

# The Emerging Pathogen *Candida metapsilosis*: Biological Aspects, Virulence Factors, Diagnosis, and Treatment

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**Abstract:** Fungal infections represent a constant and growing menace to public health. This concern is due to the emergence of new fungal species and the increase in antifungal drug resistance. Mycoses caused by *Candida* species are among the most common nosocomial infections and are associated with high mortality rates when the infection affects deep-seated organs. *Candida metapsilosis* is part of the *Candida parapsilosis* complex and has been described as part of the oral microbiota of healthy individuals. Within the complex, this species is considered the least virulent; however, the prevalence has been increasing in recent years, as well as an increment in the resistance to some antifungal drugs. One of the main concerns of candidiasis caused by this species is the wide range of clinical manifestations, ranging from tissue colonization to superficial infections, and in more severe cases it can spread, which makes diagnosis and treatment difficult. The study of virulence factors of this species is limited, however, proteomic comparisons between species indicate that virulence factors in this species could be similar to those already described for *C. albicans*. However, differences may exist, taking into account changes in the lifestyle of the species. Here, we provide a detailed review of the current literature about this organism, the caused disease, and some sharing aspects with other members of the complex, focusing on its biology, virulence factors, the host-fungus interaction, the identification, diagnosis, and treatment of infection.

**Keywords:** antifungal resistance, candidiasis, *Candida parapsilosis* complex, emerging pathogens, virulence factors

## Introduction

The *Candida* genus groups fungal species of yeast-like organisms, and from the approximately 200 species belonging to it, only 17 species are the most frequently isolated from clinical specimens.<sup>1</sup> Collectively, these species are the etiological agents of candidiasis, a medically relevant fungal infection that can affect superficial or deep-seated organs. *Candida albicans* is the most common organism that causes candidiasis in humans and thus is the species whose pathogenesis, virulence, epidemiology, and susceptibility to antifungal drugs have been thoroughly studied. However, nowadays the emergence of new species in the clinical setting is widely recognized and other *Candida* species, such as *Candida parapsilosis*, *Candida orthopsilosis*, *Candida metapsilosis*, *Candida krusei*, *Candida lusitanae*, *Candida glabrata*, and *Candida tropicalis*, have recently attracted attention.<sup>1–6</sup>

Candidiasis, colloquially known as thrush, shows a wide range of infections throughout the body and is responsible for about 96% of all mycoses in immunocompromised patients.<sup>7,8</sup> With no doubt, the highest mortality toll is associated with candidemia,<sup>1,4,6</sup> which implies the fungal dispersion to different organs by the bloodstream. On the contrary, superficial candidiasis is often an infection of mucosal (buccal, vulvovaginal, esophageal) or cutaneous (skin and nails) tissues, with benign or mild symptoms that can affect both immunocompetent and immunocompromised patients.<sup>9</sup> It is estimated that up to 75% of women suffer from vulvovaginal candidiasis caused by *Candida* spp. at least once in their life.<sup>10</sup>

*C. parapsilosis sensu lato* is the second most found *Candida* isolate in bloodstream samples from Latin America and Asia, and the third overall *Candida* species isolated worldwide.<sup>11</sup> From those isolates, it has been reported that around 90% were identified as *Candida parapsilosis sensu stricto*; while the rest as *C. orthopsilosis* and *C. metapsilosis*.<sup>12</sup>

*C. metapsilosis* is ubiquitous and has been reported as part of the oral mycobiome of healthy individuals.<sup>13,14</sup> This species is reported as the least common and virulent species of the complex, given that just around 1.1 to 8.4% of the infections caused by *C. parapsilosis sensu lato* are attributed to *C. metapsilosis*.<sup>15,16</sup> Even though it is considered the least important species of the complex, its prevalence has increased over the years and has also been linked to challenging clinical failures.<sup>17,18</sup> Although many aspects of this organism, are still unclear, here we offer a literature revision of the known information, which we consider will be helpful for the understanding of this emerging pathogen.

## Biological Aspects

### Morphology

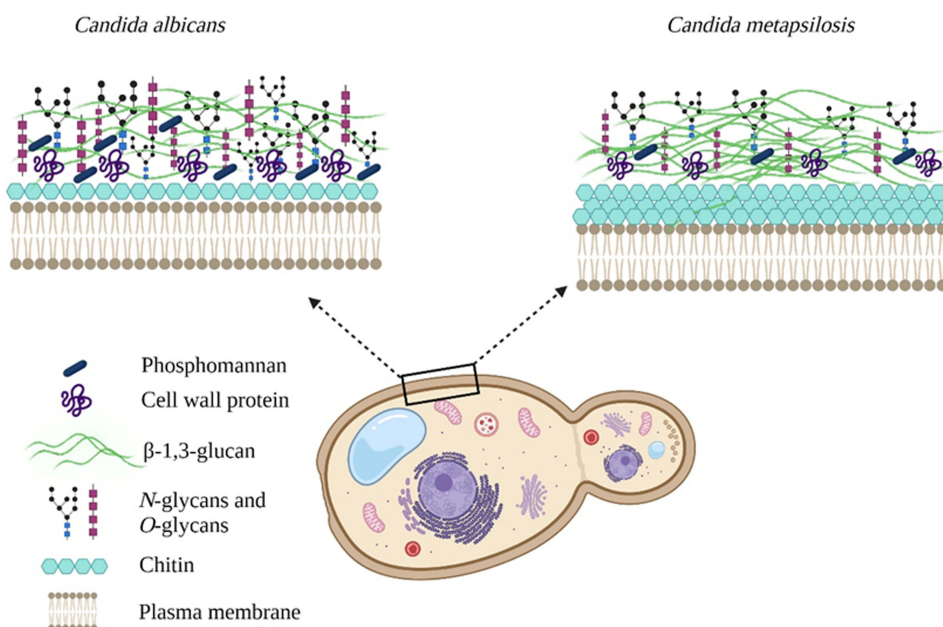
The members of the *C. parapsilosis* complex are phenotypically indistinguishable from each other but genetically different, with differences in epidemiology, virulence, and antifungal susceptibility.<sup>19</sup> The *C. parapsilosis* species have the selective advantage of growing in high glucose concentration media, and in these rich growing conditions, the fungus grows like white creamy colonies of subglobose cells, with a size of 4×3-6 µm.<sup>20</sup> It was previously thought that, like *C. parapsilosis*, *C. metapsilosis* was capable of forming pseudohyphae,<sup>21</sup> but a study of 93 different *C. parapsilosis sensu lato* isolates demonstrated that *C. metapsilosis* does not produce this morphology, being killed more efficiently by the host macrophages.<sup>16</sup> This aspect remains contradictory though, since several studies report the formation of pseudohyphae in *C. metapsilosis*.<sup>22</sup> The discrepancies shown in the literature could be related to the fungal strain analyzed, since genetic variations between strains may affect phenotypic traits, including morphological switch. Another explanation could be related to the culture conditions, factors such as temperature, pH and the composition of the culture medium could also affect dimorphism. However, most of the studies indicate that *C. metapsilosis* can form pseudohyphae.

### Cell Wall

Regarding cellular structures, thus far the cell wall is the most studied organelle and currently, there are well-described models of the *C. albicans* wall,<sup>23-25</sup> but limited information about this structure in other *Candida* species. A comparative analysis of the cell wall composition of *C. albicans*, *C. parapsilosis*, *C. orthopsilosis*, and *C. metapsilosis* revealed that the arrangement of this component is different among the species, although with a similar wall composition.<sup>26</sup> When compared to *C. albicans*, the species from the *C. parapsilosis* complex showed lower mannan content, similar protein levels, and an increase in chitin and β-1,3-glucan exposure on the cell wall (Figure 1).<sup>26</sup> Indeed, it has been showed that *C. parapsilosis* has shorter *N*-linked mannans than *C. albicans*,<sup>27</sup> an observation likely extrapolated to *C. metapsilosis*, and that could explain the overexposure of chitin and β-1,3-glucans in *C. parapsilosis sensu lato* cell surface, as a compensatory mechanism. In addition, the *C. metapsilosis* cell wall has a higher porosity and reduced phosphomannan content, which can also be explained by shorter mannan chains (Figure 1).<sup>26</sup> It was also found that there are subtle differences in the distribution of structural polysaccharides among the *C. parapsilosis* complex species, being the most remarkable that *C. orthopsilosis* exposes all of its β-1,3-glucan and chitin at the cell wall surface, which might suggest that cell wall proteins have even shorter mannans than *C. parapsilosis* and *C. metapsilosis*, or that these proteins contain fewer glycosylation sites, making the inner wall polysaccharides more accessible.<sup>26</sup>

### Genome

To determine the *C. metapsilosis* evolution and the genetic and molecular basis of its pathogenesis, the genome of 11 clinical isolates from different locations was sequenced and compared to each other, and against *C. parapsilosis* and *C. orthopsilosis*. The *C. metapsilosis* genome assembly revealed a total size of 13.3 Mbp distributed in 7 putative chromosomes, with 5973 protein-coding genes, different from what has been reported for *C. parapsilosis* (5752 protein-coding genes) and *C. orthopsilosis* (5784 protein-coding genes), a GC percentage of 38.1, and 17 pseudogenes.<sup>16</sup> These predicted chromosomes presented ends enriched in telomere repeats (GGTTAGGATGTCCAAAGTATTGA) that



**Figure 1** Comparison between the *Candida albicans* and *Candida metapsilosis* cell walls. *C. metapsilosis* cell wall contains a higher percentage of chitin and  $\beta$ -1,3-glucan, but less amount of mannans, phosphomannans, and proteins than *C. albicans*. Due to these differences, the *C. metapsilosis* cell wall porosity is significantly higher than in *C. albicans*. Created with BioRender.com.

correspond to the telomerase template domain.<sup>16</sup> The analysis of the *C. metapsilosis* genome revealed that this organism is highly heterozygous, with a divergence between alleles in heterozygous regions of 4.5%, which facilitated the delimitation of heterozygous and homozygous regions. It was found that around 50 and 60% of the genome from the different strains are heterozygous regions, much higher than that reported for *C. orthopsilosis* (17%).<sup>16</sup> This finding could mean two things: (i) the *C. metapsilosis* hybridization is a more recent event, or (ii) *C. orthopsilosis* lost its heterozygosity faster.<sup>16</sup> The heterozygous regions' sizes had identical boundaries, which might suggest that all 11 *C. metapsilosis* strains derived from the same primary hybridization event.<sup>16</sup> Due to the lack of a homozygous *C. metapsilosis* strain and a clear parental mapping, an indirect approach was used to obtain an idea of this organism's parental lineage, based on the identification of two haplotypes in a determined homozygous region. The first haplotype represented the reference assembly, while the second the remaining strains. This procedure provided an estimate of 50% for the first haplotype and 40% for the second haplotype in homozygous regions, which cannot be assigned to a specific parental genome but that might suggest a balanced presence of both parents in these regions from the *C. parapsilosis* genome and no preference for either parental strain.<sup>16</sup>

It was also shown that the 11 *C. metapsilosis* strains showed diploidy, which suggests that mating between two haploid cells not very divergent is the possible hybridization mechanism for this organism. In addition, most of the *C. metapsilosis* strains contain the *MTLa* and *MTLa* idiophorms, which supports the mating event in *C. metapsilosis*, species that was thought to be asexual.<sup>16</sup> Also, it was found that the 11 strains, isolated from different locations, had quite different evolutionary relationships, which suggests a lineage relatively ancient that spread worldwide a long time ago, unlike what has been seen with *C. orthopsilosis*, which shows stable similarities among isolates from different locations.<sup>16</sup>

Finally, the sequencing of the *C. metapsilosis* genome allowed a comparison among the *C. parapsilosis sensu lato*, which showed a conserved synteny between the three species, being *C. parapsilosis* and *C. orthopsilosis* closer to each other, with 98% of conserved synteny. In addition, phylogeny analysis revealed that *C. metapsilosis* has a basal position in this complex.<sup>16</sup>

The *C. parapsilosis* mitochondrial genome has also been assembled, resulting in a single 21 kb contig,<sup>28</sup> and although the species from the *C. parapsilosis* complex usually have this genome as linear, some *C. metapsilosis* and

*C. orthopsilosis* isolates have circular mitochondrial DNA (mtDNA).<sup>29–31</sup> In addition, the *C. metapsilosis* mtDNA is compact and encodes the same number of genes, arranged in the same order as *C. parapsilosis* and *C. orthopsilosis*, with the only difference that *C. metapsilosis* mtDNA has one intronic sequence, while the other two species have seven and two introns, respectively.<sup>32</sup> Although the mtDNA architecture is not considered a taxonomic criterion,<sup>29</sup> these differences might suggest biodiversity.<sup>20,30,31</sup>

The *C. parapsilosis* complex is a member of the CTG clade, which means that the CTG codon codes for serine instead of leucine,<sup>33</sup> and includes other clinically relevant species, like *C. albicans* and *C. tropicalis*. However, unlike these two species, *C. parapsilosis sensu lato* independently evolved their virulence.<sup>16</sup>

## General Aspects

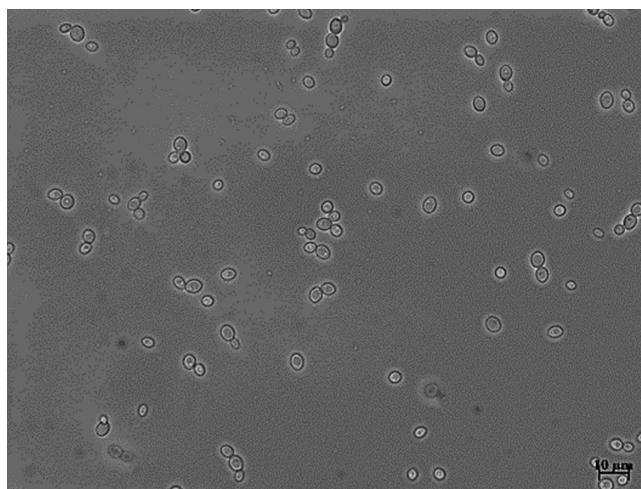
As mentioned, the *C. parapsilosis* complex species cannot be differentiated by phenotypical or biochemical analyses, since they all share similar characteristics, such as (i) macro and microscopic cellular morphology, presenting colonies of different colorations that vary from white to pink, and oval, round or cylindrical yeast cells (Figure 2),<sup>30</sup> (ii) biochemical markers, like their ability to grow on high glucose concentrations,<sup>34</sup> and a strong activity for proteinase,<sup>30</sup> and (iii) fermentation profiles, like their ability to ferment maltose, D-ribose, and D-gluconate.<sup>20,34</sup> However, a recent study found subtle differences in the growth kinetics among the three species when grown under different salinity and pH levels, being *C. orthopsilosis* the species with a lower number of viable cells when grown at 10 and 15% of NaCl and at pH 7.<sup>35</sup>

Some studies have shown that *C. metapsilosis* can ferment some sugars such as glucose, maltose, D-ribose, D-gluconate, galactose, sucrose, trehalose, N-acetyl-glucosamine, 2-K-D-gluconate, and glycerol.<sup>36</sup> In addition, this organism can assimilate melezitose, ribitol, glucose, galactose, D-xylose, and D-mannitol.<sup>29,36</sup> Recent work found that *C. metapsilosis* can significantly reduce the content of alkaloids and increase the content of aromatic components in tobacco leaves.<sup>37</sup>

## Putative Virulence Factors in *Candida metapsilosis*

Although *C. albicans* and *C. metapsilosis* are two organisms found within the same genus, both species show notable differences concerning biological aspects and virulence. The study of *C. metapsilosis* virulence is limited if compared with the large amount of information available for *C. albicans* and its closest species *C. parapsilosis sensu stricto*.<sup>38</sup> This section will provide information about *C. metapsilosis* virulence factors and a proteomic comparison to predict possible orthologs already described in *C. albicans*.

Cell adhesion plays an important role in numerous biological processes. Many fungi, such as those of the *Candida* genus, have cell wall glycoproteins known as adhesins, which provide distinctive adhesion properties.<sup>39</sup> Adhesion is the



**Figure 2** Microscopic morphology of *Candida metapsilosis*. Yeast cells growing at 28 °C in Sabouraud medium, with the typical round or cylindrical cells.

first step during *Candida* infection. The ability of *Candida* spp. to bind to host tissues or abiotic surfaces, such as catheters or medical devices is achieved through the biological products of genes that encode several adhesin families that are found in the cell wall.<sup>38</sup> One of the groups involved in this process is the Als family (agglutinin-like sequence). Although its importance as a virulence factor in *C. albicans* is known, little information is available on *C. metapsilosis*.<sup>38,40</sup> A comparative genomics analysis identified the presence of several *ALS* genes in *C. orthopsilosis* (3 genes), *C. parapsilosis* (5 genes), and *C. metapsilosis* (4 genes).<sup>41–43</sup> The proteins encoded by these genes are similar to the *C. albicans* Als proteins, and have a signal peptide, an N-terminal domain with a peptide-binding cavity, an amyloid-forming region, repeat sequences, and a C-terminal site for the addition of the glycosylphosphatidylinositol anchor.<sup>42</sup> Molecular modeling revealed the presence of unique features, including differences in shape and charge in different peptide-binding cavities.<sup>43</sup> Previous reports have indicated that *C. metapsilosis* invades reconstituted human oral and epidermal epithelial tissues; however, unlike *C. parapsilosis* and *C. orthopsilosis*, this species does not cause significant cell damage.<sup>38</sup> Furthermore, tests carried out with human bronchial epithelial cells showed that *C. metapsilosis* has a reduced adherence capacity.<sup>41,44</sup> This low adhesion phenotype could be correlated with the low virulence of this species, as has been reported in different studies.<sup>17,26</sup> Members of the *ALS* gene family, *EAP1*, *ECM33*, *IFF4*, *INT1*, *MP65*, and *PHR1*, encode the main *C. albicans* adhesins,<sup>45</sup> and although little is known about these in *C. metapsilosis*, putative orthologs could be identified within its genome, indicating that adhesion may occur through these adhesins (Table 1).

Biofilm development has also been described as an important virulence factor in *Candida* species.<sup>46</sup> It is known that biofilm formation can contribute to resistance to antifungal drugs and immune evasion.<sup>47</sup> Biofilms formed by the *C. parapsilosis* complex members have gained important attention because isolates have been obtained from a wide variety of both biotic and abiotic surfaces.<sup>46,48</sup> To date, there have been few investigations of these properties in clinical isolates of the *C. parapsilosis* complex, especially in *C. metapsilosis*. Some studies have shown that clinical isolates of

**Table 1** Prediction of Some Important Virulence Factors in *Candida metapsilosis*

Virulence factor	<i>Candida albicans</i> protein	<i>Candida metapsilosis</i> protein*	<i>Candida orthopsilosis</i> protein*	<i>Candida parapsilosis</i> protein*	E-value **	Similarity (%) **
<b>Adhesins</b>	AlsI-Als6	I9VW82_003103	CORT_0C04220	CPAR2_404790	0	68
	EapI/HwpI	I9VW82_002101	CORT_0E03560	CPAR2_502140	1e-18	61
	Ecm33	I9VW82_001077	CORT_0B09650	CPAR2_108560	0	82
	Iff4	I9VW82_001243	CORT_0C02740	CPAR2_806410	3e-138	74
	IntI	I9VW82_005436	CORT_0F00820	CPAR2_100700	0	61
	Mp65	I9VW82_002854	CORT_0C06750	CPAR2_407410	4e-150	79
	PhrI	I9VW82_003994	CORT_0E04260	CPAR2_302140	0	76
<b>Biofilms</b>	BcrI	I9VW82_003611	CORT_0D06130	CPAR2_205990	4e-13	61
	BrgI	I9VW82_001022	CORT_0B09130	CPAR2_107940	2e-43	83
	EfgI	I9VW82_003895	CORT_0G01760	CPAR2_701620	7e-99	67
	Hsp90	I9VW82_003264	CORT_0G03310	CPAR2_703150	0	96
	Ndt80	I9VW82_005636	CORT_0A00190	CPAR2_800080	4e-146	56
	RobI	I9VW82_003013	CORT_0C05180	CPAR2_405770	3e-14	45
	CsrI	I9VW82_003436	CORT_0H02190	CPAR2_600610	6e-177	60

(Continued)

Table 1 (Continued).

Virulence factor	<i>Candida albicans</i> protein	<i>Candida metapsilosis</i> protein*	<i>Candida orthopsilosis</i> protein*	<i>Candida parapsilosis</i> protein*	E-value **	Similarity (%) **
Dimorphism	Cph1	I9VW82_005089	CORT_0A07880	CPAR2_208600	2e-166	61
	Hgc1	I9VW82_005679	CORT_0B11290	CPAR2_110180	1e-144	70
	Nrg1	I9VW82_004271	CORT_0F01430	CPAR2_300790	5e-42	93
	Tup1	I9VW82_001169	CORT_0B10590	CPAR2_109520	0	79
Immune evasion	Hgt1	I9VW82_001057	CORT_0B09440	CPAR2_108370	0	90
	Msb2	I9VW82_004931	CORT_0A09600	CPAR2_210290	5e-92	70
	Pra1	No found	No found	No found	-	-
Phospholipase and proteinase	Lip5-8	I9VW82_003269 I9VW82_002019	CORT_0G03370 CORT_0B01380	CPAR2_703200 CPAR2_501220	0	74
	Sap1-3	I9VW82_000494	CORT_0B03690	CPAR2_102410	5e-116	64
	Sap5	I9VW82_000510	CORT_0B03820	CPAR2_102410	1e-97	57
	Plb1	I9VW82_001712	CORT_0F02750	CPAR2_601760	6e-72	73
	Plb2	I9VW82_001713	CORT_0F02740	CPAR2_601730	0	76
	Plb3-4	I9VW82_003857	CORT_0G01310	CPAR2_701140	0	78
	Plb5	I9VW82_000341	CORT_0A04690	CPAR2_804680	0	74
Thermotolerance	Hsp60	I9VW82_002499	CORT_0D03130	CPAR2_203040	0	89
	Hsp104	I9VW82_002374	CORT_0D04420	CPAR2_204330	0	91
	Ssa1	I9VW82_000927	CORT_0A13190	CPAR2_106940	0	93

**Notes:** \*Protein nomenclature corresponds to accession codes of the National Center for Biotechnology Information database (<https://www.ncbi.nlm.nih.gov/>). The putative protein sequence encoded by the *C. albicans* gene was subjected to a standard protein BLAST analysis at <https://www.ncbi.nlm.nih.gov/>. \*\*When comparing the encoded protein of *C. metapsilosis* with the putative ortholog in *C. albicans*. The best hit is reported in the *C. metapsilosis* protein column, and this was scored with the lowest E value. The similarity column refers to the comparison of amino acid sequences from the *C. albicans* encoded protein and the best hit.

this species are classified as weak and moderate biofilm producers.<sup>48</sup> The isolates that were considered strong biofilm producers were *C. parapsilosis sensu stricto*.<sup>48</sup> Analysis of biofilm production and scanning electron microscopy revealed that 65 clinical isolates obtained from complex species were able to form biofilms. However, *C. parapsilosis* and *C. orthopsilosis* exhibited greater metabolic activity at 24 hours, compared to *C. metapsilosis*.<sup>49</sup> Taking into account the microscopic observations, *C. metapsilosis* biofilms showed different surface topography when compared to other species of the complex.<sup>49-51</sup> Other essays have shown that *C. metapsilosis* is the lowest biofilm producer when compared with other *Candida* species.<sup>22,52,53</sup> Although *C. metapsilosis* biofilms exhibit different characteristics than *C. parapsilosis*, it is still concerning this virulence trait, in particular in the clinical setting. *C. metapsilosis* can be isolated from hospital environments, representing a potential risk of infection/reinfection for patients.<sup>46</sup> Taking into account the bioinformatic analysis, it is possible to predict that *C. metapsilosis* has seven genes involved in biofilm formation (Table 1). However, more research is required to assess the function of these genes in this organism.

Biofilms are associated with the dimorphism. As reported, *C. metapsilosis* cannot form true hyphae, only pseudohyphae, which implies changes in the expression of some virulence factors that are morphology-specific.<sup>54,55</sup> The yeast-to-hypha transition in *Candida* is a key virulence determinant that allows the organism to invade host tissues and evade the host immune response.<sup>56</sup> Dimorphism in *C. metapsilosis* is poorly studied; however, work carried out with the *Galleria*

*mellonella* model indicates that *C. metapsilosis* has a reduced ability to produce pseudohyphae. After 48 hours of incubation, none of the *C. metapsilosis* isolates managed to produce hyphae or pseudohyphae, which could be correlated with the reduced virulence of this species.<sup>17</sup> In the case of infection in the invertebrate *Caenorhabditis elegans*, the appearance of *C. metapsilosis* pseudohyphae was demonstrated after 24 and 48 hours, and distention of the nematode intestine was observed after ingesting fungal cells.<sup>57</sup> Although the death of some individuals was evident, this was lower than that observed in animals infected with *C. parapsilosis*.<sup>57</sup> Taking into account the bioinformatic analysis (Table 1), *C. metapsilosis* has putative functional orthologs of these genes coding for dimorphism regulators; however, the process that controls the dimorphism could be regulated differently in *C. metapsilosis* and *C. albicans*.

The secretion of hydrolytic enzymes is considered an important virulence factor in *Candida* spp. These enzymes fulfill important functions such as facilitating adhesion, internalization into tissues, and, therefore, invasion into host cells.<sup>58</sup> Among these enzymes, the most studied and best characterized are phospholipases and proteinases; however, esterase activity has also been described in *Candida* species.<sup>20,59</sup> In non-*albicans* species, such as *C. metapsilosis* these virulence factors have not been widely described. Enzyme activity assays of 12 *C. metapsilosis* clinical isolates obtained from cutaneous and mucocutaneous samples demonstrated the presence of phospholipase activity.<sup>60</sup> Furthermore, 10 of these isolates were positive for proteinase, and 9 out of the 10 showed to be strong producers of this enzyme.<sup>60</sup> When the esterase activity was evaluated in *C. metapsilosis*, it was found that 2 of the clinical isolates were producers of this enzyme, after an incubation period of 10 days.<sup>60</sup> These findings correlate with other reports working with isolates from different geographical origins.<sup>61</sup> Recent work has shown that *C. metapsilosis* is a proteinase producer and only some isolates synthesize phospholipases, which is of great interest since the production of these enzymes is not common in this species.<sup>48,62</sup> In vivo tests with *G. mellonella* demonstrated that *C. metapsilosis* has a reduced capacity to produce hydrolytic enzymes, however, strains of this species showed esterase activity.<sup>17</sup>

These results indicate that both phospholipases and proteinases prevail in *C. metapsilosis*, despite being the least virulent species of the complex. Taking into account that it is a species that is frequently isolated from abiotic surfaces, these enzymes can facilitate the survival and adaptation of the fungus to different niches. Furthermore, although it is considered a non-invasive species, it can also be lethal, since it has also been isolated from patient blood cultures.<sup>12,60,63,64</sup> Table 1 shows the putative orthologs of *LIP*, *SAP*, and *PLB* found in *C. metapsilosis*, genes encoding for esterase, proteinase, and phospholipase activities, respectively, although their function remains poorly understood.

In *Candida* spp., other virulence factors are known to play important functions, such as immune evasion and thermotolerance. Thermotolerance contributes to growth, dimorphism, and tissue colonization. This virulence factor plays an important role in fungal adaptation processes to stressful environments.<sup>56,65</sup> Bioinformatic analysis suggests that the *C. metapsilosis* genome contains putative orthologs of the thermotolerance genes *HSP60*, *HSP104*, and *SSA1* (Table 1). Immune evasion is considered a mechanism that involves other cellular processes, such as the formation of biofilms, morphological changes, secretion of proteases, and synthesis of proteins that contribute to evading oxidative stress.<sup>65</sup> Some of these processes have already been mentioned earlier in this review. The *C. albicans* genes involved in immune evasion, *HGT1*, and *MSB2*, were found in *C. metapsilosis* (Table 1). However, no ortholog of the *PRA1* gene was found. This finding is consistent with other non-*albicans* species, such as *Candida lusitanae*.<sup>65</sup>

Based on the bioinformatic analysis carried out, it is possible to hypothesize that, despite the genomic similarities between *C. metapsilosis* and the virulence factors previously described in *C. albicans*, there could be a differential expression of these factors in *C. metapsilosis*. This could be related to the lifestyle of this species since several authors suggest that it can also behave as an environmental pathogen.<sup>20</sup>

## *Candida metapsilosis*-Host Interaction

The *C. parapsilosis* complex members have become as important as *C. albicans* in the clinical setting.<sup>26</sup> To better understand the clinical relevance of these species, it is necessary to elucidate how they interact with host immune cells. Currently, the best described and studied interaction with the host immunity is that of *C. albicans*; however, although there are specie-specific variations, the elemental recognition process of *Candida* cells is mediated by pattern recognition receptors (PRRs) and pathogen-associated molecular patterns (PAMPs).<sup>66</sup> Furthermore, both innate and adaptive immune responses play an important role in the control of these pathogens.<sup>56,66–68</sup>

The fungal cell wall is a relevant structure during immune sensing. This has very specific functions, among which are structural support, signal transduction, and molecular scaffold to display virulence factors. It is also involved in the first events of interaction with the host cells, containing most of the PAMPs recognized by the PRRs in innate immune cells.<sup>23,24,69</sup>

A comparative study of *C. parapsilosis* complex species with *C. albicans* revealed that the cell wall composition of *C. parapsilosis*, *C. metapsilosis*, and *C. orthopsilosis* is similar, however, the organization of wall components may have some differences, which impacts on the ability to activate human peripheral blood mononuclear cells (PBMCs).<sup>26</sup> In contrast to *C. albicans*, *C. metapsilosis* stimulated higher and similar levels of the cytokines TNF $\alpha$ , IL-1 $\beta$ , IL-6, and IL-10. When yeast cells were subjected to different cell treatments, such as heat inactivation and  $\beta$ -elimination, *C. metapsilosis* was found to stimulate higher levels of TNF $\alpha$ , IL-6, and IL-10 in both types of treatments, contrasting with *C. albicans* that only stimulated higher cytokine levels upon heat inactivation.<sup>26</sup>

The cytokine stimulation by *C. metapsilosis* has a strong dependence on the dectin-1, at the difference of *C. albicans*. This observation is in line with the higher  $\beta$ -1,3-glucan content at the *C. metapsilosis* cells surface.<sup>26</sup> In the case of the TLR4 receptor, it is known to be the only receptor that recognizes O-linked mannan in *C. albicans*,<sup>70</sup> and probably also plays an important role in the recognition of *C. metapsilosis* wall components. Blockade of the TLR4 receptor resulted in reduced TNF $\alpha$  stimulation by *C. metapsilosis*, but IL-1 $\beta$ , IL-6, and IL-10 production was not affected, suggesting a modest contribution of this receptor for cytokine stimulation.<sup>26</sup> Contrary to these observations, the mannose receptor, the main PRR for N-linked mannans,<sup>70</sup> plays an important role during the stimulation of proinflammatory cytokines by *C. metapsilosis*.<sup>26</sup> These observations are relevant, as they allow proposing the costimulation hypothesis, which has already been observed in other fungal species.<sup>67,71,72</sup>

An in vitro infection model using microglial cells was employed to investigate the pathogenic potential of clinical isolates of the *C. parapsilosis* complex species.<sup>73</sup> *C. metapsilosis* isolates were found to be more susceptible to microglia-mediated antifungal activity compared to the other two species. Isolates of this species are less phagocytosed, however, phagosomes internalizing *C. metapsilosis* yeasts showed a higher degree of acidification compared to phagosomes containing other species of the *C. parapsilosis* complex.<sup>73</sup> This may indicate that this species is less susceptible to phagocytosis, but when phagocytosed, it is less effective in counteracting the host's intracellular antimicrobial defenses.<sup>73</sup> When the secretory response of microglia to infection was assessed, comparable levels of macrophage inflammatory protein-1 (MIP-1 $\alpha$ ) were observed in all species of the complex. On the other hand, *C. metapsilosis* did not cause any apparent damage to microglial cells, unlike *C. parapsilosis* and *C. orthopsilosis*.<sup>73</sup> Cytotoxicity was measured using the enzyme lactate dehydrogenase, and it was found that microglial cells infected with *C. metapsilosis* released low levels of the enzyme, compared to the other species of the complex.<sup>73</sup> All these results suggested that *C. metapsilosis* is the least virulent species of the complex. Tests on female mice confirmed *C. metapsilosis* reduced virulence. When they were infected with cells of this species, the fungal load found during the first stages of infection was lower than that found with *C. parapsilosis* and *C. orthopsilosis*.<sup>44</sup> Tests with human buccal epithelial cells showed that *C. metapsilosis* strains have reduced adhesion capacity to these cells.<sup>44</sup> When the virulence, hemocyte density, and phagocytic activity were evaluated in *Galleria mellonella* larvae, it was found that the larvae managed to survive infection by this pathogen, compared to the other species of the complex, where greater mortality was documented.<sup>17</sup> During the hemocyte counting, two hours after the yeast infection, a significantly greater increase was observed in the larvae infected by *C. metapsilosis*.<sup>17</sup> Work carried out with the invertebrate model *C. elegans* demonstrated that unlike *G. mellonella*, all species of the complex can kill *C. elegans*. However, the time to death when infected with *C. metapsilosis* was longer (6 days), compared to *C. parapsilosis* (4 days).<sup>57</sup> To assess the defense mechanisms that are involved during *C. metapsilosis* infection, five antimicrobial peptides were evaluated, which are thought to have antifungal activity in vivo.<sup>57</sup> After infection, the expression of *cnc-4*, *cnc-7*, and *fipr22/23* was demonstrated, showing that *C. elegans* has a specific defense response against *C. metapsilosis*.<sup>57</sup>

## Candidiasis Associated with *Candida metapsilosis*

The *C. parapsilosis* complex members play an important role in human infections. These species can cause a wide range of clinical manifestations, ranging from superficial to disseminated infections.<sup>74,75</sup> These species are highly prevalent in neonates, especially *C. parapsilosis*.<sup>18</sup>

In Brazil, there are few reports on the prevalence of the three species of the *C. parapsilosis* complex. In 2014, a study was conducted to determine whether candidemia in neonates and children was related to these species.<sup>76</sup> A total of 3 cases of fungemia identified in the Brazilian Pediatric Hospital, in São Paulo, Brazil, were reported, the persistent symptomatology in these patients was high fever, chills, rapid breathing, and tachycardia.<sup>76</sup> The treatment was based on fluconazole and amphotericin B, and at the time of distinguishing between species, the isolates were sequenced, resulting in the species *C. metapsilosis* and *C. orthopsilosis*.<sup>76</sup> For the case of *C. metapsilosis*, the sample was isolated from a single child, and those of *C. orthopsilosis* were isolated from the blood of a newborn and a child.<sup>76</sup> Even though the patients were treated immediately, infection by both pathogens was lethal, as these three strains were resistant to fluconazole, which was used as a first-line treatment.<sup>76</sup>

The incidence of *C. metapsilosis* and *C. orthopsilosis* infections has increased since 2004, with prevalence rates ranging from 0.9 and 6.9% to 2.3 and 9%, respectively.<sup>12</sup> However, there are still poorly identified risk factors to learn more about these species, along with limited data about their prevalence in hospitals.

In Spain, during the years 2009 to 2010, 1356 cases of fungemia were analyzed, and *C. parapsilosis* was isolated in 400 of these cases, which corresponds to an incidence of 29%.<sup>15</sup> Although this species was found in a higher proportion of men, statistical analysis did not determine significant differences between genders. Of the 400 isolates, 330 corresponded to *C. parapsilosis sensu stricto*, 30 to *C. orthopsilosis*, and 4 to *C. metapsilosis*.<sup>15</sup> Infections caused by these fungi were mainly observed in patients older than 15 years; candidemia in newborns was not related to *C. orthopsilosis* and *C. metapsilosis*, as reported in Brazil.<sup>15</sup> It was also found that these species were associated with different hospital wards, with *C. parapsilosis* being found more frequently in the hematology, pediatrics, and neonatology departments, while *C. metapsilosis* was found in the adult and elderly hospitalization areas.<sup>15</sup>

In Asia, the frequency of *C. metapsilosis* is 2.6% of the total fungal isolates, but in China, this species represents 10.7% of candidiasis cases.<sup>60</sup> The reason for this is not clear yet; however, when reviewing the clinical records, it was hypothesized that a high incidence of this species could be related to a unique distribution pattern in China. However, this hypothesis has not yet been validated.<sup>60</sup>

## Identification and Diagnosis of *Candida metapsilosis*

Accurate identification of *Candida* species in the clinical setting has become essential, given the increasing frequency of candidiasis in recent years. Over time, various strategies have been developed for *Candida* species identification. However, these strategies may vary considering the similarity that exists among species.

For *C. metapsilosis*, it is not possible to make its specific identification with biochemical and morphological techniques, due to its similarity with the other members of the *C. parapsilosis* complex.<sup>77</sup> When clinical samples are inoculated on CHROMagar *Candida* medium for 24 and 48 hours, the colonies show a light brown color, which does not provide relevant information to distinguish them from the species of the complex.<sup>78</sup> When Sabouraud dextrose agar is used, all cultures are identified as *C. parapsilosis*, without discriminating between *C. metapsilosis* and *C. orthopsilosis*,<sup>78</sup> ie, this medium does not provide informative results for species identification.<sup>29,74,79</sup> API 20C AUX carbohydrate assimilation test has been used most frequently for *C. parapsilosis* identification, with 100% efficiency.<sup>80</sup>

Considering that biochemical and morphological approaches do not yield much information, molecular and biophysical methods have been proposed to significantly accelerate *C. metapsilosis* identification in laboratories.<sup>29,61,81,82</sup> Molecular strategies are based on the amplification of the *SADH* gene by PCR, with oligonucleotides that amplify a specific region of this locus.<sup>29</sup> The amplicons are digested with *BanI* and the digestion products are separated by electrophoresis.<sup>74</sup> For *C. metapsilosis*, there are three cleavage sites at positions 96, 469, and 529, while for *C. parapsilosis* and *C. orthopsilosis*, the cleavage is at position 196, demonstrating that by this analysis it is possible to differentiate between the complex species.<sup>29</sup>

To differentiate between species, 32 isolates were sequenced and divided into groups (I, II and III), using this technique and sequence polymorphism data for *ACPL*, *ACPR*, *COX3*, *GAL1*, *L1A1*, *LIP1*, *SADH*, *SAPP3*, *SYA1*, *TOP2* and *URA3* genes, it was found that *C. metapsilosis* had no amplification product for *ACPL*, *ACPR*, *L1A1*, *LIP2*, *TOP2*, and *URA3* genes, unlike *C. parapsilosis* and *C. orthopsilosis*. In addition, for *GAL1* and *SAPP3* genes, higher amplicons

were observed than those obtained in *C. parapsilosis*.<sup>29</sup> The analysis of the rRNA and ITS regions is another of the tools that have been improved for the *C. metapsilosis* identification. It has been found that the *ITS1* sequence similarity between *C. metapsilosis* and *C. parapsilosis* is 82.5% and with *C. orthopsilosis* 86.1%.<sup>29</sup> Thus, there is a significative dissimilarity between them.

Multiplex PCR assays have been performed using primers targeting genes such as *HWPI* and ITS regions (*ITS1*, *ITS2*) of different *Candida* species, including *C. metapsilosis*.<sup>82,83</sup> These assays, together with techniques such as matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) and DNA sequencing have been able to reproducibly differentiate between *Candida* species, including *C. metapsilosis*.<sup>83</sup> MALDI-TOF-MS, a technique that can be useful for the correct identification of *C. metapsilosis*, can be performed in a few minutes and is mainly based on the analysis of the proteins contained in a crude cell extract of the different isolates. The instrument's database provides the references to compare the spectra obtained and thus conclude the specific identity of the sample. This technique discriminates between the three species of the *C. parapsilosis* complex.<sup>81,84</sup> Among all the proposed techniques, this has proved to be the most effective for timely diagnosis.<sup>81</sup>

Real-time PCR techniques based on mtDNA, using as a target a polymorphic region of the *NADH5* gene, have contributed to a precise identification of each member of the *C. parapsilosis* complex, due to the species-specific variability of this gene.<sup>85</sup>

## Antifungal Resistance and Therapeutic Options

The treatment for different types of candidiasis is based on azoles, polyenes, echinocandins, and pyrimidine analogs, such as flucytosine.<sup>3</sup> These drugs are effective against a wide range of *Candida* spp; however, several studies have demonstrated that non-*albicans* *Candida* species may become resistant or less susceptible to different antifungal classes, such as echinocandins.<sup>29,82,86</sup>

*C. metapsilosis* is known to show higher minimal inhibitory concentrations (MICs) for echinocandins, and although no significant clinical failures have been reported, there is concern that strains of this species may be predisposed to higher levels of resistance to these drugs.<sup>87</sup> Echinocandins inhibit  $\beta$ -1,3-glucan synthases, which are important for cell wall glucan biosynthesis, by targeting the Fks1 catalytic subunit.<sup>88,89</sup> When the in vitro activity of different echinocandins, such as caspofungin, micafungin, and anidulafungin was evaluated on *C. parapsilosis*, *C. metapsilosis*, and *C. orthopsilosis*, it was found that the MIC of anidulafungin and micafungin was 20 to 50 times higher than the control strains. The antifungal caspofungin showed a minor 3.6-to-7.8-fold increase, however, this was still statistically significant.<sup>82</sup> Echinocandin resistance has been linked to amino acid substitutions in two conserved regions of Fks1 or Fks2.<sup>90</sup> In *C. metapsilosis*, and the other species of the complex, a variation in alanine at position 660 in Fks1 has been observed, replacing proline. This substitution has not been observed in other species; however, other types of substitutions have been identified in species such as *C. guilliermondii*, which are also related to reduced susceptibility to echinocandins.<sup>82,91</sup> These data further suggest that mutations within the Fks1 regions may alter the in vitro sensitivity of the glucan synthase enzyme and alter its catalytic capacity.

Previous reports have revealed that the complex species are susceptible to azoles and polyenes, and the MIC of caspofungin for *C. metapsilosis* is lower than that found for *C. parapsilosis*.<sup>92</sup> Caspofungin is the most active echinocandin against these species and is known to generate significant therapeutic effects for the treatment of systemic infections. On the other hand, micafungin is more efficient against *C. metapsilosis*.<sup>92</sup> A multicenter study in Spain showed that all *C. orthopsilosis* and *C. metapsilosis* isolates were susceptible to nine antifungal agents tested, including amphotericin B, flucytosine, fluconazole, itraconazole, voriconazole, posaconazole, anidulafungin, caspofungin, and micafungin.<sup>15</sup> Other studies have indicated that *C. orthopsilosis* and *C. metapsilosis* isolates can be inhibited by concentrations >1mg/l of amphotericin B.<sup>93</sup> Considering the results showed in different studies, it is possible to observe variation concerning antifungal susceptibility in the different species of the complex. This could be related to the clinical isolate and the methodology used to determine MIC values.<sup>15</sup>

Tests in neutropenic mice have shown that when intraperitoneal treatment with caspofungin is initiated 24 hours after infection with *C. metapsilosis*, the antifungal exerts a fungistatic response of  $\geq 2$ -8  $\mu\text{g/mL}$  in vitro against this fungus. This indicates that at clinically achievable concentrations this antifungal is effective against *C. metapsilosis*.<sup>94</sup> For

*C. metapsilosis*, the available information about antifungal treatment is scarce, however, there is no consensus regarding treatment, so it is normal to empirically administer amphotericin B, azoles, flucytosine, and echinocandins.<sup>20</sup> Although amphotericin B is the most commonly used therapy, it is usually associated with some side effects, such as renal affectations.<sup>20</sup> Fluconazole is often used in patients with candidiasis associated with members of the *C. parapsilosis* complex; however, it has been observed that prolonged exposure to it often leads to drug resistance.<sup>95,96</sup> The choice of antifungal and the duration of treatment may vary according to the severity of the infection and the area affected.

Considering that *C. metapsilosis* is a poorly studied but frequently occurring species, it is necessary to determine which are the appropriate antifungal therapies that contribute to treating infection caused by this species.

## Concluding Remarks

In recent years, there has been increasing interest in research on candidiasis caused by non-*albicans* species. Most studies have focused on understanding the biological, epidemiological, and clinical aspects of species such as *C. parapsilosis*, *C. orthopsilosis*, *C. lusitaniae*, and *C. krusei*. *C. metapsilosis* has been largely overlooked due to its low frequency of isolation in medical settings, and because its diagnosis is often confused with its closest species *C. orthopsilosis* and *C. parapsilosis*. However, it is important to note that infections caused by this species can be fatal when spread to the bloodstream. Additionally, *C. metapsilosis* can develop resistance to some antifungal drugs, making it a difficult species to treat.<sup>87</sup>

The findings of this work highlight the lack of substantial information about this species, which allows carrying out more detailed research that will provide the scientific community with information that may help to clarify fundamental aspects of the biology of *C. metapsilosis*. Although there are already phenotypic and molecular strategies for the identification of *C. metapsilosis*, the implementation of options that are more efficient and economical is sought.

The bioinformatics analyses included here may increase the information to understand basic aspects of this species. Sequence analyses allow the generation of genetic predictions, which facilitate the creation of working models to identify general and species-specific virulence factors.

## Acknowledgments

This work was supported by Consejo Nacional de Ciencia y Tecnología (ref. Ciencia de Frontera 2019-6380). The funding source that supported this work did not have any involvement in the design, acquisition, and analysis of data and writing of the manuscript.

## Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis, and interpretation, or all these areas; took part in drafting, revising, or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

## Disclosure

The authors declare no conflict of interest.

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