii et al., 2015; Shu et al., 2016). Several factors are considered as an advantage for the utilization of a model of infection using larvae, including an easier manipulation and lower maintenance cost (de Souza et al., 2015; Ishii et al., 2015). The Silkworm—*Bombyx mori*, (Lepidoptera: Bombycidae) produces a large enough larvae for antifungal drugs distribution studies (Nwibo et al., 2015; Uchida et al., 2016). *B. mori* larvae were used as a *C. tropicalis* model of infection in order to evaluate the effective dose of both fluconazole and amphotericin B (Hamamoto et al., 2004). When *C. tropicalis* was inoculated into the larval hemolymph followed by antifungal drugs administration, it was obtained an effective dose for 50% of them (ED 50%) of 1.8 μ g/g of larvae for amphotericin B and fluconazole, being in agreement with previous animal models using mice previously performed (Hamamoto et al., 2004). Therefore, it confirms its possible use for *C. tropicalis* virulence studies.

Drosophila melanogaster larvae (Diptera: Drosophilidae), known as fruit flies also have been used as an animal model to study microbial interactions with innate immune response (Alarco et al., 2004). Zanette and Kontoyiannis satisfactorily used this model to investigate *C. tropicalis* strains with or without paradoxical growth (Zanette and Kontoyiannis, 2013).

Galleria mellonella larvae (Lepidoptera: Pyralidae) have been used as another invertebrate model to investigate fungal and

host interactions (Champion et al., 2016), in systemic studies of antimicrobial efficiency (Wei et al., 2016), evaluation of virulence in immunosuppressive models (Torres et al., 2016), immunomodulatory response (Fuchs et al., 2016), and antifungal resistance (Souza et al., 2015).

G. mellonella infection by *C. tropicalis* was used to investigate cross resistance to azoles or multidrug resistance among them and amphotericin B (Forastiero et al., 2013). Two hours after infection with *C. tropicalis*, different antifungal drugs were applied (fluconazole, voriconazole, amphotericin B, and anidulafungin). In this study, 80% of the untreated infected larvae died between day 3 and 4 of infection, while better survival rates were observed for animals inoculated with susceptible strains (10 mg/kg/day of voriconazole and 9 mg/kg/day of fluconazole). When the larvae were infected with strains resistant to the azoles with the same therapeutic doses, survival rates were equivalent to the group that was untreated. This study demonstrates the reliable application of the use of the *G. mellonella* model for the study of infection by *Candida* as well as for the evaluation of antifungal action (Forastiero et al., 2013).

SUPERFICIAL AND SYSTEMIC INFECTIONS

C. tropicalis belongs to the normal human microbiota and is present on the skin, gastrointestinal, genitourinary, and respiratory tracts of humans (Basu et al., 2003; Oksuz et al., 2007; Negri et al., 2010). This yeast has been associated with superficial and systemic infections all over the world, specifically in neutropenic patients, or in individuals with a reduction of the microbiota by antimicrobial use or presenting damage in gastrointestinal mucosa (Colombo et al., 2006).

C. tropicalis is classified as the third or fourth NCAC species more commonly isolated in the clinical practice (Pfaller et al., 2010; Peman et al., 2012), but it is considered the most prevalent yeast in Asia (Chakrabarti et al., 2009; Kothavade et al., 2010; Adhikary and Joshi, 2011) and the second or the third more isolated species in Brazil and other Latin America countries (20.9 and 13.2%, respectively) (Pfaller et al., 2010). The expressive increase in isolation of this yeast in cases of both superficial and systemic infections in different casuistic all over the world emphasizes its emergent character.

The clinical aspects of *Candida* infections may vary according with the body site affected. Oral candidiasis, VVC, and onychomycosis are superficial mycoses caused by this genus, while systemic candidiasis involves blood and deep-seated organs such as the lungs and gastrointestinal tract (Jacobs and Nall, 1990).

Oral candidiasis is an opportunistic infection caused by *Candida* commonly found in the elderly (due to low immunity caused by age), HIV patients, malnourished individuals and those submitted to systemic steroid therapy, denture wearers, and people with xerostomia (Muadcheingka and Tantivitayakul, 2015). Clinical manifestations are divided into white and erythematous forms. The white form is characterized by whitish lesions and includes pseudomembranous candidiasis and hyperplastic candidiasis. The erythematous form presents

with red lesions, including acute atrophic candidiasis, chronic atrophic candidiasis, median rhomboid glossitis, angular cheilitis, and linear gingival erythema. There are also three forms which are not classified into these two clinical categories, which are chronic mucocutaneous candidiasis, cheilocandidiasis, and chronic multifocal candidiasis (Millsop and Fazel, 2016).

In Brazil, a study performed by da Silva-Rocha et al. (2014) investigated *Candida* species distribution of isolates obtained from the oral cavity of kidney transplant recipients from two geographic regions of Brazil (Northeast and South). The authors found that *C. tropicalis* was the second most prevalent species, corresponding to 4.5% of the isolates.

A prevalence study of *Candida* species obtained from oral candidiasis was carried out in Thailand with 250 strains isolated from 207 patients and *C. tropicalis* was the third most isolated species (10.4%) (Muadcheingka and Tantivitayakul, 2015). Similarly, in a study conducted in the northwest of Ethiopia with 215 oral cavity isolates from HIV positive patients, this yeast was also the third most prevalent species, with a percentage of isolation equal to 14.1%. More interestingly, 8% of them were resistant to fluconazole and 4% to ketoconazole, itraconazole, and flucytosine (Mulu et al., 2013). Another Indian study concluded that there was a significant increase in *Candida* infections in oral cancer patients who underwent chemotherapy or radiotherapy, where NCAC species predominated, mainly *C. tropicalis*, occurring in 42.8% of cases (Jain et al., 2016).

VVC is an infection of the vulva and vagina caused by different *Candida* spp. (de Medeiros et al., 2014; Sobel, 2016). *C. tropicalis* is generally described as the third most prevalent *Candida* species in VVC, preceded by *C. albicans* and *C. glabrata* in most of the studies (Dias et al., 2011; Kanagal et al., 2014; Rangunathan et al., 2014).

A study developed in India by Vijaya et al. (2014) with 300 women of reproductive age with clinical signs of VVC reported *C. tropicalis* as the second most prevalent *Candida* species, corresponding to 26.4% of the isolates. Of these, 42.9% were resistant to fluconazole and 14.3% to voriconazole.

An investigation in Iran with 67 *Candida* isolates obtained from vaginal secretion samples from patients with VVC found that *C. tropicalis* was present in 5.9% of cases, with 100% resistance to fluconazole, 50% resistance to clotrimazole, 25% to ketoconazole, and 75% against terbinafine. In addition, all isolates showed dose-dependent susceptibility (DDS) to itraconazole (Salehei et al., 2012).

Nevertheless, *C. tropicalis* is reported to a lesser degree in cases of onychomycosis in relation to other species such as *C. albicans* and *C. parapsilosis* species complex, promoting paronychia infection mainly in immunosuppressed patients and individuals in extreme age (elderly and children) (Aghamirian and Ghiasian, 2010; Cambuim et al., 2011).

A study developed in South Korea reports the prevalence of the *Candida* genus in 59% of cases of onychomycosis in pediatric patients. The authors obtained 39 isolates, where only 2.6% of them belonged to *C. tropicalis* (Kim et al., 2013). Another study developed in Mexico analyzing 166 samples of dystrophic nails reports *C. tropicalis* as one of the less prevalent *Candida* species in onychomycosis, corresponding to 4.2% of the isolates.

However, 14.2% were resistant to fluconazole, itraconazole, and ketoconazole (Manzano-Gayosso et al., 2011), reinforcing its clinical importance.

However, contradictory results were found in a Brazilian study with 200 *Candida* isolates obtained from nail infections that reported a prevalence of 26% for *C. tropicalis*. These authors also observed high antifungal drugs resistance in these isolates, including 30.6% resistance to fluconazole, 25% to itraconazole, 9.6% to ketoconazole, and 96.2% resistance to terbinafine (Figueiredo et al., 2007).

According to McCarty and Pappas (2016), invasive infection by *Candida* species is commonly associated with medical care, where they may be the third or fourth cause of bloodstream infection (BSI). Risk factors for systemic candidiasis are well-known and include the presence of central venous catheter (CVC), the exposure to broad spectrum antibacterial agents, prolonged staying in the ICU with or without mechanic ventilation (more than 3 days), complex surgery, the presence of necrotizing pancreatitis, hemodialysis, and immunosuppressive conditions (McCarty and Pappas, 2016). Candidemia caused by *C. tropicalis* infection has a greater association with skin petechia than other *Candida* species (Manzano-Gayosso et al., 2011), and this species was described as the most common etiological agent of invasive infection associated with the hospital environment in India (Giri and Kindo, 2012).

According to Kontoyiannis et al. (2001), *C. tropicalis* produces more persistent systemic infections than *C. albicans*, leading to a longer stay in the hospital environment. Other studies have associated *C. tropicalis* infections with a higher mortality rate when compared to other NCAC species, even when compared to *C. albicans* (Krcmery et al., 1999; Kontoyiannis et al., 2001; Eggimann et al., 2003). This factor may be related to the known higher virulence of both species as well as to a higher antifungal resistance by *C. tropicalis*.

Recently, an important multicenter study was carried out in 29 Spanish hospitals, where *C. tropicalis* was isolated in 7.6% of a total of 781 cases of candidemia and 20% of them were resistant to azoles (Guinea et al., 2014). Another multicenter study in China with 389 isolates from patients with candidemia admitted to intensive care units found *C. tropicalis* as the third most isolated species (17.2%), while resistance to fluconazole was observed in 37.3% of isolates of this species, as well as 10% of them were resistant to voriconazole (Liu et al., 2014).

A research carried out in Malaysia with 82 bloodstream isolates and peritoneal fluid reports *C. tropicalis* as responsible for 18.3% of the isolates obtained. Resistance to ketoconazole was observed in 20.9% of the clinical strains, in addition to 13.4% resistance to itraconazole (Santhanam et al., 2013). A study performed by Chang et al. (2013) with isolates from 152 cases of candidemia in Taiwan reported a prevalence of 19.7% for *C. tropicalis*.

In Brazil, a study conducted by Oliveira (2011) investigated candidemia in a pediatric hospital in Sao Paulo from 2007 to 2010. *C. tropicalis* was the second most isolated *Candida* species (24%), only preceded by *C. albicans*. More recently, a multicenter surveillance study involving 16 public and private hospitals in the five Brazilian regions (North, Northeast, Center-West, Southeast

and South) was conducted, which investigated 137 episodes of systemic infections. NCAC species were responsible for 65.7% of the total infections and *C. tropicalis* was the third most isolated *Candida* species (15.3%) (Doi et al., 2016).

It is known that candidemia is the most common form of invasive candidiasis, but there are other less frequent clinical manifestations with *C. tropicalis* as an etiological agent (McCarty and Pappas, 2016). For example, a case of acute disseminated candidiasis in a pediatric patient with aplastic anemia (Fong et al., 1988); the formation of fungal vegetation in a mitral valve prosthesis, causing endocarditis (Nagaraja et al., 2005); and the development of septic arthritis in a cancer patient on chemotherapy with diabetes secondary to corticosteroid therapy that had a negative outcome (Vicari et al., 2003).

Another unusual clinical form of candidiasis is endophthalmitis, which is considered an important indicator of systemic infection in hospitalized patients (Donahue et al., 1994). *C. tropicalis* seems to be an important etiological agent of this infirmity, being classified as the fourth species of the genus to promote ocular infection in adult and pediatric patients attended at two medical centers in the USA (Dozier et al., 2011).

Disseminated chronic candidiasis is another condition of low occurrence characterized by the presence of histopathological evidence of candidiasis in the spleen, liver, and kidneys, or radiological evidence of hepatosplenic or renal candidiasis (Al-Anazi and Al-Jasser, 2006). Xu et al. (2010) described the isolation of *C. tropicalis* in a patient with acute leukemia whose computerized tomography showed multiple hypodense lesions in the liver and spleen. This yeast was also isolated from the kidneys of a patient diagnosed with acute lymphocytic leukemia (Sun et al., 2006) and was associated with a higher mortality rate than other *Candida* species involved in this disease (Al-Anazi and Al-Jasser, 2006).

Finally, *C. tropicalis* is more rarely found as an etiological agent of respiratory tract infections (Garczewska et al., 2016). This was the second most common yeast species in patients with cystic fibrosis, preceded only by *C. albicans*. Similarly, another study reports *C. tropicalis* as the second most common *Candida* species in cases of pulmonary co-infection with *Mycobacterium tuberculosis* (Kali et al., 2013).

ANTIFUNGAL SUSCEPTIBILITY

The high incidence of severe infections caused by *C. tropicalis* has attracted attention, especially considering the evident increase in the reports of resistance of this yeast to antifungal drugs, which is a serious therapeutic problem.

Resistance to azoles in this species has been extensively reported, especially to fluconazole. In this respect, Anil and Samaranyake (2003) argue that the increasing global use of this drug is one of the main causes for the dominant tendency of infections caused by NCAC species to the detriment of *C. albicans*. It is known that there are several factors involved in the development of *Candida* spp. antifungal resistance in clinical settings, including indiscriminate antifungal therapy use in nosocomial infections (Joseph-Horne and Hollomon, 1997). However, studies on the molecular mechanisms underlying this phenomenon are still necessary.

With regard to azoles, the action target of these compounds is the enzyme 14 α -lanosterol demethylase (Erg11p), a product of the *ERG11* gene (Table 5), which is part of the ergosterol biosynthesis pathway (Lupetti et al., 2002). Ergosterol is the predominant component of the cell membrane of fungi, and influences various cellular functions such as membrane fluidity and integrity, as well as the adequate activity of various enzymes anchored to it, such as proteins related to nutrient transport and chitin synthesis. Therefore, the azoles cause depletion of ergosterol and accumulation of 14 α -methyl steroids harmful to cells, inhibiting growth of fungal cells (Lupetti et al., 2002).

Sanglard and Odds (2002) report different mechanisms that may lead to resistance to azoles. The first is the action of multidrug transporters or efflux pumps, which leads to a decrease in drug concentration within the fungal cell (Pfaller, 2012). The positive regulation of *MDR1* ("multidrug resistance gene") and *CDR1* ("*Candida* drug resistance") genes (Table 5), are related to the active efflux of azoles in several *Candida* species, including *C. tropicalis* (Marie and White, 2009; Morschhäuser, 2010). The induction of efflux caused by *CDR* genes tends to affect all azoles. In contrast, efflux pumps encoded by *MDR* genes in *Candida* are normally selective for fluconazole (Pfaller, 2012).

Another pathway leading to azole resistance is the occurrence of amino acid substitutions in Erg11p, which is the target of these drugs, generating changes in protein conformation (Forastiero et al., 2013). Increased *ERG11* gene expression results in the production of a large amount of 14 α -lanosterol demethylase, favoring the continuous synthesis of ergosterol, and the maintenance of cell integrity, which allows the fungus to resist the action of the drugs (Manastir et al., 2011). This factor may occur as a function of a point mutation in *ERG11* (Kelly et al., 1993). Pam et al. (2012) detected this point mutation in a *C. tropicalis* isolate with DDS to fluconazole and demonstrated increased *ERG11* expression.

Eddouzi et al. (2013) studied the molecular mechanisms of drug resistance in a clinical isolate of *C. tropicalis* with multidrug resistance to fluconazole, voriconazole, and amphotericin B, obtained from a hospital in Tunisia. Analysis of sterol production by mass spectrometry and gas chromatography revealed accumulation of 14 α -methylfecosterol, 4,14 α -dimethylzimossterol, and 14 α -methyl-3 β , 6 α -diol, indicating change in Erg3p (Table 5). Another study reported the occurrence of *ERG3* mutation in a *C. tropicalis* isolate, with substitution of a phenylalanine for serine in portion 258, a residue that is absolutely conserved in this protein (Vincent et al., 2013).

A study conducted in five Chinese hospitals investigated resistance to these drugs in 52 clinical isolates of *C. tropicalis* (Jiang et al., 2013). Resistance to fluconazole was observed in 34.6% of the isolates, while 40.4% were resistant to itraconazole and only 7.7% to voriconazole. The authors suggest that voriconazole has a more potent activity against the clinical isolates of *C. tropicalis* than the other drugs tested.

Despite the number of studies involving strains of *C. tropicalis* resistant to azoles, there are still relatively less studies regarding the resistance of this species to other drugs, such as amphotericin B. This compound is the third most commonly used antifungal in clinical practice (Seneviratne et al., 2016) and is part of the

TABLE 5 | Genes involved with antifungal resistance mechanisms in *Candida tropicalis*.

	Gene	Gene product in <i>C. tropicalis</i>	Biological function	References
Azoles	<i>ERG11</i>	Erg3p	Ergosterol biosynthesis pathway	Eddouzi et al., 2013; Vincent et al., 2013
	<i>ERG3</i>	Erg11p	Ergosterol biosynthesis pathway	Kelly et al., 1993; Manastir et al., 2011; Pam et al., 2012
	<i>MDR1</i>	Mdr1p	Energy-dependent transportation	Marie and White, 2009; Morschhäuser, 2010
	<i>CDR1</i>	Cdr1p	Energy-dependent transportation	
Amphotericin B	<i>ERG3</i>	Erg3p	Ergosterol biosynthesis pathway	Lupetti et al., 2002; Forastiero et al., 2013
	<i>ERG6</i>	Erg6p	Ergosterol biosynthesis pathway	Vandeputte et al., 2007
	<i>ERG11</i>	Erg11p	Ergosterol biosynthesis pathway	Forastiero et al., 2013
Echinocandins	<i>FKS1</i>	Fks1p	Catalytic action	Park et al., 2005; Garcia-Effron et al., 2008; Chen et al., 2011; Beyda et al., 2012; Jensen et al., 2013
	<i>FKS2</i>			
	<i>FKS3</i>			
	<i>RHO1</i>	Rho1p	Regulation of β -1,3-D-glucan biosynthesis and other cellular processes	

class of polyenes. Its fungicidal activity comes from the ability to selectively bind to the ergosterol of the fungal cell, inducing the formation of pores in the plasma membrane, resulting in intense osmotic imbalance and rapid collapse of the cell (Brajtburg et al., 1990). A recent study reported that the production of reactive oxygen species is also part of the fungicidal mechanism of action of amphotericin B (Forastiero et al., 2013).

Amphotericin B resistance seems to be a rare phenomenon in yeasts, but Woods and Bard in 1974 demonstrated the development of resistance to this drug in two isolates of *C. tropicalis* obtained from the urine of a patient with pyelonephritis (Woods et al., 1974). A subsequent study of these strains revealed the existence of a mutation in the ergosterol of the cell membrane, exactly at the binding site of amphotericin B (Drutz and Lehrer, 1978). Also in the 1970s, Merz and Sandford reported the isolation of eight strains of *C. tropicalis* resistant to amphotericin B, obtained from urine of transplanted patients, with the same mutation in ergosterol (Merz and Sandford, 1979).

Reports of isolation of *C. tropicalis* resistant to this drug have been progressively increasing over the years. In 1988, Powderly et al., reported that the development of resistance to amphotericin B is most observed in patients with some kind of immunosuppression and who frequently use this drug (Powderly et al., 1988). Resistance to this polyene is believed to result from changes in ergosterol, or changes in the plasma membrane itself (Seneviratne et al., 2016).

Lupetti et al. (2002) postulated that resistance to amphotericin B in *Candida* species generally occurs due to defects in ergosterol biosynthesis and most likely results from mutations in the *ERG3* gene. In addition to the *ERG3* gene, mutations in *ERG6* can generate resistance to polyenes, a phenomenon already described in *C. tropicalis* (Vandeputte et al., 2007). A study conducted by Forastiero et al. (2013) showed that concomitant mutations in the *ERG11* and *ERG3* genes lead to multidrug resistance between amphotericin B and azoles (fluconazole and itraconazole) in *C. tropicalis*.

In addition to amphotericin B, echinocandins have been increasingly used in the treatment of invasive infections, being the first new class of echinocandins that target the fungal cell wall, blocking β -1,3-D-glucan synthase (Perlin, 2007). It has been described that these drugs have an excellent range of action against the main *Candida* species, including *C. tropicalis* (Pfaller et al., 2008).

A surveillance study carried out in 2008 by Pfaller et al. (2008) with 5,346 *Candida* spp. isolates obtained from candidemia infection showed a 12% prevalence for *C. tropicalis*, and all of them were 100% susceptible for the three echinocandins tested (casposfungin, anidulafungin, and micafungin).

Despite the extensive use of these drugs for more than a decade, the incidence of resistance in the *Candida* genus remains very low (Beyda et al., 2012). More recent surveillance studies have indicated an incidence of 2.9–3.1% *Candida* spp. resistance to echinocandins (Arendrup et al., 2010; Castanheira et al., 2010). However, recently Garcia-Effron et al. (2010) reported the isolation of three strains of *C. tropicalis* resistant to casposfungin obtained from patients with hematological malignancies. A study by Eschenauer et al. (2014) with 185 isolates of *C. tropicalis* reports 1.4% resistance to casposfungin, anidulafungin, and micafungin.

Another study described the presence of the paradoxical effect (or paradoxical growth) in 15 isolates of *C. tropicalis* in the presence of high concentrations of echinocandins (Soczo et al., 2007). This phenomenon was first documented by Stevens et al. (2004) for casposfungin in *C. albicans*, and is defined as fungal growth in the presence of echinocandin concentrations above MIC in broth microdilution susceptibility tests performed according to the guidelines of the Clinical Laboratory Standards Institute (CLSI, previously NCCLS) (Melo et al., 2007).

The target enzyme of echinocandins, called glucan synthetase, possesses at least two subunits: Fks1p (encoded by the *FKS1*, *FKS2* and *FKS3* genes) and Rho1p (Table 5; Beyda et al., 2012). Fks1p has a catalytic action and Rho1p is a regulatory protein of several cellular processes, including the biosynthesis

of β -1,3-D-glucan (Chen et al., 2011). In general, the reduction of *C. tropicalis* susceptibility to echinocandins occurs by response to adaptive stress or mutations in the *FKS* genes.

With regard to adaptive responses, it is known that the fungal cell wall is a dynamic structure that presents a compensatory mechanism to increase the production of one or more components that are eventually inhibited, such as that produced by the action of echinocandins. A study by Chen et al. (2014) investigated the role of calcineurin in *C. tropicalis*, which is one of the main signaling pathways for the compensatory increase of chitin synthesis in *C. albicans*. Calcineurin is a phosphatase that regulates numerous stress response processes in fungi, including stress promoted on the cell wall (Cowen, 2009). The study demonstrated that, in fact, calcineurin is responsible for this effect on *C. tropicalis* against micafungin, since it promotes the thickening of the chitin layer of the cell wall as a function of β -1,3-D-glucan depletion.

In the case of mutations in the *FKS1* gene, it is already well-established that substitutions in specific gene regions cause reduced susceptibility to echinocandins, being quite associated with therapeutic failure (Perlin, 2007). Mutations in the *FKS1* gene in *C. tropicalis* were already described (Park et al., 2005). Garcia-Effron et al. (2008) demonstrated that 7.5% (3/40) of clinical isolates of *C. tropicalis* showed resistance to caspofungin because of amino acid substitutions in Fks1p. Jensen et al. (2013) also performed an investigation alterations in *FKS1*, with isolates of *C. tropicalis* from patients with acute lymphoblastic leukemia and found that after 8–8.5 weeks of treatment with caspofungin, two isolates showed resistance to the three echinocandins. Multilocus sequencing of *FKS1* revealed progressive development of heterozygosity, and finally the presence of homozygous mutation, leading to substitutions of amino acids S80P and S80S.

The low level of resistance of *C. tropicalis* to echinocandins and lower side effects, since they target the wall of the fungal cell, make them vital in cases of resistance to fluconazole and amphotericin B, with a broad spectrum of action against *C. tropicalis* (Pfaller et al., 2008).

NATURAL PRODUCTS WITH ANTIFUNGAL PROPERTIES AGAINST *CANDIDA TROPICALIS*

Several groups have been dedicated to the study of products of natural origin with antifungal action, in order to identify and isolate compounds with effective activity, safety in use and low toxicity against pathogenic fungi (Correia et al., 2016).

There are several parts of the plants used to search for biological activity, with emphasis on antifungal action, such as leaves (Morais-Braga et al., 2016), stem bark (Mendes de Toledo et al., 2015), roots, seeds, and essential oils (Asdadi et al., 2015), that may be isolated used or in synergism with synthetic antifungal drugs, such as fluconazole (Mendes de Toledo et al., 2015).

The use of essential oils of *Vitex agnus* was used in a study by Asdadi et al. (2015) in clinical strains of *Candida* isolated

from hospital infection. The extraction product was tested against *Candida* isolates using the principle of disc diffusion and broth macrodilution, according to the standardization of CLSI (Salari et al., 2016). It was observed that for the isolates of *C. tropicalis*, 10 μ l of essential oils produced halos of inhibition of growth of 58 mm, superior to the halos of control drugs such as amphotericin B (8 mm), and fluconazole (21 mm) (Salari et al., 2016).

Salvia rhytidia Benth., A plant belonging to the family Lamiaceae, typical in Iran was used to evaluate the antifungal activity in several *Candida* isolates by Salari et al. (2016). A total of 96 clinical isolates of *Candida*, including 11 *C. tropicalis* strains were tested using broth microdilution with the methanolic extract, according to CLSI protocols (Salari et al., 2016). It was observed that for *C. tropicalis* the MIC range had a variation of 100–6.26 μ g/ml. Similarly, Siqueira et al. found biological activity using a red propolis alcoholic extract, with an MIC range of 64–32 μ g/ml (Siqueira et al., 2015) for this species.

In relation to plants found mainly in Brazilian territory, Correia et al. (2016) carried out an important study with different plants found in the Brazilian Cerrado, a region with an important number of species used in popular medicine, mainly in studies of essential oils with anti-*Candida* activity (Nordin et al., 2013; Correia et al., 2016).

In a study conducted by Morais-Braga et al. (2016) the interaction of aqueous and hydroethanolic extracts of *Psidium brownianum* was observed in association with fluconazole. The IC 50 values for fluconazole were 68.10 μ g/ml for *C. tropicalis* CTINCQS 40042 and 41.11 μ g/ml for *C. tropicalis* CTLM 23, obtained by broth microdilution. When in combination with fluconazole, the aqueous and hydroethanolic extracts of *P. brownianum* showed a significant reduction in IC 50 values, ranging from 37.2–3.10 μ g/ml for CTINCM 40042 and 13.66–6.94 μ g/ml for CTLM 23.

All these studies involving the evaluation of vegetal products with biological activity, especially against *C. tropicalis*, has reinforced the great importance and necessity of the emergence of alternative and less toxic sources of treatment, alone or in combinations with different antifungal drugs in commercially available.

C. TROPICALIS OSMOTIC STRESS RESPONSE AND BIOTECHNOLOGICAL APPLICATIONS

Several virulence attributes are expressed by fungi in response to stress conditions induced by the environment (Brown et al., 2014), and some yeasts can tolerate high salt concentrations, developing physical and genetic mechanisms to neutralize the two main deleterious effects of osmotic stress, which are toxicity and loss of water and cellular turgidity (Garcia et al., 1997; Beales, 2004).

A study conducted by Rodriguez et al. (1996) reported the gene isolation involved with osmotic adaptation in *C. tropicalis*, a true homolog of *HAL3*, called Ct*HAL3*. In fact, *C. tropicalis* is able to grow in culture medium with more than 10–15%

sodium chloride and has been isolated from the hypersaline environment for the first time from Dead Sea samples (Butinar et al., 2005). Bastos et al. (2000) reported the isolation of this yeast from a sample of Amazonian forest enriched with high salt concentration.

Garcia et al. (1997) carried out one of the few studies investigating the mechanisms of osmotic adaptation of *C. tropicalis*, analyzing ion extrusion. The results showed that Na⁺/K⁺-ATPase transporters are activated immediately after exposure to hypersaline environment, promoting rapid efflux of ions and restoring intracellular osmotic equilibrium.

Exposure to sodium chloride (NaCl) leads to high osmotic stress in fungal cells, promoting rapid loss of water that leads to reduced size and loss of cellular turgidity (Kühn and Klipp, 2012).

With regard to *C. tropicalis*, Garcia et al. (1997) reported that the accumulation of glycerol necessary for the restoration of a normal cellular physiology occurred only after the stationary phase. In addition, they found that there is a preponderant role of efflux pumps in the osmotic adaptation of *C. tropicalis* to the detriment of the nonionic compensatory mechanisms of water loss and turgor. Besides, the accumulation of intracellular glycerol seems to be less efficient than the activation of the Ion efflux pumps (Garcia et al., 1997).

Therefore, *C. tropicalis* is considered an osmotolerant yeast, since it can grow well in environments with high osmotic pressure, but this condition is not essential to its survival (Tokuoka, 1993). Such property is often associated with its use in industrial and biotechnological practices.

In the food industry, osmolytic strains of *C. tropicalis* improve xylitol production (Kwon et al., 2006; Misra et al., 2012). Rao et al. (2006) used *C. tropicalis* strains in hypersaline solution to produce xylitol from corn fiber and sugarcane bagasse. Another example of industrial application of this species is the production of ethanol from algae (Ra et al., 2015).

C. tropicalis is still widely used in bioremediation processes. Al-Araji et al. (2007) reported the use of this yeast in the commercial recovery of petroleum spillage. In 2011, Farag and Soliman reported the high degradability of crude oil and hydrocarbons by *C. tropicalis* (Farag and Soliman, 2011). Benard and Tuah (2016) also evaluated this property under conditions simulating sea water. In addition, Yan et al. (2005) demonstrated the high potential for degradation of phenol by *C. tropicalis* in saline medium. Microorganisms with this capacity are called biosorbents, found to correct pollution processes without causing damage to ecosystems (Leitão et al., 2007).

Halotolerance also provides a longer permanence of *C. tropicalis* in the coastal environment, allowing greater opportunity for contamination of bathers. Prolonged persistence in the marine environment may also lead to adaptation to high concentrations of other ions and UV light. This whole process

can be reflected in genetic alterations that results in selection pressure (Krauke and Sychrova, 2008).

Recently, our group was involved in the investigation of osmotolerance and its relation to virulence expression *in vitro* with *C. tropicalis* isolated from the coastal environment. We found that these strains can fully express virulence attributes and may show a high persistence capacity on the coastal environment, because they all tolerated high salt concentration. In addition, they showed high MICs to several antifungal drugs used in current clinical practice, demonstrating that environmental isolates may have pathogenic potential and suggesting that the persistence of yeasts in the sand environment may have led to the overexpression of efflux pumps, that may partially explain the reason why *C. tropicalis* isolates not previously exposed to antifungal drugs had high levels of resistance to azoles and amphotericin B (Zuza-Alves et al., 2016).

CONCLUDING REMARKS

In conclusion, this review highlights important aspects of *C. tropicalis* biology and clinical relevance. This species may be easily identified by classical taxonomy, commercial, proteomics, and molecular methods and no cryptic sibling species has been discovered. This asexual yeast closely related to *C. albicans* may be considered of high virulence, which can be verified in animal models of superficial and systemics infections, plus its ability to form true hyphae and complex biofilm *in vitro*, besides the ability to secrete proteinases, phospholipases and hemolysins. *C. tropicalis* is classified as the third or fourth NCAC species more commonly isolated in the clinical practice, while may be the second more frequently isolated *Candida* species in Latin America and Asia. Several mechanisms of antifungal resistance have been elucidated, including *ERG* and *FKS* gene families' mutations and efflux pumps. Some natural products have also been investigated as new potential use for future development of antifungal compounds active against *C. tropicalis*. This species is considered osmotolerant and this characteristic has been recently demonstrated to influence the expression of virulence factors and primary antifungal resistance. This ability to survive to high salt concentrations is a property that explains *C. tropicalis* potential use for biotechnological processes, including ethanol production through the fermentation of sea algae. Therefore, for all the factors previously described, *C. tropicalis* may be indubitably considered one of the most important *Candida* species.

AUTHOR CONTRIBUTIONS

DZ and WS prepared the manuscript. GC designed all topics and revised the manuscript. All authors approved the final manuscript.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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