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# *Candida parapsilosis* and the neonate: epidemiology, virulence and host defense in a unique patient setting

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## Abstract

Invasive candidiasis is a common problem in premature infants that leads to high morbidity and mortality. Although *Candida albicans* has historically been the most prominent species involved in these infections and has therefore been the subject of the most study, *Candida parapsilosis* is increasing in frequency, and neonates are disproportionately affected. This article reviews unique aspects of the epidemiology of this organism as well as strategies for prophylaxis against invasive candidiasis in general. Additionally, important differences between *C. parapsilosis* and *C. albicans* are coming to light related to virulence determinants and interactions with components of host immunity. These developments are reviewed while highlighting the significant gaps in our understanding that remain to be elucidated.

### Keywords

antifungal prophylaxis; antifungal resistance; *Candida albicans*; *Candida parapsilosis*; echinocandins; fluconazole; host defense; lactoferrin; virulence factors

*Candida* spp. are the leading cause of invasive fungal infections in premature infants, leading to death or severe neurodevelopmental impairment in a large majority of those affected [1]. Among non-*albicans* species, *Candida parapsilosis* is increasingly being recognized as an important cause of invasive candidiasis in this population, and in some centers has overtaken *Candida albicans* as the leading species in invasive disease [2,3]. This diploid yeast is closely related to *C. albicans*, but has a number of unique attributes that are the focus of this review. *C. parapsilosis* was formerly divided into Groups I, II and III based on a variety of observed genetic differences. Further study published in 2005 demonstrated that these groups are sufficiently different such that they warrant designation as three

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distinct but closely related and phenotypically indistinguishable species [4]. Thus, *C. parapsilosis* Groups II and III are now designated as *Candida orthopsilosis* and *Candida metapsilosis*, respectively. Although the majority of clinical isolates appear to be *C. parapsilosis sensus stricto*, *C. orthopsilosis* and *C. metapsilosis* have been identified in collections of clinical isolates at frequencies that vary based on geographic region [5,6]. Because these definitions are relatively recent, and careful attention to defining these species is not universal in the literature, the term '*C. parapsilosis'* will be used to discuss these organisms for the remainder of this review.

## Epidemiology

### Colonization & infection

Rates of infection with *C. parapsilosis* vary from region to region throughout the world. The incidence of candidemia in different regions and the distribution of *Candida* spp. by age group was reviewed recently [7]. *C. parapsilosis* accounts for a wide range of *Candida* isolates, ranging from as little as 4% (Switzerland and one center in Brazil) to as high as 44% of all isolates (Saudi Arabia). Colonization with yeast is an important predisposing factor for developing invasive disease [8]. *C. parapsilosis* in particular was noted to colonize neonates later than *C. albicans* by several weeks [9], which is consistent with observations that *C. parapsilosis* is a rare cause of neonatal early-onset sepsis. In a study of 82 premature infants in the USA, four infants were found to have early stool colonization with *C. parapsilosis* between 1 and 2 weeks of age. All four infants developed *C. parapsilosis* candidemia [10]. A study in India showed similar trends, and added that central venous catheters were a risk factor for colonization. In this study, *Candida tropicalis* was the most common organism [8]. A French study suggested that a delayed time to total enteral nutrition is associated with *C. parapsilosis* colonization. Other suspected risk factors, including number of central venous catheters, duration of central venous catheters, days of antibiotics and mechanical ventilation were not significantly associated with invasive infection in this study, perhaps owing to a relatively low number of patients [11].

Because of its association with invasive infection, the source of colonization has also been an area of interest. Multiple studies have demonstrated horizontal transmission from environmental sources in outbreak settings, and hands of healthcare workers are commonly implicated [12,13]. Molecular techniques have confirmed identity between strains recovered from patients and strains recovered from colonizing sources [14]. Vertical transmission has been implicated as a source of neonatal colonization with *C. albicans*, with vaginal delivery as an independent risk factor. However, one study that used molecular methods to study route of colonization in premature neonates found *C. parapsilosis* colonization to be much more prevalent in neonates than in their mothers and no cases of vertical transmission were documented [15].

*C. parapsilosis* infections have been reported in virtually every sterile body site in neonates, including blood, lung, urine, retina and ascites. Unlike *C. albicans*, however, *C. parapsilosis* meningitis is relatively uncommon and is mainly associated with indwelling medical devices [16]. Clinical manifestations of disease in neonates are difficult to distinguish from other organisms causing neonatal sepsis. One study compared neonates with *C. albicans* fungemia and *C. parapsilosis* fungemia, and found that infants with *C. parapsilosis* were less likely to have bradycardia, hypoxemia or respiratory distress, and more likely to have hypothermia. However, the *C. albicans* group was more likely to have positive sterile site cultures other than blood, suggesting that involvement of other organs may have contributed to their symptoms [17].



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### Risk factors

Several studies have examined risk factors for development of candidemia in neonates, but few have examined *C. parapsilosis* in particular. Risk factors for candidemia are numerous and are listed in Table 1. While some or all of these risk factors are likely to apply to infants with *C. parapsilosis*, few studies focus on neonates. Those that have examined this issue compared infants with invasive *C. albicans* to those with invasive *C. parapsilosis.* Factors that have been reported to increase risk for invasive *C. parapsilosis* infection include use of cephalosporins [18], presence of an indwelling central venous catheter [19,20] and receipt of parenteral nutrition [20]. Factors that favor *C. albicans* infection over *C. parapsilosis* include development of oral thrush or diaper dermatitis [19]. These studies should be interpreted with caution, as each series may have been underpowered to detect significant associations. These same studies reported that *C. parapsilosis* was more likely to be found in the peripheral blood, and less likely to be found in the urine, on central venous catheter tips or in deep-seated infections [18]. Further investigation is warranted to clarify potentially unique risk factors for *C. parapsilosis* infections.

### Species prevalence

Several surveillance programs exist to monitor fungal infections regionally, nationally and internationally. Overall, *C. albicans* is typically the leading fungal pathogen, followed by *Candida glabrata* [21,22]. In some settings, *C. tropicalis* may be the dominant fungal pathogen [23]. By contrast, studies that examined neonates or subsets of neonates, such as extremely low birth-weight infants, found *C. parapsilosis* as the second most common fungal pathogen (Figure 1) [1,24,25]. In fact, *C. parapsilosis* has surpassed *C. albicans* to become the dominant fungal pathogen in children and neonates in some centers [26,27]. These authors suggested that the shift may be due to a high number of patients undergoing gastrointestinal surgery and/or receiving parenteral nutrition, particularly at home [26]. As medical technology becomes more widespread and more common in out-of-hospital settings, clinicians should be aware of potential shifts in epidemiology of fungal infections.

## Antifungal prophylaxis

The use of prophylactic medications, both systemic and mucosal, for prevention of invasive fungal infections has received a great deal of attention in recent years. Studies have focused on infants with low birthweight (<2500 g) or very low birthweight (<1500 g). The best-studied agent in systemic prophylaxis studies is fluconazole. In a Cochrane review last updated in 2009, eight randomized, placebo-controlled trials were included [28]. Since this update, Aydemir *et al.* have published another randomized trial comparing fluconazole, nystatin and placebo [29]. While these studies did not examine *C. parapsilosis* specifically, it was included in the aggregate end point. Limiting discussion to randomized trials included in the Cochrane review, 758 low birth weight or very low birth weight infants were included in these trials. Most (656) were included in trials of intravenous fluconazole versus placebo. The pooled effect sizes estimated that the number of infants needed to treat with fluconazole is 11 to prevent one episode of invasive candidiasis [28]. However, the reviewers noted that this estimate was driven by locations with high prevalence of invasive disease, and cautioned that the number needed to treat is considerably higher in low-prevalence locations. Indeed, the incidence of invasive candidiasis varied markedly among centers, making a unified strategy for prophylaxis difficult to develop [24]. In addition, no overall benefit to all-cause mortality was noted in the pooled studies [28].

The efficacy of mucosal antifungal prophylaxis to prevent invasive candidiasis is promising, but suffers from a lack of rigorous trials. Another Cochrane review examined the potential use of nonsystemic antifungal agents such as nystatin and miconazole for the prevention of

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invasive fungal infections [30]. The reviewers included four randomized studies. The pooled analysis of 1625 infants showed a protective effect for invasive fungal infection, needing to treat five infants to prevent one infection. Similar to fluconazole trials, such numbers may have been driven by large trials (948 infants) in high-prevalence areas. The results should be interpreted cautiously owing to methodological flaws. Some studies were unblinded, and one study was performed in a setting where resources did not allow for interventions such as ventilator support for infants with birthweights less than 1250 g. Similar to systemic prophylaxis, no difference was found in all-cause mortality prior to discharge [30]. A recent randomized trial comparing systemic fluconazole and oral nystatin to placebo in preterm neonates provided additional insight into this issue [29]. This study of 278 infants revealed similar decreases in invasive fungal infections, including *C. parapsilosis*, in both intervention groups compared with placebo. The rate of invasive infection was 16.5% in the placebo group, compared with 3.2% in the fluconazole group and 4.3% in the nystatin group. Again, there was no difference among the groups in mortality before discharge. This study suggests that nonsystemic prophylaxis may be comparable to systemic prophylaxis in reducing invasive fungal infections in very low birthweight infants.

Although the benefit of antifungal prophylaxis in reducing the incidence of invasive candidiasis in infants is well documented in settings where the baseline infection rate is high, concern regarding the possibility of inducing antifungal resistance remains. Studies in the USA and Italy have shown that there was no selection for intrinsically azole-resistant *Candida* spp. during periods of routine use of fluconazole [24,31]. By contrast, a national study in Finland showed an association of increased use of fluconazole with an increased proportion of the intrinsically azole-resistant *C. glabrata* [32]. However, this study was not limited to neonatal populations. Another Finnish study did not find a similar shift in a neonatal intensive care unit setting, but did uncover a single strain of *C. parapsilosis* with increasing resistance to fluconazole over a 10-year period [33]. Data on colonization from one of the early randomized controlled trials of fluconazole prophylaxis were also not entirely reassuring in this regard. The prevalence of colonization with the more azole-sensitive *C. albicans* ($MIC_{90}$ = 0.5 μg/ml) was reduced from 43 to 7%, but colonization was replaced with the more resistant non-*albicans* strains, primarily *C. parapsilosis* ($MIC_{90}$ = 8 μg/ml), which increased from 45 to 83% [34]. In another set of vulnerable patients, recipients of hematopoietic stem cell transplants and patients with acute myelogenous leukemia, Mann *et al.* demonstrated a shift in colonizing yeast to intrinsically resistant *Candida* spp. in patients receiving azole prophylaxis [35]. There was also a fourfold increase in MIC to azole drugs in colonizing strains that became invasive infections. Finally, development of resistant *C. parapsilosis* in the setting of fluconazole prophylaxis in an animal neonatal intensive care unit has also been reported [36].

Lactoferrin, a component of mammalian milk, is well known to have anti-infective properties. The utility of bovine lactoferrin administration for prevention of sepsis in premature neonates has received recent attention with promising results. A multicenter study conducted in Italy randomized 472 infants to lactoferrin alone or in combination with the probiotic *Lactobacillus rhamnosus* GG compared with placebo. Significant reductions in both late-onset sepsis and invasive *Candida* infections were seen in both lactoferrin groups relative to placebo [37]. Interestingly, there was no effect on fungal colonization.

Systemic prophylaxis appears to be safe and effective in reducing the incidence of invasive fungal infection. Studies involving mucosal therapy are encouraging; however, more carefully controlled studies are needed. The cost–benefit ratio of administering prophylactic antifungals depends largely upon the local prevalence of invasive fungal infection. If a center opts to use routine antifungal prophylaxis, surveillance of resistance patterns should occur to identify emergence of resistant organisms. Lactoferrin is a promising new strategy

for prophylaxis that avoids the use of conventional antifungal agents and could avoid the potential complication of emerging resistance.

## Treatment of neonatal candidiasis

The treatment of neonatal candidiasis is a continuously evolving field. A summary of available antifungal agents and dosing recommendations is listed in Table 2. Guidelines have been published that cover treatment of candidiasis across all ages and body sites [38] and pharmacotherapy in neonates has recently been reviewed [39]. We will focus our discussion on the newest class of antifungal agents, the echinocandins. These medications inhibit the synthesis of 1,3-β-D-glucan, a component of fungal cell walls. Member drugs include caspofungin, micafungin and anidulafungin. Although no controlled trials have compared efficacy against other antifungal classes, successful therapy with caspofungin and micafungin in neonates for invasive fungal infections, including *C. parapsilosis*, has been reported [40,41]. There are no data available on the use of anidulafungin in neonates. Because of lack of data, reserving use of echinocandins for situations of treatment failure, intolerable or toxic side effects or adverse events with other antifungal medications is recommended [38]. Adverse events are largely unknown in neonates, but may include hepatitis and electrolyte abnormalities.

### Patterns of resistance

Resistance patterns vary from location to location, and while multinational studies have shown that widespread antifungal resistance is not yet emerging, local data may show the opposite. For example, while some studies have shown that *C. parapsilosis* has retained the susceptibility to amphotericin B [42], other reports have shown resistance of *C. parapsilosis* to this agent [40,43]. Although surveillance has not yet identified emerging azole resistance in the setting of fluconazole prophylaxis [31], ongoing monitoring is important as these trends can take many years to detect. In locations where fluconazole is used routinely for prophylaxis, systematic monitoring should be in place to determine if strains of *C. parapsilosis* and other *Candida* spp. are developing resistance to fluconazole.

Concerns have also been raised over echinocandin resistance in *C. parapsilosis* isolates [44], which may be associated with acquired mutations of the *fks* gene encoding β-D-glucan synthase [45]. It is recognized that *C. parapsilosis* strains tend to have decreased susceptibility to echinocandins without displaying *fks* gene mutations; however, these differences do not appear to be clinically significant in most cases [46]. Indeed, worldwide surveillance of invasive *Candida* species, including *C. parapsilosis*, has shown very low rates of resistance with updated Clinical Laboratory and Standards Institute guidelines [46]. Other studies have shown very low incidence of non-wild-type *C. parapsilosis,* suggesting overall low incidences of mutation or acquisition of mutant *fks* genes [47].

## Virulence factors

The focus thus far has been on clinical aspects of these important infections in a neonatal population. Clinical disease is the measurable end point of a complex interplay involving properties of the microbe that contribute to its ability to cause disease in a human host and a failure of host defense mechanisms that have evolved to prevent these infections and their consequences. Because of its historical importance and its unique virulence attributes, *C. albicans* has been the subject of the vast majority of work related to *Candida* biology and interactions with the host in health and disease. Abundant knowledge has been gained as a result of these studies. However, as increasing attention has been paid to the non-*albicans* species in recent years, unique aspects of the virulence and host–pathogen interactions of these fungi have come to light, and significant differences from *C. albicans* are emerging.



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These observations underscore the need to study host–pathogen interactions from a narrow focus, as even closely related pathogens can yield very different results and may therefore be amenable to unique therapeutic strategies.

Virulence factors increase a microbe's pathogenic potential by augmenting its ability to colonize and invade its host, avoid immune detection and ultimately increase morbidity and mortality. Virulence factors associated with *C. parapsilosis* include its ability to adhere to a wide array of biological and prosthetic surfaces, the ability to form biofilms on implanted medical devices and secretion of hydrolytic enzymes capable of causing significant tissue damage. Recent studies have also highlighted the importance of fatty acid synthesis and storage in the role of *C. parapsilosis* pathogenesis. This section reviews the current information regarding these attributes of *C. parapsilosis*.

## Adhesion & biofilm formation

*Candida* biofilms are fungal communities of yeast associated with biological and prosthetic surfaces and are often associated with implanted medical devices [48]. Their clinical significance is underscored by the observation that *C. parapsilosis* biofilms are resistant to antifungal treatment and host immune responses and are associated with increased mortality [49–51]. In comparison to *C. albicans* biofilms, the architecture of *C. parapsilosis* biofilms is much simpler [52]. *C. parapsilosis* biofilms are not as thick as *C. albicans* biofilms and are often observed as monolayers or aggregates of yeast. Additionally, unlike *C. albicans* biofilms in which production of filamentous forms including true hyphae and pseudohyphae plays a prominent role, *C. parapsilosis* does not produce true hyphae, and instead exists in either yeast or pseudohyphae cell morphologies. Interestingly, biofilm formation has been linked to cell and colony morphology, where in general, *C. parapsilosis* pseudohyphae are more biofilm proficient than yeast [53]. The mechanisms behind *C. parapsilosis* pseudohyphae production are unclear although the presence of specific amino acids has been identified to play a role [54]. Finally, although *C. albicans* biofilms produce an extracellular matrix that is protein rich, the extracellular matrix produced by *C. parapsilosis* is predominately carbohydrate [52]. Despite these differences in biofilm structure, *C. parapsilosis* has a much higher affinity for prosthetic material than *C. albicans*, especially in the presence of high glucose and lipid media [55,56]. These properties may relate to the association of *C. parapsilosis* infection with parenteral nutrition.

## Hydrolytic enzyme production

*Candida* species secrete hydrolytic enzymes that are associated with virulence, including secreted aspartyl proteases (SAPs), phospholipases, lipases and hemolysins. In reconstituted human tissue models of *C. parapsilosis* infection, inhibition of these enzymes decreased tissue damage [57,58]. In general, SAP production seems to be higher in *C. albicans* than *C. parapsilosis*, although there are some discrepancies among studies [59–61]. *C. parapsilosis* has three SAP genes: *SAPP1*, *SAPP2* and *SAPP3*. *SAPP1* has undergone gene duplication and has been referred to as *SAPP1a* and *SAPP1b* [62]. The proteolytic activity of *SAPP1* and *SAPP2* has been examined, with *SAPP1* having an increased expression and a broader substrate specificity than *SAPP2* [62,63]. The role of SAP production in *C. parapsilosis* systemic infections remains unclear, although SAP activity does play an important role in establishing mucocutaneous infection. Interestingly, non-blood isolates had higher protease activity than blood isolates but activity can increase during infection [58,64,65]. SAP production also plays a role in establishing vaginal infections, as non-SAP producers were incapable of causing long-term vaginitis in a rat model [66,67]. *C. parapsilosis* SAP production may also have a role in immune evasion, as deletion of *SAPP1a* and *SAPP1b* resulted in increased susceptibility to human serum and increased phagocytosis and killing by macrophages (Figure 2A) [62].

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Phospholipases are important virulence factors for many fungal pathogens and contribute significantly to host damage [68]. Phospholipase activity of *C. parapsilosis* appears to be strain dependent as a large range of phospholipase activity has been reported [60,61,64,69–71]. The role of phospholipase in virulence remains unclear, although increased phospholipase activity is associated with increased adherence to epithelial cells (Figure 2b) [64]. Lipase production, however, is recognized to play an important role in *C. parapsilosis* infection. In addition to being biofilm deficient, *C. parapsilosis* mutants lacking lipases *Cplip1* and *Cplip2* also exhibited decreased virulence in a murine model of infection [72]. Interestingly, mutants exhibited decreased virulence when animals were infected by the intraperitoneal route, but not when introduced intravenously. In comparison, neonatal rats infected with *C. parapsilosis* lipase mutants intravenously, intragastrically and intraperitoneally had lower fungal burdens than rats infected with wild-type *C. parapsilosis* [73]. *Cplip1* and *Cplip2* double mutants also had a reduced ability to cause epithelial damage, and were phagocytosed and killed more efficiently by macrophage-like cells [72] and by dendritic cells (DCs) (Figure 2A) [74].

Hemolytic activity for *C. parapsilosis*, in general, has been reported to be absent or very low, although this may be a result of small sample sizes [61,75]. One study, however, looked at the hemolytic activity of 34 *C. parapsilosis* isolates, the majority of which exhibited weak hemolytic activity [65]. This study noted that tracheal isolates had increased hemolytic activity compared with blood isolates, indicating that perhaps hemolytic ability plays a role in colonization of specific sites. However, the role of hemolytic activity in *C. parapsilosis* pathogenesis remains poorly understood and more research into this area is needed.

### Fatty acid synthesis & storage

Recent studies have highlighted the importance of fatty acid synthesis and storage in *C. parapsilosis* virulence. Surprisingly, these genes play a role in the immune evasion, biofilm formation and enhanced virulence in murine models of infection. When *FAS2* (fatty acid synthase type 2) was deleted, mutant cells failed to form biofilms, were hypersensitive to human serum, and were phagocytosed and killed more efficiently by effector cells (Figure 2A) [76]. When *OLE1* (stearoyl-coenzyme A desaturase 1) was deleted, mutant cells failed to produce pseudohyphae and were hypersensitive to osmotic and oxidative stress, as well as human serum [77]. In addition, *ole1* mutants were phagocytosed and killed more efficiently by macrophages and exhibited decreased virulence in a murine model of infection (Figure 2A). Disruption of *FIT2* (fat storage-inducing protein type 2) also increased *C. parapsilosis* sensitivity to osmotic stress and resulted in decreased virulence in a murine model of infection [78].

## Host defense

In recent years, the number of studies focusing on host–pathogen interactions with *C. parapsilosis* has increased but this relationship is still poorly understood. The majority of recent studies have focused on this interaction from the pathogen's perspective; that is, how *C. parapsilosis* genes or morphological traits influence interactions with the host. Very few studies have focused on the host receptors involved in the recognition and response to *C. parapsilosis.* Improved knowledge of these interactions will identify unique aspects of the host response to this species and could inform novel therapeutic strategies.

### Receptors

To defend against pathogenic fungi, the host's immune system must first recognize and respond to the invading pathogens by recognizing specific fungal pathogen-associated molecular patterns (PAMPs). These PAMPs are recognized by pattern recognition receptors

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(PRRs), which are found on a wide array of effector cells. Fungal PAMPs are generally components of the carbohydrate-rich fungal cell wall and include chitin, β-glucan and various mannan structures. PRRs include several different members of the C-type lectin receptor family and Toll-like receptors. Although recent work has defined many interactions between *C. albicans* PAMPs and PRRs of the innate immune system (reviewed in [79]), similarities and differences specific to *C. parapsilosis* remain poorly defined.

## Neutrophils

Neutrophils are key mediators against fungal infection, as both neutropenic mice and humans have increased susceptibility to systemic *Candida* infections [80,81]. Human neutrophils underwent phagocytosis and killing of *C. parapsilosis* much more efficiently than *C. albicans* yeast, suggesting that these interactions have properties that are unique among different *Candida* spp. [82]. Despite the increased susceptibility of preterm neonates to infection with *C. parapsilosis*, neutrophils from these patients responded to *Candida* in a similar fashion to adult cells [83]. Neutrophil phagocytosis of *C. albicans* is mediated in part via β-glucan recognition by dectin-1 and complement receptor 3 [84–87], although there is an ongoing controversy about the contribution of each receptor. Neutrophil phagocytosis of *C. parapsilosis*, however, does not appear to be mediated by dectin-1 or complement receptor 3, as neutrophil treatment with blocking antibodies against both receptors did not inhibit phagocytosis [82]. Current work from our group has demonstrated that the lectin galectin-3 plays an important role in the increased efficiency of neutrophil phagocytosis of *C. parapsilosis* versus *C. albicans* [Linden *et al.*, The role of galectin-3 in the human neutrophil response to candida and protection against disseminated candidiasis (2012), Submitted]. Our data suggest that *C. parapsilosis* induces galectin-3 secretion and that secreted galectin-3 acts as an autocrine or paracrine signal on neutrophils, augmenting phagocytosis. Interestingly, our group and others have recently found a relative deficiency of galectin-3 in neonates and decreased expression associated with lower gestational age [88]. Based on these observations, it is tempting to speculate that impaired galectin-3 expression in neonates may contribute to their enhanced susceptibility to *C. parapsilosis* and potentially other infections as well, but this hypothesis requires further study.

## Monocytes & macrophages

Macrophages play an important role against disseminated *Candida* infections [89]. Interestingly, *C. parapsilosis* possesses several enzymes that are protective against macrophage effector functions. *C. parapsilosis* mutant strains deficient in Sapp1a and Sapp1b were phagocytosed and killed more efficiently by human monocytes and macrophages than their wild-type counterparts [62]. Deletion of genes involved in fatty acid synthesis, including *OLE1* and *FAS2* also increased phagocytosis and macrophage killing of the mutant *C. parapsilosis* strain compared with the wild-type (Figure 2A) [76,77,90].

## Dendritic cells

Located in the skin and mucosae, DCs are an important link between innate and adaptive immunity during infection. DCs protect the host as antigen-presenting cells capable of mounting Th1, Th2 or Th17 responses against *C. albicans* infection [91–94]. DCs also act as ‘nonprofessional phagocytes’ capable of ingesting and killing *Candida* yeast. In response to *C. parapsilosis*, DCs produced pseudopodial protrusions called fungipods [95]. Interestingly, DCs produced more fungipods in response to *C. parapsilosis* than in response to *C. tropicalis* or *C. albicans*. Fungipod formation was mediated by recognition through the mannose receptor, not dectin-1 or DC-SIGN, suggesting that *C. parapsilosis* mannan may have unique inflammatory properties. The fungipod was suggested to aid in DC effector functions by improving particle binding and retention, possibly augmenting phagocytosis by size discrimination. Work by another group demonstrated that *C. parapsilosis* lipases provided

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protection from the immune system by inhibiting DC effector function [74]. Lipase-deficient *C. parapsilosis* mutants were phagocytosed and killed more efficiently by both immature and mature DCs, possibly via increased lysosome maturation (Figure 2A). Lipase activity also reduced cytokine production by DCs, as the lipase mutants induced more cytokine production than wild-type *C. parapsilosis*.

## Epithelial cells

Adherence to host cells is a critical aspect in *Candida* pathogenesis, and adhesion to epithelial cells is probably important for colonization and transmission of *C. parapsilosis*. The majority of work focusing on *C. parapsilosis* interactions with epithelial cells has used oral epithelial cells, including carcinoma cell lines, primary cell lines and reconstituted oral human epithelial tissue. Adhesion of *C. parapsilosis* to buccal epithelial cells has been positively correlated with many factors including specific cell surface carbohydrates [96]. Although *C. parapsilosis* is capable of adhering to oral epithelial cells, it does not have the same invasive qualities associated with production of hyphae by *C. albicans*. Despite its poor ability to invade, *C. parapsilosis* was capable of causing extensive tissue damage in a model of reconstructed human oral epithelial tissue [58]. Colonization and invasion was strain dependent and did not depend on cell morphology (yeast vs pseudohyphae). Another group using models of both reconstituted oral epithelium and reconstituted epidermal tissue saw similar results including strain-to-strain variability, poor invasion and extensive tissue disruption and damage [57]. Interestingly, a strain producing pseudohyphae was noted to be more invasive and destructive in the oral epithelium model than yeast isolates. Because of the low number of strains used in each of these studies, the relationship of cell morphology and adherence to epithelial cells remains uncertain. Tissue destruction is mediated by *C. parapsilosis* production of SAP and lipases, as inhibition of these enzymes reduced epithelial damage but not invasion (Figure 2B) [57,58]. These results were confirmed using mutant strains of *C. parapsilosis* deficient in the lipase genes *CpLIP1* and *CpLIP2* [72].

Epithelial cells can discriminate between *C. albicans* yeast and hyphae by activation of NF-κB and different stages of the biphasic MAPK response. Epithelial cells responded to both yeast and hyphae by activation of the first MAPK phase via c-Jun activation. In response to hyphae, however, epithelial cells activated the second MAPK phase via *MKP1* and c-Fos activation, which correlates with proinflammatory responses [97]. Although *C. parapsilosis* was capable of activating NF-κB, it failed to activate the second MAPK phase responsible for hypha recognition [98]. Notably, the *C. parapsilosis* strain used failed to form pseudohyphae. *C. parapsilosis* also induced less secretion of the proinflammatory cytokines G-CSF, GMSF and IL-6 when compared with *C. albicans. C. parapsilosis* was capable of causing epithelial damage and inducing secretion of the damage-associated cytokine IL-1α (Figure 2B). By contrast, another study using a filament-producing environmental strain of *C. parapsilosis* demonstrated that infection caused an increase in epithelial gene expression of *TLR2*, *TLR4* and *TLR6*. Interestingly, these epithelial cells inhibited fungal growth, presumably by increased expression of IL-1β, TNF-α and IFN-γ and secretion of human β-defensins [99]. Taken together, these studies suggest that epithelial cells may respond differently to differing *C. parapsilosis* morphologies.

## Endothelial cells

Interactions between *Candida* and endothelial cells play a critical role in the pathogenesis of disseminated candidiasis. In hematogenously disseminated disease, endothelial cells provide a barrier between pathogens circulating in blood vessels and uninfected host tissue. Though much work has focused on understanding how *C. albicans* escapes the vascular lumen, little work has examined the interaction of *C. parapsilosis* with endothelial cells. *C. albicans* was capable of inducing its own endocytosis by human umbilical vein endothelial cells

(HUVECs) by binding of Als3 and Ssa1 to N-cadherin [100–102]. *C. albicans* invaded human brain micro-vascular endothelial cells (HBMECs) by again inducing its own endocytosis by endothelial recognition of Als3 and Ssa1. In comparison to HUVEC, however, HBMEC recognized Als3 using heat-shock protein GP96, a receptor expressed by HBMEC but not HUVEC [103]. Ssa1-mediated endocytosis by HBMEC occurred through an undefined receptor. The extent to which *C. parapsilosis* exhibits similar properties is essentially unexplored.

## Expert commentary & five-year view

Systemic candidiasis remains a common problem among the immunocompromised. Disseminated disease occurs in the setting of bone marrow transplant recipients, patients undergoing chemotherapy for solid organ and hematologic malignancies, and in cases of mucosal compromise such as gastrointestinal surgery or severe burns. Neutropenia or defects in neutrophil function are known to be important risk factors in the development of invasive candidiasis, as are common components of ICU care including endotracheal intubation, central venous and urinary catheters, parenteral nutrition, broad-spectrum antibiotics and corticosteroid use. Despite the availability of current antifungal agents, the morbidity and mortality related to these infections remains unsuitably high.

Premature infants constitute another group of patients at high risk for this disease and its complications. Although they share some of the risk factors common to ICU care in other patient populations, their susceptibility to these infections is also likely to be related to unique mechanisms of compromised immunity. Despite sharing many risk factors with other patient populations that develop candidiasis, the high frequency with which *C. parapsilosis* is the causative organism in this population is quite unique, suggesting that features of this particular species and the neonatal host make infection with this organism more likely to occur. We have reviewed a number of features related to the pathogenesis of *C. parapsilosis* that differ from *C. albicans*, supporting the notion that this species brings a specific set of properties to the host–pathogen interface that may affect the likelihood of disease in a specific host setting. However, our understanding of how these virulence properties manifest *in vivo*, particularly in a neonatal host, is in its earliest stages with much work still to be done.

The development and function of the innate and adaptive arms of the neonatal immune system have been the subject of much study, and many specific mechanisms that contribute to the enhanced susceptibility to infection in neonates have been described. Considerably less attention has been focused on the specific developmental deficiencies in immune function that increase the risk for fungal disease. Because quantitative or qualitative defects in neutrophil function, for example, are known to contribute to invasive fungal infection, yet neonates who develop these infections are rarely neutropenic, our group tested specific adult and infant neutrophil functions when confronted with *C. parapsilosis* and *C. albicans* [82,83]. No significant differences among preterm infant, term infant or adult neutrophils were detected in these studies, but unexpected, dramatic differences in phagocytosis efficiency of these two species were observed. Our more recent finding of a role for galectin-3 in neutrophils confronting *C. parapsilosis* coupled with a relative deficiency of galectin-3 in neonatal serum suggests that other mechanisms that affect neutrophil function may be at play [Linden *et al.* The role of galectin-3 in the human neutrophil response to candida and protection against disseminated candidiasis (2012), Submitted]. This example is meant to illustrate that gaining an understanding of how susceptibility of a specific patient population relates to a specific pathogen is likely to require study tailored to that interaction. Although important and instructive, more general observations of immune function, or



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responses of a host to a closely related species of pathogen need to be confirmed in the specific host–pathogen interaction of interest.

Treatment of fungal infections has relied on conventional chemotherapeutic agents for decades. The echinocandins represent the newest class of antifungal drugs, and the first to target fungal cell wall synthesis. These agents are proving to be a useful addition to the antifungal armamentarium, but they have yet to be studied rigorously in neonates. However, even with available antifungal therapies, once these infections are detected in premature infants, the result is either death or neurodevelopmental sequelae in the vast majority of infected infants [1]. The most effective method to improve these outcomes is prevention. Indeed, antifungal prophylaxis as described above has been well studied and shown to be effective, particularly in high-incidence settings. Although not observed so far, concern remains regarding the emergence of resistance or replacement of current species with fungi that carry intrinsic resistance, potentially resulting in resistance patterns that preclude the use of an entire class of antifungals. With limited options, new drug development should be encouraged well before this eventuality. Newer strategies that avoid antifungal drugs and their selective pressures are being investigated, such as lactoferrin [37]. The promising results suggest that continued investigation aimed at augmenting or replacing components of innate immunity in patients at risk may offer new strategies that are safe and effective in prevention. When prophylactic strategies fail, outcomes may also be improved by more accurate and timely diagnosis of fungal infection. Clinical signs of fungal infection are nonspecific and require a high index of suspicion. Additionally, culture-based methods are insensitive, which can lead to costly delays in initiation of appropriate therapy. Rapid diagnostics are an area of intense interest, and if developed will probably facilitate timely and accurate detection and treatment and improve outcomes.

As infections with non-*albicans Candida* species have increased in prevalence, so has interest in the unique properties of these organisms and how they manifest in the human host. Likewise, mechanisms of antifungal host defense that are poorly developed in neonates are beginning to be elucidated. Future work in these areas is likely to not only improve our understanding of these basic mechanisms and interactions, but inform the development of newer, more effective preventive, diagnostic and therapeutic strategies. Future innovations will not only improve the outcomes of premature infants, but will hopefully extend to broader groups of patients at risk.

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## Key issues

- Although *Candida albicans* is the most common species associated with invasive candidiasis, incidence of infection with *Candida parapsilosis* has increased and varies by geographic region and age group. Premature neonates have the highest reported incidence of infection with this species relative to other patients at risk.
- Antifungal prophylaxis with systemic or mucosal agents in premature infants is effective at reducing rates of invasive candidiasis, particularly in high-prevalence settings. Although not evident to date, concerns about emerging resistance with these strategies remain.
- Echinocandins are a useful addition to available antifungal agents, but there are few data available on their use in neonates.
- Virulence properties of *C. parapsilosis* include its tendency to grow as a biofilm, which increases its resistance to immune cells and antifungal agents, and production of hydrolytic enzymes. Gene products involved in fatty acid synthesis and storage appear to have particular importance in *C. parapsilosis* virulence.
- Interactions of *C. parapsilosis* with host cells are beginning to be defined and contrasted to prior studies with *C. albicans*. Neutrophils undergo phagocytosis of *C. parapsilosis* yeast with much higher efficiency than *C. albicans* yeast and galectin-3 is involved in this process. Dendritic cells undergo unique morphological changes (‘fungipods’) in response to *C. parapsilosis* relative to other *Candida* species. Epithelial cells respond to *C. parapsilosis* differently than *C. albicans*. These observations underscore the need to study these interactions in a species-specific manner.

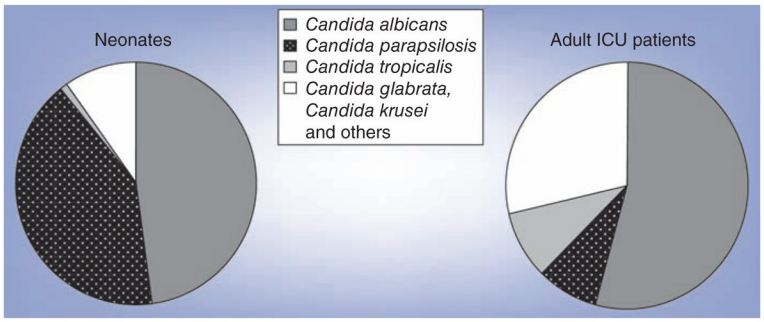


**Figure 1. Distribution of *Candida* species by age**

ICU: Intensive care unit.

Data taken from [1,21].

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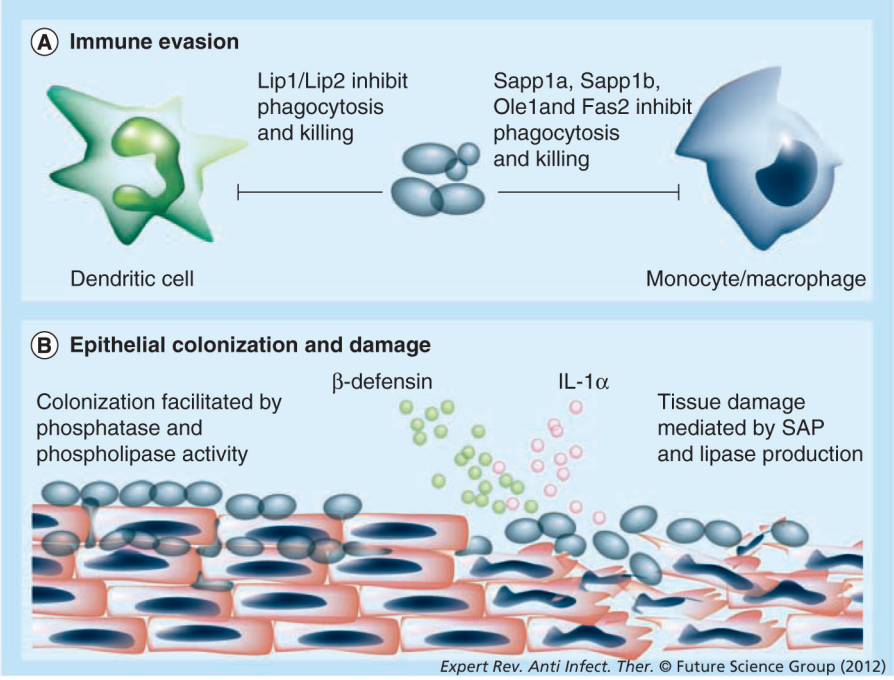


**Figure 2. Immune response to *Candida parapsilosis.***

Factors involved in **(A)** immune evasion and **(B)** epithelial colonization and damage by Candida parapsilosis. See text for further details.

Lip: Lipase; SAP: Secreted aspartyl protease.



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## Table 1

Risk factors for candidemia in neonates.

| Risk factor | Ref. |
|---|---|
| Extreme prematurity † | [104–106] |
| Vaginal delivery | [105] |
| Colonization with *Candida* | [107] |
| Apgar score <5 at 5 min | [104] |
| Shock | [104] |
| Antibiotic number >2 | [104] |
| Longer duration of antibiotics | [108] |
| Use of a third-generation cephalosporin | [106,109] |
| Parenteral nutrition >5 days | [104] |
| Lipid use >7 days | [104] |
| Central venous catheter | [104] |
| Use of H2 blockers | [104] |
| Length of stay >7 days | [104] |
| Intubation | [104] |
| Early dexamethasone | [108] |
| Abdominal surgery | [105] |
| Thrombocytopenia | [106] |

† Increase in risk is inversely proportional to gestational age in weeks.

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## Table 2

Medications for use in invasive *Candida* infections in neonates.

| Drug class | Drug | Recommended dosing | Comments |
|---|---|---|---|
| Polyenes | Amphotericin B deoxycholate | 1–1.5 mg/kg iv. every 24 h | |
| | Amphotericin B liposomal formulation | 5–7 mg/kg iv. every 24 h | |
| | Amphotericin B lipid complex | 5–7 mg/kg iv. every 24 h | |
| Triazole | Fluconazole | Loading dose: 12–25mg/kg; maintenance dose: 6–12 mg/kg/dose | Interval of dosing varies by gestational age |
| | Voriconazole | No data in neonates | Use with caution in renal insufficiency |
| | Itraconazole | No data in neonates | |
| | Posaconazole | No data in neonates | |
| Echinocandins | Caspofungin | 25mg/m $^2$ iv. every 24 h or 2 mg/kg iv. every 24 h | |
| | Micafungin | 7–10 mg/kg iv. every 24 h | Higher dosing warranted in infants less than 27 weeks' gestational age and less than 14 days of life |
| | Anidulafungin | No data in neonates | |
| | Flucytosine | 12.5–37.5 mg/kg enterally every 6 h | Not for use in monotherapy<br>No parenteral formulation<br>Carries a US FDA black box warning to use with caution in renal insufficiency |

iv.: Intravenous.