Candida parapsilosis: a review of its epidemiology, pathogenesis, clinical aspects, typing and antimicrobial susceptibility

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Abstract
The Candida parapsilosis family has emerged as a major opportunistic and nosocomial pathogen. It causes multifaceted pathology in immuno-compromised and normal hosts, notably low birth weight neonates. Its emergence may relate to an ability to colonize the skin, proliferate in glucose-containing solutions, and adhere to plastic. When clusters appear, determination of genetic relatedness among strains and identification of a common source are important. Its virulence appears associated with a capacity to produce biofilm and production of phospholipase and aspartyl protease. Further investigations of the host-pathogen interactions are needed. This review summarizes basic science, clinical and experimental information about C. parapsilosis.

Keywords: Candida parapsilosis, epidemiology, strain differentiation, clinical aspects, pathogenesis, antifungal susceptibility

Introduction

Candida bloodstream infections (BSI) remain an exceedingly common life-threatening fungal disease and are now recognized as a major cause of hospital-acquired infection (Douglas 2003; Tortorano et al. 2006; Tortorano et al. 2004). It is now the fourth most common organism recovered from blood cultures among hospitalized patients in the USA (Hobson 2003; Pfaller et al. 1998b, Rangel-Frausto et al. 1999; Schaberg et al. 1991).

Some years ago Candida albicans accounted for 70–80% of the Candida isolates recovered from infected patients (Banerjee et al. 1991; Beck-Sague & Jarvis 1993; Fidel et al. 1999). However, infections due to non-albicans species have emerged over the past two decades, and a shift from C. albicans to species such as Candida glabrata, Candida parapsilosis, and Candida tropicalis has occurred (Fidel et al. 1999; San Miguel et al. 2005). C. parapsilosis is now the second or third most common cause of candidiasis, behind C. albicans.

The organism was first described in 1928 (Ashford 1928), and early reports of C. parapsilosis described the organism as a relatively non-pathogenic yeast in the normal flora of healthy individuals that was of minor clinical significance (Weems 1992). Important factors that have contributed to the increasing incidence of C. parapsilosis are the use of life support systems, such as parenteral nutrition, or central venous catheters (Eggimann et al. 2003, Krcmery & Barnes 2002). The increased incidence of candidemia due to C. parapsilosis also is associated with extended hospital stay, which leads to increased cost of medical care. Its spectrum of clinical manifestations include fungemia, endocarditis, peritonitis, arthritis, and endophthalmitis. This species displays many interesting biological features that are presumed to be directly related to its virulence, such as its selective adherence to prosthetic materials and formation of biofilms on plastic surfaces (Branchini et al. 1994; Pfaller 1995), secretion of extracellular proteases (Fusek et al. 1993, Merkerova et al. 2006, Pichova et al. 2001),
colonization of human hands (Bonassoli et al. 2005), proliferation in high concentration of glucose and lipids (Branchini et al. 1994), phenotypic switching (Laffey & Butler 2005, Lott et al. 1993), and resistance to drugs and inhibitors (Camougard et al. 1986). Unfortunately, we have much to learn about the virulence of *C. parapsilosis* and even more about the host defenses directed against the organism. Therefore, studies increasing our knowledge about this pathogen are needed.

Since a previous review was written (Weems 1992), *C. parapsilosis* has only increased its standing as a pathogen. It therefore is highly relevant to review the recent literature on *C. parapsilosis*. While this article was in preparation, last year an updated useful review of many of the aspects of *C. parapsilosis* was presented (Trofa et al. 2008). We review the literature on *C. parapsilosis*; specific topics discussed include its epidemiology, the molecular epidemiology, clinical perspectives, pathogenesis, and antimicrobial susceptibility and treatment.

### Epidemiology

*C. parapsilosis* is a ubiquitous microorganism in the natural environment. It is not only isolated easily from soil, seawater, and plants, but also can be isolated from mucosal surfaces, skin and nails, where it belongs to the benign commensal flora of humans and mammals (De Bernardis et al. 1999; Kuhn et al. 2004; Sanchez et al. 1993; Weems 1992). The epidemiology of *C. parapsilosis* in the hospital environment is unique among *Candida* species, because it is frequently isolated from physical surfaces. It is a frequent cause of opportunistic infection, associated with high morbidity and mortality rates in hospitalized immuno-compromised patients (Girmenia et al. 1992; Jarvis 1995). Although *C. parapsilosis* is an opportunistic pathogen, the majority of patients who develop disseminated candidiasis due to *C. parapsilosis* are not immunosuppressed in the classical sense (Spellberg & Edwards 2002). Rather, the predominant risk factors for disseminated candidiasis due to *C. parapsilosis*, which are held in common among afflicted patients, are iatrogenic and/or nosocomial factors (Spellberg et al. 2006). This species has been particularly associated with BSI in very low birth weight neonates (Campbell et al. 2000; da Silva et al. 2001; Damjanovic et al. 1993; Huang et al. 1998; Huang et al. 1999; Sarvikivi et al. 2005; Saxen et al. 1995; Solomon et al. 1986; Welbel et al. 1996), but is also seen frequently in patients with catheter-associated candidemia and/or with intravenous hyperalimentation (Cano et al. 2005; Huang et al. 2004; Levin et al. 1998; Levy et al. 1998; Marais et al. 2004).

Although *C. albicans* continues to predominate, several surveillance programs report the emergence of non-*albicans* species worldwide as a cause of BSI (Pfaller & Diekema 2002). The percentage of isolates of non-*albicans* species varies considerably from region to region (Pfaller et al. 2006a; Pfaller et al. 2006b). In a previous study (Wingard 1995), spanning a period from 1952 to 1992, *C. parapsilosis* accounted for only 7% of candidemia in cancer patients. Weems (1992) gave a summary of *C. parapsilosis* fungemia cases described before 1992. From 1962 to 1986, 11 different studies reported between 3% and 27% prevalence of *C. parapsilosis* among Candida fungemia. Since 1990 *C. parapsilosis* showed an increase in incidence and is the second or third most common yeast species isolated from the blood in Asia and Latin American countries (Pfaller et al. 2008a; Pfaller et al. 2000; Sandven 2000), and has been commonly found in Europe as well (Pfaller et al. 1999; Sandven 2000). Pfaller et al. (2002) reported the role of sentinel surveillance studies of candidemia and demonstrated differences among studies done between 1992 and 2001. The overall incidence in six different studies showed a prevalence between 7 and 21 percent of *C. parapsilosis* causing candidal BSI. They further showed that the distribution of *C. parapsilosis* causing BSI in adults was 5–12% while the distribution in neonates was 24–45%. *C. parapsilosis* was most prevalent in patients less than 1 year of age.

Kao et al. (1999) conducted a prospective, active population-based surveillance for candidemia in two United States cities, Atlanta and San Francisco, during 1992 to 1993. *C. parapsilosis* was the second most common *Candida* species, and was recovered from 21% of isolates from different patient populations. *C. parapsilosis* was recovered from 45% of the cases of candidemia in neonates.

The National Epidemiology of Mycosis Survey (NEMIS) performed an 18-month prospective study, in surgical intensive care units (SICUs) and neonatal intensive care units (NICUs), from several centers in the United States (Rangel-Frausto et al. 1999). *C. parapsilosis* was isolated from the blood in 7% of the cases in the SICU, whereas a prevalence of 29% of *C. parapsilosis* was identified in the NICU.

A 10-year study, from 1992 through 2001, recorded the distribution of BSI isolates of different *Candida* species (Pfaller & Diekema 2004). Isolates were collected from 250 medical centers in 32 nations worldwide. *C. parapsilosis* accounted for 13% and was the third most common *Candida* species isolated from BSI. Pfaller et al. (1998a) previously reported *C. parapsilosis* had a higher prevalence in Europe, Canada and Latin America compared to the United States. In 1997 and 1998 the SENTRY Antimicrobial Surveillance Program reported 12 months of BSI surveillance in the United States, Canada and Latin America (Diekema et al. 1999; Pfaller et al. 2000). Of 634 BSI, 8.4% was due to *C.
parapsilosis. There was a difference of frequency of BSI due to C. parapsilosis between countries and difference in years. There was a decline in frequency of C. parapsilosis fungemia in both Canada and Latin America between 1997 (23%; 38% respectively) and 1998 (7%; 19%). An increase in BSI due to C. parapsilosis in the United States was noted from 9% in 1997 to 15% in 1998 (Diekema et al. 1999; Pfaller et al. 2000). The SENTRY Antimicrobial Surveillance Program studied candidemia in 20 European medical centers (13 nations) in 1997 (Pfaller et al. 1999). C. parapsilosis was, with 21%, the second most common Candida species causing BSI in Europe. The greatest prevalence of C. parapsilosis was noted in the Latin American hospitals. Over a 3-year period, from 1995 to 1998, Edmond et al. (1999) reported C. parapsilosis (21%) as the third most common Candida species causing nosocomial BSI, in data obtained from 49 hospitals across the United States (1999).

A decreasing trend in the rate (overall decrease, 10 to 11%) of C. albicans isolation was noted over a 6.5 years period (1997–2003) from the ARTEMIS DISK Global Antifungal Surveillance Study including 121 different institutions (Pfaller et al. 2005c). In contrast, C. parapsilosis showed an increase of 3% frequency of isolation over this time period. From 1997 to 1998, C. parapsilosis accounted for 4%, and in 2003 for 7% (Pfaller et al. 2005c).

Diekema et al. (2002) performed prospective surveillance for candidemia at 16 hospitals in the State of Iowa between 1998 to 2001. C. parapsilosis was identified in 7%, the fourth most common Candida species. However, the percentage of candidemia caused by C. parapsilosis trended higher among infants less than 1 year of age (17%) compared to other age groups (6%) (Diekema et al. 2002).

Between 2001 and 2004 the distribution of Candida species of clinical isolates (blood or sterile-site) were tested among 91 medical centers worldwide (Asia-Pacific, Europe, Latin America, and North America) and C. parapsilosis accounted for 14% (Pfaller et al. 2006b). In another report, during the 2001–2002 study period, a prevalence of 14% was found for C. parapsilosis isolates obtained from 54 different centers worldwide (Pfaller et al. 2005b). Nucci et al. (2001) reported a study of C. parapsilosis and C. albicans candidemia in a tertiary hospital in Brazil. Patients with a candidemia of C. parapsilosis were less ill, more had cancer in complete remission, fewer had antibiotics, were less likely to be hypotensive and showed a lower mortality compared to patients with a candidemia caused by C. albicans (Nucci et al. 2001).

In 2004 and 2005 the Global Surveillance study reported species distribution of Candida species (clinical isolates from blood or sterile site) among 60 medical centers worldwide, and reported a 14% prevalence of C. parapsilosis (Pfaller et al. 2005c). C. parapsilosis and C. tropicalis were predominant in the Asia-Pacific and Latin American regions but was less so in Europe, Canada and the United States.

In the surveillance study performed by the European Confederation of Medical Mycology (ECMM), species distribution of Candida bloodstream infections of 2089 cases were documented by 106 institutions in seven European countries from Sept 1997–Dec 1999 (Tortorano et al. 2006, Tortorano et al. 2004). In a range of patient populations, C. parapsilosis accounted for 13% of the cases of candidemia, with a range of 6–30%, being the third most common Candida species after C. albicans (56%) and C. glabrata (14%) (Tortorano et al. 2004). Variation in prevalence was noted and has been attributed to the different patient populations hospitalized in the different units (Tortorano et al. 2004). In addition, there was variation between countries noted (Tortorano et al. 2006). C. parapsilosis was identified in 15% in cancer patients (Tortorano et al. 2004). This study reported C. parapsilosis the most frequent Candida species identified in premature neonates (29%). In another study, the ECMM performed an epidemiological prospective survey of candidemia in one Italian region, Lombardy, for a 28-month period (September 1997–December 1999) (Tortorano et al. 2002). Fifteen percent of the cases of candidemia were caused by C. parapsilosis, and it was the second most common cause of candidemia, behind C. albicans (59%). C. parapsilosis was isolated with the highest frequency in premature neonates and hematological patients, comprising 44 and 24% of isolates. Another study from the ECMM, in the same time period from several hospitals widely distributed throughout Spain, showed a prevalence of 22% of C. parapsilosis isolates from adults (Peman et al. 2005). In pediatric patients C. parapsilosis was the most prevalent species (50%).

The advent of new medical therapies and procedures to treat cancer, such as steroids, the increase in surgical procedures, widespread use of broad spectrum antibiotics and prolonged hospital stay are all linked to the increase of Candida bloodstream infections (Eggimann et al. 2003; Sofair et al. 2006). The shift in the epidemiology towards C. parapsilosis infections may be especially linked to the increase, which occurred in the past two decades, in incidence of blood stream infections associated with intravascular devices (San Miguel et al. 2005). The majority of the C. parapsilosis infections are due to exogenous acquisition. An important study that recognized this showed that a series of 13 patients with C. parapsilosis fungemia did not have the organism isolated from other sites before the fungemia (Meunier-Carpentier et al. 1981). This is in contrast with fungemia due to other Candida species. Further to this point, there is a significantly increased risk of systemic
disease associated with intravascular catheters, prosthetic devices and parenteral nutrition (Levin et al. 1998; Tortorano et al. 2006; Zancope-Oliveira et al. 2000). *C. parapsilosis* has the ability to produce an extracellular polysaccharide or slime, and this property is believed to aid adherence and biofilm formation on plastic devices (Levin et al. 1998; Ramage et al. 2005). The source of the infection by this yeast species is not always apparent.

In many neonatal intensive care units (NICU), *C. parapsilosis* has emerged as the predominant pathogen causing invasive infection, and significantly increases the morbidity and mortality of severely ill infants who require care in a NICU (Kao et al. 1999; Krcmery et al. 1999; Kuhn et al. 2004; Levy et al. 1998; Roilides et al. 2004; Saiman et al. 2001; Stamos & Rowley 1995). The proportion of infections due to *C. parapsilosis* has increased among neonates. *C. parapsilosis* comprised less than 10% of isolates in the 1980s and early 1990s (Baley & Silverman 1988; Faix 1984; Huttova et al. 1998a). More recently, studies reported proportions of 50–60% of *C. parapsilosis* infections among neonates (Benjamin et al. 2000; Kossoff et al. 1998; Levy et al. 1998; Rodriguez et al. 2006). The proportion of infections due to *C. parapsilosis* has increased among neonates. As neonates mature, the incidence of *C. parapsilosis* decreases (Pfaller & Diekema 2002; Tortorano et al. 2006). Changes in neonatal intensive care practices decreases (Pfaller & Diekema 2002; Tortorano et al. 2006). Changes in neonatal intensive care practices may be related to the emergence of *C. parapsilosis* as an important pathogen in very low birthweight infants (Clerihew et al. 2007). Cases can persist in units for years (Sarvikivi et al. 2005) and the case fatality rate can be high (83%) (Saxen et al. 1995). Cross-transmission between patients, especially among neonates, through the hands of healthcare workers plays an important role in the spread of *C. parapsilosis*, contributing to the high isolation rate of *C. parapsilosis*. *C. parapsilosis* is a frequent fungal colonizer of the subungual space of healthy volunteers (Abi-Said et al. 1997; McGinley et al. 1988; Weems 1992). Several reported outbreaks have been caused via direct interaction between healthcare workers and the newborn, through the placement or manipulation of catheters, the site through which *C. parapsilosis* entered the bloodstream, as well as liquid glycerin suppositories and topical ointments (Barchiesi et al. 2004; Campbell et al. 2000; Huang et al. 1999; Kuhn et al. 2004; van Asbeck et al. 2007; Welbel et al. 1996). Hypoxemia, bradycardia, respiratory distress requiring incubation and dissemination to secondary sites are less common with *C. parapsilosis* fungemia in newborns, compared to *C. albicans* fungemia (Huang et al. 2000).

Other outbreaks have been reported, such as outbreaks of *C. parapsilosis* caused by contamination of hyperalimentation solutions, intravascular pressure monitoring devices, ophthalmic irrigating solutions and glucose-containing solutions (Clark et al. 2004; Deresinski et al. 1995; Levy et al. 1998; Postero et al. 2004; Solomon et al. 1986; Solomon et al. 1984; Weems et al. 1987).

With the use of azoles there has been an epidemiological shift of hematogenous candidiasis to those caused by non-albicans *Candida* species, especially *C. krusei* and *C. glabrata* (Abi-Said et al. 1997; Kao et al. 1999). The rise of *C. parapsilosis* correlates with increased use of caspofungin and voriconazole (Forrest 2008). Girmenia et al. (1996) showed an overall decrease of isolation of *C. albicans* with a concomitant increase in isolation of *C. parapsilosis* among adult patients with cancer. This indicates that even among older patients the incidence of *C. parapsilosis* may be on the rise. The crude mortality rate associated with *C. parapsilosis* infection has been estimated to be 30%, lower than that reported for invasive infection with *C. albicans* (79%) or other non-*albicans Candida* spp. (mean 78%) (Horn et al. 1985; Nucci et al. 2001; Pappas et al. 2003).

**Molecular epidemiology**

Biochemical methods have traditionally been relied on to identify *C. parapsilosis* in the clinical microbiology laboratory, although occasionally these can lead to misidentification (Fenn et al. 1994; Heelan et al. 1998; Pfaller et al. 2008a; Ramani et al. 1998; Wadlin et al. 1999). Due to problems in the traditional classification of yeast within the genus *Candida*, molecular techniques are needed to classify and identify medically important yeast species. This will help us to understand the evolutionary relationships between these organisms. The application of molecular procedures has already resulted in major reclassification of many species. Comparative studies with chromosomal DNA have been very helpful to differentiate *Candida* species from each other and also to delineate different strains within species. The ideal method for differentiating *C. parapsilosis* at a subspecies level should be able to discriminate between strains that are epidemiologically unrelated, should be highly reproducible both intra- and inter-laboratory, should take minimal time, should be capable of processing a large number of strains, should require a minimum of specialized equipment, and be relatively inexpensive (McCullough et al. 1996). It is unclear whether the emergence of pathogenic isolates of *C. parapsilosis* in varying clinical institutions is due to the genetic diversity and the virulence of some strains, or to widespread favorable environmental conditions persisting in compromised hosts (Dassanayake & Samaranayake 2000). Detailing the genotypic characteristics are not only important for a more appropriate taxonomy, it could also greatly contribute to our understanding of the epidemiology and
the pathogenic mechanisms of this emerging pathogen (Weems 1992). Hospital outbreaks have been associated with specific strains of C. parapsilosis by use of the various methods we will discuss (Shin et al. 2001; Barchiesi et al. 2004; Clark et al. 2004; Garcia San Miguel et al. 2004; Marais et al. 2004). Molecular typing also has the utility of discerning “pseudo-outbreaks,” contaminated processing materials leading to false conclusion of a case cluster (Deresinski et al. 1995; Schar et al. 1990).

Genetic analysis of C. parapsilosis has been hindered by the lack of characterized sexual cycle (Logue et al. 2005), ploidy (aneuploid and/or diploid) of the organism (Fundyga et al. 2004) and the unavailability of appropriate molecular genetic tools (Nosek et al. 2002a). Various DNA typing method studies report that C. parapsilosis can be divided into three groups based on isoenzyme analysis, internally transcribed spacer (ITS) sequences of DNA within the ribosomal cassette, RAPD profiles, RFLP typing, electrophoretic karyotype patterns, multilocus enzyme electrophoresis, morphotyping, multilocus sequence typing (MLST), and DNA relatedness (Lehmann et al. 1992; Lin et al. 1995; Lott et al. 1993; Roy & Meyer 1998; Scherer & Stevens 1987; Tavanti et al. 2005). Although division into three groups can be achieved, when subtyping within the three groups is performed, it was noted that a large number of the isolates are indistinguishable.

Scherer and Stevens (1987) described a useful method for the extraction of DNA from the yeast of Candida spp., followed by digestion and restriction endonucleases and electrophoresis of DNA fragments. Seven C. parapsilosis isolates typed in this manner could be placed in 3 major DNA groups with, initially, 5 subtypes. As more isolates were studied, greater genotypic diversity became apparent, for example, Deresinski et al. (1995) found a unique RFLP subtype, VII-3, identified in a common source outbreak, and a recent study done by van Asbeck et al. (2008a) examined the molecular epidemiology of the global and temporal diversity of C. parapsilosis. In this study 15 new subtypes were observed, with one dominant subtype, VII-1 (82% of 536 C. parapsilosis isolates). Dividing the isolates into VII-1 versus non-VII-1 showed temporal variation for the USA pre-1995 versus post-1995 (p < 0.0001) and versus Europe pre-1995 (p < 0.0001). Genotype distribution differed among localities (p < 0.0001); Mexico was unique (p = 0.05) due to the high proportion of non-VII-1. The prevalence of C. parapsilosis RFLP type VII-1 apparently has risen in the USA and current isolates show some variation in distribution of types in some non-USA locales compared to the USA. There were no differences in distribution of types comparing babies versus adults, or blood stream isolates versus colonizing or environmental isolates. In contrast to the conserved restriction fragment profiles in strains from ITS groups I and III, a polymorphism within group II was observed (van Asbeck et al. 2008a).

Roy and Meyer (1998) studied the DNA relatedness and RFLP patterns of whole-cell DNA and the general properties of 14 C. parapsilosis isolates and observed unrelatedness of the three previously described groups at the species level. The low degree of the DNA relatedness, less than 25% DNA identity, among the three different DNA groups of C. parapsilosis suggested that they represent distinct species. Various studies supported this idea of possible separation of C. parapsilosis isolates (Cassone et al. 1995; De Bernardis et al. 1999; Kurtzman & Robnett 1998; Lin et al. 1995; Pontieri et al. 2001). The internally transcribed spacer (ITS) regions flanking the 5.8S rDNA in yeasts have greater heterogeneity than the 5.8S region itself. Using this observation, for example, Lin et al. (1995) concluded there were 3 different genotypes in C. parapsilosis based on sequence analysis.

Based on their ITS (mostly ITS1) region sequences, Tavanti et al. (2005) proposed to recognize Candida orthopsilosis and Candida metapsilosis as species separate from C. parapsilosis. Thus far, these sibling species are phenotypically indistinguishable from C. parapsilosis sensu stricto. DNA sequence analysis showed conformity of C. parapsilosis with previous group I, C. orthopsilosis with group II and C. metapsilosis with group III (Lin et al. 1995; Roy & Meyer 1998; Tavanti et al. 2005). Based on sequence analysis of four gene fragments, nine C. orthopsilosis isolates showed nucleotide sequence diversity. This suggests great heterogeneity among the C. orthopsilosis isolates, compared to the mainly clonal C. parapsilosis. Lack of diversity among group I isolates had been suggested by karyotyping (Carruba et al. 1991; Lott et al. 1993; Shin et al. 2001). The sequence homogeneity in group I isolates suggests they emerged more recently than the other types. Some hospital outbreaks have been related specifically to group II (Lin et al. 1995; Zancupe-Oliveira et al. 2000), although most would appear to be related to group I.

Our laboratory compared two predominant typing methods, RFLP and MLST, and reported C. parapsilosis sensu stricto appear to be nearly identical to the predominant RFLP subtype VII-1, and cannot be subtyped by RFLP and MLST (van Asbeck et al. in press). However, C. orthopsilosis can be subtyped by both MLST and RFLP (van Asbeck et al. in press).

Tavanti et al. (2007) used amplification fragment length polymorphism (AFLP) to identify C. orthopsilosis at the species level and efficiently delineate intra-specific genetic relatedness because of the high percentage of polymorphic bands resulting from the AFLP. Clonal reproduction and recombination both contribute to C. orthopsilosis genetic population structure. This
study also reported the clinical relevance of *C. orthopsilosis*. Screening a large collection of *C. parapsilosis*, 5% of the infections/colonizations were identified as *C. orthopsilosis*.

Recently, Gomez-Lopez et al. (2008) describe the prevalence of the two newly described species *C. metapsilosis* and *C. orthopsilosis* (described below (Tavanti et al. 2005)) causing bloodstream infections in Spain. The prevalence of these species appeared to be important, representing the fifth and sixth most common species (1.7% and 1.4% for *C. metapsilosis* and *C. orthopsilosis* respectively) (Gomez-Lopez et al. 2008). This study also provided evidence that these species may behave as human pathogens. A more recent paper also studied geographical distribution of the sister species (Lockhart et al. 2008a). *C. orthopsilosis* comprised 6.1% and *C. metapsilosis* 1.8% of 1,929 isolates. *C. orthopsilosis* was most common in South America. Somewhat in contrast to our observations (van Asbeck et al. 2008a), *C. orthopsilosis* appeared to have increased in recent years. Mexico had a high frequency of *C. orthopsilosis*, likely a reflection of the same trends as our increase of non-VII-1 types in that country (van Asbeck et al. 2008a).

Lockhart et al. (2008b) reported that an apparently closely related species, *Lodderomyces elongisporus*, is mislabelled by routine clinical laboratory methods as *C. parapsilosis*. Once erroneously considered to be the teleomorph of *C. parapsilosis* (van der Walt 1966), *L. elongisporus* can be distinguished phenotypically from *C. parapsilosis* as they produce different colored colonies on ChromAgar medium. On cornmeal agar, *L. elongisporus* forms short pseudohyphae indistinguishable from *C. parapsilosis*. On sporulation medium, only *L. elongisporus* forms ascospores. The species differentiation is supported by DNA/DNA homology and sequencing of large-subunit rRNA genes. In their large population studied (Lockhart et al. 2008b), *L. elongisporus* could be differentiated as 10/542 isolates from a collection ostensibly all *C. parapsilosis*; all of the former were blood stream isolates.

Camougrand et al. (1988) used restriction analysis of mitochondrial DNA of *C. parapsilosis* reference strains and showed great differences between them. By probing the restriction patterns with *S. cerevisiae* mitochondrial DNA fragments, further differentiation could be made. Nosek et al. (2004) determined the complete mitochondrial DNA (mtDNA) sequence of *C. parapsilosis*, represented by linear DNA molecules terminating with arrays of tandem repeats of a 738 bp unit, varied in number and size, termed mitochondrial telomeres. Recently, Kosa et al. (2006) studied the complete sequence of mitochondrial DNA of the sibling species and observed *C. metapsilosis* likely diverged from a common ancestor prior to the split of *C. orthopsilosis* and *C. parapsilosis sensu stricto*. All three sibling species genomes are highly compact and encode the same set of genes arranged in an identical order. However the size of *C. orthopsilosis* and *C. metapsilosis* mtDNA represents about two-thirds that of the *C. parapsilosis sensu stricto*. Earlier, Rycovska et al. (2004) assessed the polymorphism at the level of mtDNA and indicated that group II isolates all have a circular mtDNA, whereas the mtDNA in group I and III is mostly linear. Kosa et al. (2006) showed *C. orthopsilosis* and *C. metapsilosis* differ in the molecular form of the mitochondrial genome, circular—versus linear—mapping, whereas *C. parapsilosis sensu stricto* possesses a linear mitochondrial genome.

Mitochondrial telomeres are unique among *C. parapsilosis* compared to related species (Nosek et al. 2002b). Thus, mtDNA enables us to discriminate between the groups of *C. parapsilosis* and it represents a powerful tool to distinguish species.

Similarly, Iida et al. (2005) found that most of the *C. parapsilosis* strains from Japan and Brazil tested for ITS sequences belonged to one of the three genetically distinct groups (I, II, and III). However, they found that 5 strains showed differences in ITS sequence from those already reported, and based on this, suggested the presence of a new major DNA group (IV) among the genetic groups within the *C. parapsilosis* family.

Dominance of these *C. parapsilosis* group I isolates, and its geographic spread, might be due to the clonal mode of reproduction, shown by the low nucleotide variability observed within the species (Fundyga et al. 2004; Tavanti et al. 2005; Tavanti et al. 2007).

A new DNA probe, Cp3-13 for DNA fingerprinting, has been described, which not only discriminated differences between strains, but also identified changes in a clonal population after a few hundred generations in vitro or changes that have occurred in vivo in infected persons (Clark et al. 2004; Enger et al. 2001; Sofair et al. 2006). Recently, a microsatellite method, with high discrimination and reproducibility, has been reported that further differentiates group I *C. parapsilosis* isolates with high discriminatory power (Lasker et al. 2006). Microsatellites are short 2- to 10 bp multiple tandem repeats, and have a high mutation rate. This method makes it possible to detect microevolutionary variations of isolates obtained from different body sites, and may facilitate detection of outbreaks.

Kosa et al. (2007) constructed a collection of replicative shuttle vectors, a system for genetic transformation of the yeast *C. parapsilosis*. These vectors, which allow expression of the cloned genes, intracellular localization of protein products or monitoring of a promoter activity, may contribute to the investigation of diverse biological features related to *C. parapsilosis* (2007).

A sequencing project of *C. parapsilosis* nuclear genome would be important for better understanding
the biology of this pathogenic yeast species at the molecular level. Understanding the genetic diversity of the yeast requires further study and may lead to insight in the pathogenesis of fungal infections, and ultimately may make it possible to define proper preventive measures (Roy & Meyer 1998).

Clinical aspects

C. parapsilosis is an important opportunistic pathogen that causes infections ranging from thrush to invasive disease such as fungemia, endocarditis, endophthalmitis, arthritis, and peritonitis, all of which usually occur in association with invasive procedures or prosthetic devices. We will describe only the most frequent infections caused by C. parapsilosis.

Superficial Infections

Frequent isolation of C. parapsilosis from pathological lesions of nails and skin, with a distal subungual type of onychomycosis often occurs with this organism (De Bernardis et al. 1999; Figueiredo et al. 2007; Mujica et al. 2004; Pfaller et al. 1995; Segal et al. 2000; Strausbaugh et al. 1994). Risk factors significantly associated with colonization in patients are prolonged antibiotic therapy, parenteral nutrition, duration of stay in the hospital, surgery, indwelling devices, diabetes, obesity, malignancy, elderly, and neonates of very low birth weight (Bendel 2003; Dorko et al. 2005; Figueiredo et al. 2007; Jautova et al. 2001).

C. parapsilosis is second to C. albicans as the most common Candida causing onychomycosis (Brilhante et al. 2005; Dorko et al. 2002a; Khoatrvi & Mansouri 2001). However, some studies observed an even higher prevalence of onychomycosis caused by C. parapsilosis. Segal et al. (2000) reported a high prevalence of C. parapsilosis among onychomycosis patients obtained from two different centers in Israel. C. parapsilosis was the predominant Candida species, recovered from both fingernail and toenail infections (Segal et al. 2000). Figueiredo et al. (2007) reported 41% of the 200 samples recovered from fingernails of Candida species were C. parapsilosis, which is in agreement with Rodrigues-Soto et al. (1993), who identified C. parapsilosis as the prevalent species in onychomycosis involving the fingernails. In addition, Oliveira et al. (2006) reported C. parapsilosis as the Candida species most frequently isolated from onychomycosis lesions. Recently, in a study among Algerian military personnel, C. parapsilosis was the predominant yeast species causing both superficial fungal infection and onychomycosis of the foot (Djeridane et al. 2006; Djeridane et al. 2007). In addition, C. parapsilosis was the most common yeast causing onychomycosis in Malta (Vella Zahra et al. 2003). One case of onychomycosis caused by C. parapsilosis, involving both the toenail and the fingernail, in a 35-day-old patient has been reported (Koklu et al., 2007). With all positive cultures from nails, supporting histopathological evidence of infection is needed to distinguish infection from colonization. Folliculitis due to C. parapsilosis has been described by Li et al. (1988).

C. parapsilosis has been associated with disease of the external or middle ear (Dorko et al. 2004; Garcia-Martos et al. 1993; Vennewald et al. 2003). The risks appear to be ocean swimming, trauma and prior antibacterial therapy.

Mucosal

The primary mucosal infection due to C. parapsilosis is vaginal. In the majority of women with Candidal vaginitis it is due to C. albicans, which causes 80% to 92% of all cases of Candida vaginitis worldwide (Singh et al. 1972; Trama et al. 2005). In immunodeficient women, vaginal colonization by non-albicans species is more frequent (Trama et al. 2005). Weems (1992) concluded that vaginitis caused by C. parapsilosis is infrequent. However, an increase in frequency of vaginitis caused by C. parapsilosis has been observed among healthy women (Nyirjesy et al. 2005; Trama et al. 2005). C. parapsilosis can be responsible for vulvovaginal symptoms, such as itching, burning, dyspareunia and vaginal discharge, but can also appear asymptotically (Nyirjesy et al. 2005). The change of species distribution, non-albicans species such as C. glabrata, C. parapsilosis, and C. tropicalis becoming more frequent, can be related to factors that might change the vaginal environment and flora (Trama et al. 2005). These factors could be increasing use of azole drugs, an aging population, increased exogenous use of hormones to manage menopause, and/or more medical and hospital contact in the patients sampled (Trama et al. 2005). Cassone et al. (1995) showed that ~10% of vaginal candidiasis is caused by C. parapsilosis. Nyirjesy et al. (2005) reported 9% of vaginitis cases were caused by C. parapsilosis. C. parapsilosis, like C. albicans, produces acid proteinases, enzymes which are associated with vaginopathic potential (Agatensi et al. 1991; Nyirjesy et al. 2005). Secretion of aspartyl proteinases is elevated in vaginitis caused by these vaginopathic strains. Women suffering multiple episodes of vulvovaginitis, and with theoretically compromised integrity of the vaginal mucosa, may be more susceptible to infection with C. parapsilosis because of the acid proteinase activity of the organism (Nyirjesy et al. 2005). Vaginitis caused by this pathogen seems to respond to a variety of antifungals (Nyirjesy et al. 2005).

Unlike other Candida species, catheter-related fungemia caused by C. parapsilosis has a higher rate of spontaneous clearance and a much lower rate of establishment at secondary sites (Levy et al. 1998; Weems 1992).

Breakthrough fungemias during antifungal treatment appear especially common in cancer patients and are associated with an infected foreign body, such as catheters, and insufficient antifungal treatment (Safdar et al. 2002). Krcmery et al. (1999) reported breakthrough fungemia among neonates during fluconazole therapy and prophylaxis. The presentation of C. parapsilosis fungemia in neonates can be subtle including thrombocytopenia and leucopenia (Huang et al. 1999). C. parapsilosis fungemia has a lower mortality than bloodstream infections due to other Candida species.

Endocarditis

As a cause of endocarditis, C. parapsilosis has demonstrated a tendency to persist, even despite suppressive therapy, and recrudescence if suppression is discontinued. This can be misleading, in that patients with C. parapsilosis endocarditis are reported in the literature while still receiving suppressive therapy (and sometimes with short follow-up post-surgery, and without attempts to evaluate fungemia), resulting in a false impression of cure (Galgiani & Stevens 1977). Serologic follow-up during and after therapy may be useful (Galgiani & Stevens 1977). Weems (1992) described some patients with endocarditis caused by C. parapsilosis before 1992. In the past two decades fungal endocarditis has increased in incidence, carrying with it a high risk of mortality (Garzoni et al. 2007; Lopez-Ciudad et al. 2006); this is one of the most serious manifestations of candidiasis. A long duration of illness has been reported in patients suffering non-albicans endocarditis, so prolonged treatment is required to avoid grave consequences (Khan et al. 2007).

Clusters of cases involving prosthetic valves have been reported (Diekema et al. 1997). Garzoni et al. (2007) described intraoperative contamination (e.g. cardiac bypass equipment, tears in surgical gloves) to be the most common source of infection in outbreaks of C. parapsilosis infective endocarditis (Diekema et al. 1997; Johnston et al. 1994). The most prevalent predisposing factors for fungal endocarditis caused by C. parapsilosis are prosthetic valves, then, in this order, intravenous drug use, parenteral nutrition, antibiotic therapy, preexisting valvular heart disease, prior episode of endocarditis and reconstructive cardiovascular surgery (Brandstetter & Brause 1980; Garzoni et al. 2007; Khan et al. 2007; Rubinstein et al. 1975; San Miguel et al. 2006; Tonomo et al. 2004; Weems 1992). Abdominal surgery and immunosuppression have also been described as risk factors. The aortic valve is the first, and mitral valve the second, most commonly involved valve in infection of native valves (Garzoni et al. 2007; Weems 1992), with intravenous drug use the most common predisposing factor (Garzoni et al. 2007). The overall mortality of these patients was high (42%). Among those treated with conventional antifungals and adjunctive surgery, mortality was lower (Johnston et al. 1994). Peripheral embolic and hemorrhagic events are the most common complications (44%) of C. parapsilosis endocarditis (Garzoni et al. 2007).

Peritonitis

Fungal peritonitis is an uncommon but potentially life-threatening complication of continuous ambulatory peritoneal dialysis (CAPD) (Amici et al. 1994; Bren 1998; Chen et al. 2004; Chen et al. 2006; Johnson et al. 1985; Kaitwatcharachai 2002). C. parapsilosis has become the most prevalent pathogen of fungal peritonitis (Chen et al. 2006). This organism, which is a common skin and subungal colonizer, has the ability, in high glucose concentrations, to adhere easily to prosthetic material by extensive biofilm formation on the surface of the plastic catheter. The high glucose content of dialysate, and catheter implantation in peritoneal dialysis patients causes this high prevalence of fungal peritonitis caused by C. parapsilosis (Chen et al. 2006). The mechanism of C. parapsilosis infections is considered to be transmission...
by skin colonization of *C. parapsilosis* via the catheter lumen (Kaitwatcharachai 2002).

An earlier study of Manzano-Gayosso et al. (2003) reported *C. albicans* and *C. parapsilosis* as the most common species among 165 patients with peritonitis receiving CAPD treatment. Other studies showed *C. parapsilosis* the most prevalent pathogen of fungal peritonitis in patients receiving peritoneal dialysis (Chen et al. 2006; Wong et al. 2000). Recently, Chen et al. (2006) reported a percentage of 86% (19 cases), of fungal peritonitis caused by *Candida* species, of these, 41% (9 cases) were *C. parapsilosis*. Fifty percent of these patients developed severe complications, with abscess formation and persistent peritonitis after catheter removal (Chen et al. 2006).

Wang et al. (2000) showed that the presence of abdominal pain, antibiotic use within 3 months before fungal peritonitis, bowel obstruction and a catheter kept in situ seems to be associated with the development of fungal peritonitis and necessitate CAPD discontinuation (removal of the catheter) (Kaitwatcharachai 2002; Wong et al. 2000). Kaitwatcharachi (2002) showed that either abdominal pain or antibiotic use in the previous three months were risk factors for fungal peritonitis prior to CAPD discontinuation. Prior gram negative bacterial peritonitis is also a risk factor for development of fungal peritonitis (Kaitwatcharachi 2002). Whereas there is no consensus whether or when peritoneal catheters should be removed, rapid catheter removal has been recommended in *C. parapsilosis* peritonitis in CAPD patients (Bren 1998; Kaitwatcharachi 2002). Chen et al. (2006) showed that patients with *C. parapsilosis* peritonitis developed more complications and have a worse prognosis than those infected with other *Candida* species in peritoneal dialysis-associated fungal peritonitis. Because of different severity and prognosis, *C. parapsilosis* peritonitis in peritoneal dialysis patients should be treated more aggressively than other *Candida* species (Chen et al. 2006).

### Endophthalmitis

*C. parapsilosis* has been known as a cause of epidemic of post-cataract endophthalmitis caused by intrinsically contaminated, intraocular lens-irrigating solution (McCray et al. 1986; O’Day 1985; O’Day et al. 1987; Stern et al. 1985). In one study 4% of patients exposed to the contaminated solution developed *C. parapsilosis* endophthalmitis (McCray et al. 1986). Although other sporadic postoperative intraocular infections with *C. parapsilosis* have been reported, hematogenous endophthalmitis appears to be rare (Feman et al. 2002; Weems 1992). Recently, Marangon et al. (2004) reported *Candida* species as the most common cause of endogenous endophthalmitis in their institution, with *C. albicans* the most frequent subspecies (30%). *C. parapsilosis* accounted for 21% of all *Candida* endophthalmitis infections.

A few cases of keratitis have been reported, with risk factors such as corticosteroid use, corneal transplantation and laser in situ keratomileusis (LASIK) (Bourcier et al. 2003; Muallem et al. 2003; Solomon et al. 2004). Since treatment, such as surgery and antifungal therapy, can save vision, evidence of intraocular infection should be recognized as early as possible (Feman et al. 2002).

### Joint diseases

*Candida* arthritis is most commonly seen among intravenous drug users (Vasquez et al. 2002). Nosocomial *C. parapsilosis* arthritis has been reported in only a few cases, involving the knee, shoulder and wrist (De Clerck et al. 1988; Diekema et al. 1997; Legout et al. 2006; Salo et al. 1990; Smith et al. 1987; Vasquez et al. 2002; Yarchoan et al. 1979) Arthritis caused by *C. parapsilosis* is associated with joint prostheses, probably due to the increased ability of this micro-organism to adhere to plastic (Brooks & Pupparo 1998; Cushing & Fulgenzi 1997; Hennessy 1996; Wada et al. 1998). Arthritis, produced by *C. parapsilosis*, can result through direct inoculation of the fungus at arthrocentesis and is sporadic in older individuals (Cuende et al. 1993). Risk factors for these sporadic incidents include diabetes mellitus, intravenous drug use, and immunosuppression (Cuende et al. 1993).

### Panreatitis

There are a growing number of reports of pancreatic infections due to *Candida* species, most commonly *C. albicans*, mainly with previous abdominal manipulation, such as previous pancreatic drainage procedures (percutaneous or surgical) (Robbins et al. 1996). Fungal pancreatitis due to non-*albicans* species has increased in incidence and is attributed to several factors, such as the use of broad-spectrum antibiotics, parenteral nutrition as well as the increase in use of immunosuppressive agents (Robbins et al. 1996). Only a few cases of pancreatic infections (infected pancreatic necrosis and pancreatic abscesses) due to *C. parapsilosis* have been described (Ibanez & Serrano-Heranz 1999; Kull et al. 1999; Gautret et al. 1998).

### Meningitis

Up to 1992, in the review article by Weems (1992) one case of meningitis caused by *C. parapsilosis* was reported (Faix 1983). Several cases of meningitis caused by this organism have since been described, especially among neonates (Dorko et al. 2002b; Huttova et al. 1998b).
Surgery, antibiotic treatment, parenteral nutrition, skin folliculitis, fungemia and the use of intravascular and intraventricular catheters are potential risk factors (Huttova et al. 1998b; Jimenez-Mejias et al. 1993; Weems 1992).

**Pathogenesis**

Although very little on this subject has been studied, in this section we will discuss what is currently known about virulence factors of *C. parapsilosis* and host defense against this organism.

**Virulence**

*C. parapsilosis* accounts for a significant proportion of nosocomial infections, with an increasing prevalence. As with other *Candida* species, invasion of *C. parapsilosis* can result in severe disease, particularly in hosts with a suppressed immune system. For the development of more effective containment measures it is necessary to understand the mechanisms that underlie pathogenicity. The lack of knowledge of virulence factors that play a role in the pathogenicity of *C. parapsilosis* may in part be due to the exclusive use of type-collection strains in most experiments (Cassone et al. 1995). The paucity of data on clinical isolates needs to be corrected.

We suspect that the lower virulence of *C. parapsilosis* compared to *C. albicans* and some other non-*albicans Candida* species is a reason for the lower mortality and morbidity rates observed in adults and neonates (Faix 1992; Sullivan et al. 1995; Weems 1992). Comparing *C. parapsilosis* with *C. albicans* has made it possible to define some putative virulence factors. These virulence factors, present in both species, include adherence to epithelial and endothelial cells, proteinase production (Cassone et al. 1987; Ross et al. 1990), pseudohypha formation (Sobel et al. 1984), phospholipase production (Ibrahim et al. 1995) and phenotypic switching (Soll 1992).

The lower virulence of *C. parapsilosis* compared to *C. albicans* can also be attributed to the lack of formation of hyphae. Most *Candida* species, including *C. parapsilosis* (Laffey & Butler 2005) exist as spherical to ovoid blastospores or yeast cells. Many *Candida* species are capable, to various degrees, of producing chains of elongated blastospores termed pseudohyphae, both in vivo, and under certain conditions, in vitro. The hyphal forms are more difficult to ingest, and the cell walls may be more resistant to digestion. In addition, hyphal forms have been defined as more adhesive than pseudohyphae (Hostetter 1994).

The ability of a micro-organism to adhere to mucosal surfaces is the critical first step for successful colonization and subsequent infection of host tissues by a potentially pathogenic *Candida* spp. *C. parapsilosis*, which is thought to be acquired from exogenous sources, adheres to indwelling devices, and after adherence invades the host (Kuhn et al. 2004). *C. albicans* and other non- *albicans Candida* species adhere to a greater extent to mucosal tissue than does *C. parapsilosis* (Klotz et al. 1983, Wingard 1995). A decreased adherence to epithelial cells by *C. parapsilosis* compared to *C. albicans* and *C. tropicalis* has been observed (Krcmery & Barnes 2002, Weems 1992) and the reduced pathogenic potential of *C. parapsilosis* related to its adhesive properties (Branchini et al. 1994). However, Panagoda et al. (2001) observed adhesion by *C. parapsilosis* skin isolates to human buccal epithelial cells noted among certain of the *C. parapsilosis* isolates used in this study might be attributed to variation in strains and culture conditions, or aggregation among yeast cells, but implies the potential for colonization of mucosal surfaces, possibly equal to that of *C. albicans* (Panagoda et al. 2001).

De Bernardis et al. (1999) found isolates from blood and skin adhered about equally to plastic, but that greater adhesion to plastic was found among isolates showing a fringed colony morphology versus a nonfringed morphology. In addition, these authors reported higher secreted aspartic protease (Sap) production among isolates from skin, which were better able to cause experimental rat vaginitis than were low Sap-producing fungemia isolates. However, the skin isolates were less virulent than were the blood isolates in a model of systemic infection in neutropenic mice (De Bernardis et al. 1999). These data indicate that different biotypes may thus differ in virulence. Sap enzymes are considered a putative virulence factor of *C. albicans* (Gokce et al. 2007; Naglik et al. 2004). They may have a role in disrupting mucosal surfaces or destroying host defense proteins. These enzymes also have been strongly associated in superficial, but not with systemic invasion, caused by *C. parapsilosis* (Cassone et al. 1995; De Bernardis et al. 1999; Gokce et al. 2007). However, Dagdeviren et al. (2005) observed a higher production of acid proteinase among blood isolates compared to non-blood isolates. A proteinase inhibitor can reduce tissue damage due to this species (Gacser et al. 2007b).

Although *C. parapsilosis* possesses fewer Sap enzymes than *C. albicans* (De Bernardis et al. 1999), Lin et al. (1995) reported differences in proteinase activity within the major DNA groups of *C. parapsilosis*. Group I showed strong to moderately strong proteinase production, whereas group II and III isolates had low proteinase activity.
One potential virulence factor for *C. parapsilosis* includes slime production. This is of special importance for adhesion to foreign body material and in formation of biofilms (microbial communities that are associated with solid surfaces such as intravascular catheters) (Nett et al. 2007), which results in the increase of catheter-related candidemia and antifungal resistance related to catheter insertion (De Bernardis et al. 1999; Hawser & Douglas 1994). The molecular mechanisms that regulate biofilm development in *C. parapsilosis* is not yet understood (Girimenia et al. 1996; Laffey & Butler 2005; Levy et al. 1998; Viscoli et al. 1999), although quorum-sensing mediated through farnesol has been implicated (Laffey & Butler 2005).

Song et al. (2005) also observed differences in biofilm formation among the three groups of *C. parapsilosis*. They only observed biofilm production among the group I *C. parapsilosis* isolates, the group preferentially associated with bloodstream isolates and those from healthcare workers. Kuhn et al. (Kuhn et al. 2004) also indicated less biofilm production among non-group I strains, and reported a greater biofilm production by outbreak isolates compared to sporadic isolates. Recently, Melo et al. (2007) demonstrated biofilm production in all three groups. Tavanti et al. (2007) reported no biofilm production among *C. orthopsilosis* (former group II *C. parapsilosis*) and 95% of the *C. metapsilosis* (formerly group III *C. parapsilosis*) were unable to produce biofilm. Differences in the results of the three studies are likely due to differences in methods of inducing biofilm production and the quantification methods (Melo et al. 2007). The failure to produce extracellular matrix could contribute to the lower frequency of group II and III (*C. orthopsilosis* and *C. metapsilosis*) in the clinical setting (Tavanti et al. 2007). Parenteral nutrition and high glucose environments seems to play a role promoting the development of biofilm production (Branchini et al. 1994; Kuhn et al. 2004). Recently, Ruzicka et al. (2007) described a discrepancy of biofilm production between *C. parapsilosis* blood isolates and *C. parapsilosis* isolated from the skin. Biofilm production was found among 59% of the bloodstream isolates and just 39% in those from the skin. Studies also reported that biofilms can also reduce susceptibility to antifungal agents (Ruzicka et al. 2007; Shin et al. 2002).

Although phenotypic switching was largely studied in *C. albicans*, recently it was determined that phenotypic switching does occur in *C. parapsilosis* (Enger et al. 2001; Lott et al. 1993). This specific phenotypic instability allows strains to switch colony phenotype without affecting the identifiable genotype (Fidel et al. 1999; Soll 1992). Laffey & Butler (2005) found a correlation in phenotypic switching and biofilm formation. The concentric phenotype generates up to twofold more biofilm than crepe or crater phenotypes. Of the four phenotypes identified, smooth phenotypes produce the least biofilm. The molecular mechanisms that regulate phenotypic switching in *C. parapsilosis* are not yet understood (Laffey & Butler 2005).

Another enzyme that seems to play an important role in the pathogenesis of *C. parapsilosis* is phospholipase. This group of enzymes affect adhesion to and penetration of host cells. Phospholipase activity was detected in 51% of *C. parapsilosis* strains in a study done by Ghannoum et al. (2000). Dagdeviren et al. (2005) described phospholipase activity among 26% of the blood isolates. As with *C. albicans*, *C. parapsilosis* isolated from blood cultures were found to be stronger phospholipase producers than isolates from other body sites (Ibrahim et al. 1995). One study indicated no statistical difference between phospholipase production and adherence, possibly due to the small sample size (Dagdeviren et al. 2005).

The enzyme lipase seems to be another important virulence factor. These enzymes affect adhesion and may deny the host nutrients. Gacser et al. (2007a) showed that lipase inhibitors significantly reduce tissue damage during infection of reconstituted human tissues. Recently, Gacser et al. (2007b) studied targeted gene deletion on the lipase genes *CpLIP1* and *CpLIP2*. Their data support the idea that lipase, secreted by *C. parapsilosis*, is involved in disease pathogenesis. Extracellular lipase proteins are important for optimal growth of *C. parapsilosis* in lipid solutions, such as lipid-rich total parenteral nutrition, frequently used for very low birth weight neonates. Another finding of the study was the correlation between biofilm formation and lipase. Biofilm formation of the *C. parapsilosis* lipase-negative mutant was decreased dramatically (Gacser et al. 2007b). This enzyme seems to be involved in survival of *C. parapsilosis* after phagocytosis by macrophages. Lipase-negative mutants were more readily phagocytosed and killed by macrophages, compared to the parental strain and reconstituted mutants (mutants of *C. parapsilosis* showing lipase activity). The lipase-negative strain of *C. parapsilosis* was tested in a murine intraperitoneal infection model and showed less virulence compared to the parental and reconstituted strains (Gacser et al. 2007b).

Cassone et al. (1995) reported that *C. parapsilosis* is markedly heterogeneous in experimental pathogenicity and made a ranking of biotype. They assessed the various differential characteristics of colony morphology (morphotype), resistance to various chemicals (resistotype), and correlated these with virulence in a systemic murine infection model. These authors were able to place isolates into groups on the basis of these traits, although virulence in murine models showed a wide range within a group. In addition, these authors found no correlation of virulence in systemic infection with Sap production (Cassone et al. 1995).
Gacser et al. (2007a) investigated pathogenicity of different *C. parapsilosis* strains in vitro using reconstituted human epidermal and oral tissues. Reconstituted epidermal human tissues are extremely useful for modeling host interactions with *C. parapsilosis* and studying virulence factors of this organism (Gacser et al. 2007a). Their results showed that *C. orthopsilosis* caused damage similar to *C. parapsilosis*, while *C. metapsilosis* was less virulent.

Earlier studies on pathogenicity may have been confusing because *C. parapsilosis* is a group of three sibling species, as we now know. Thus comparisons of traits may have been across species lines rather than within a single species. Since we are now able to distinguish these 3 species, it will be important for future studies first to identify the organism to species level, so that the appropriate comparisons can be made. Host-parasite interaction studies will benefit by recognizing the genetic diversity of strains. Extensive genetic studies (microarrays, the whole genome sequence) may make it possible to define the virulence factors and the rising importance of *C. parapsilosis* makes these studies necessary. In addition, there is a need for models that enable the study of interaction of this fungus in human tissue, because of the rising importance of *C. parapsilosis* among humans.

**Host Response**

Important to the relative virulence of *C. parapsilosis* is the host response to infections caused by *C. parapsilosis*. In contrast to the vast body of work that has been described on host defense against *C. albicans*, little is known about specific host defense mechanisms against *C. parapsilosis*. *C. parapsilosis* has been described as a relatively low-grade pathogen for humans. However, infections and death due to *C. parapsilosis* have increased steadily during the last decade, particularly among immunocompromised adults, but are also common in the non-neutropenic hosts (i.e., premature infants and surgical patients). The differences in pathogenicity in different host groups indicate that some level of host defense does indeed exist. Better understanding of the mechanisms of host defense against *C. parapsilosis* may enable us to support or deploy these mechanisms against this opportunistic pathogen and improve treatment (Marodi et al. 1991b).

Mononuclear phagocytes, macrophages and polymorphonuclear leukocytes (PMN) have been studied and shown to be a critical component of host defense that protects against candidemia and candidiasis (Aybay & Imir 1996; Marodi et al. 1991a; Marodi et al. 1991b; Roilides et al. 1995a; Takao et al. 1996; Vecchiarelli et al. 1985). *C. parapsilosis* is not as difficult for macrophages to kill compared to several other pathogenic fungi, such as *C. albicans*, Blastomyces dermatitidis and Paracoccidioides brasiliensis (Brummer et al. 1991). The mechanism of killing *C. parapsilosis* by peritoneal macrophages, activated in vitro by lymphokines or recombinant gamma-interferon was studied by Brummer et al. (Brummer & Stevens 1989). They observed killing of *C. parapsilosis* by non-activated peritoneal macrophages. Superoxide dismutase blocked and dimethyl sulfoxide partially blocked this killing, which suggested a mechanism depending on the presence of superoxide anion is involved in the killing of *C. parapsilosis* by macrophages. Killing by activated macrophages was not inhibited by superoxide dismutase or dimethyl sulfoxide, suggesting that activated macrophages probably depend on the myeloperoxidase systems. In addition, Brummer et al. (1991) observed no inhibition of killing of *C. parapsilosis* by the macrophages by the use of cycloheximide and hydrocortisone.

Takao et al. (1996) examined the role of reactive oxygen metabolites in phagocytosis and killing by murine peritoneal macrophages incubated with *C. parapsilosis*, by the use of a fluorochromatic vital staining technique. The relative contribution of reactive oxygen metabolites, depending on NADPH oxidase or xanthine oxidase, was determined using selective inhibition of each of these enzyme systems. Generation of reactive oxygen metabolites by xanthine oxidase and NADPH oxidase-dependent pathways were found to be important for phagocytic killing by murine peritoneal macrophages.

Although there is a difference in pathogenicity of the species *C. albicans* and *C. parapsilosis*, Marodi et al. (1991b) found no difference in the biochemical basis of phagocytosis by human monocytes or monocyte derived macrophages between these two *Candida* species. In a separate study, *C. parapsilosis* was killed significantly better by human monocytes than was *C. albicans*; however monocyte derived macrophages did not require the yeast to be opsonized, and killed both species to an equivalent extent (Marodi et al. 1991a). The greater killing of *C. parapsilosis* by monocytes may be due to the sensitivity of the yeast to toxic oxygen metabolites, hypochlorite, etc., which is required for the killing of *Candida* species (Marodi et al. 1991a). In addition, opsonization was required for phagocytosis by monocytes for both *C. albicans* and *C. parapsilosis*, and used both the classical pathway and alternative pathway (Marodi et al. 1991b). Potoka et al. (1998) studied phagocytosis and phagocytic killing of *C. parapsilosis* by rat Kupffer cell alone and by Kupffer cells in coculture with hepatic endothelial cell-enriched fraction of non-parenchymal cells. They also reported both the NAPD oxidase and the xanthine oxidase dependent pathways are important in Kupffer cell killing of *C. parapsilosis*. 3
PMNs damage pseudohyphae, the invasive form of *Candida* species, by attachment and secretion of oxidative burst metabolites (Rolildes et al. 1995a, Rolildes et al. 1995b). *Candida* species differ in resistance to killing by PMNs. Rolildes et al. (1995a) showed that non-opsonized pseudohyphae of *C. parapsilosis* were more resistant to PMN induced damage than were *C. albicans* or *C. tropicalis*; GM-CSF and interferon-gamma significantly enhanced the killing activity of PMN against *C. parapsilosis*. Possibly these differences in the *Candida* species susceptibility to PMNs may contribute to host susceptibility to infection (Rolildes et al. 1995a).

However, Lyman and Walsh (1994) obtained a similar percentage of phagocytosis by PMN of serum-opsonized *C. albicans*, *C. parapsilosis*, *C. tropicalis*, and *C. glabrata*. Vecchiarelli et al. (1985) showed equal killing of *Candida* species by murine PMN and bone marrow cells, however *C. guilliermondii*, *C. krusei*, and *C. parapsilosis* were killed by these phagocytic cells more rapidly and at significantly lower effector to target ratio compared to *C. albicans*, *C. tropicalis*, and *C. viswanathi*.

Cytokines, such as granulocyte-macrophage colony-stimulating factor (GM-CSF) and interferon-γ, enhance the ability of phagocytic cells to damage or kill *Candida* cells (Rolildes et al. 1995a). GM-CSF has the ability to affect monocyte complement production (Hogasen et al. 1995). Hogasen et al. (1995) determined the release of GM-CSF by monocytes after exposure to different *Candida* species and compared the stimulation of production of complement components C3 and factor B by monocytes. *C. albicans*, *C. tropicalis*, and *C. parapsilosis* were the more effective inducers of C3, factor B and GM-GSF production compared to *C. krusei*, *T. glabrata* (*C. glabrata*), *C. kefyr*, *C. guilliermondii*, and *T. candida* (Hogasen et al. 1995). Hogasen et al. (1995) concluded that monocyte responses are associated with specific yeast species, related to pathogenicity, and may explain predilection of some yeasts for particular underlying diseases.

The major components of the cell wall of *Candida* yeast are mannann, an α-linked polymer of mannose; glucan, a β-linked branched chain polysaccharide of glucose; and chitin, a cellulose-like biopolymer consisting predominantly of N-acetyl-D-glucosamine (Zhang et al. 2006). Mannose binding lectin (MBL), produced in the (human) liver is an important component of innate immunity (serum) (Jack & Turner 2003). *Candida* mannann is a likely target for MBL binding (Lillegard et al. 2006). Mannose binding protein binds to carbohydrate structures on microbial surfaces, leading to direct killing via complement activation or via enzymes linked to MBL, or via enhancing phagocytosis by acting as an opsonin or chemotactic factor (Ip & Lau 2004; Brummer & Stevens, in press). Opsonin-independent phagocytosis could be blocked by mannann, suggesting the mannose receptor may play a role in the clearance of *Candida* in opsonin-poor conditions, especially by macrophages. High avidity binding was shown between MBL and *C. parapsilosis* (van Asbeck et al. 2008c). MBL increased the deposition of C4 and C3b, and enhanced the uptake of *C. parapsilosis* by neutrophils. Recently, Zhang et al. (2006) demonstrated a protective role for human antimannan antibody-mediated immunity and of murine antimannan antibody-mediated immunity against experimental murine systemic candidiasis due to *C. albicans*. Furthermore, these authors demonstrated a mannan epitope, M1g1, which is common to *Candida* spp., including *C. parapsilosis* (Zhang et al. 2006). These data are suggestive that antimannan antibodies, while protective against *C. albicans* infection, might also be protective against infection due to *C. parapsilosis*.

**Animal models**

Experimental animal models are a critical component of understanding the pathogenesis and host resistance to infection with *Candida* spp., as well as to development of more efficacious antifungal therapies (Capilla et al. 2007). There has been very little development of animal models of *C. parapsilosis*, despite the emerging importance of *C. parapsilosis* infections, both systemic and mucosal. Those models that have been developed have included mucosal models in mouse and rat (oral or vaginal), and systemic murine models in normal or immunocompromised animals. In various experimental models, *C. parapsilosis* has been shown to be less pathogenic than *Candida albicans* and other *Candida* species (Bistoni et al. 1984, Edwards et al. 1977; Howlett 1976). However, animal models have been used to examine different aspects of infection due to *C. parapsilosis* and for preclinical antifungal trials.

**Mucosal models**

In humans, *C. parapsilosis* has been associated most often with vaginal or gastrointestinal colonization and disease and much less frequently with oral mucosal disease (Weems 1992). Several models of mucosal infection have been developed.

In an early study, Howlett et al. (1976) described the varying abilities of five different *Candida* species to invade the orthokeratinized mucosa from the dorsal surface of neonatal rat tongue, reflecting their different degree of pathogenicity. *C. parapsilosis* showed only slight invasion of the connective tissue, compared to *C. albicans*, which was the only species able to invade all the tissues present. The keratin layer of the rat tongue mucosa appeared to act as a barrier to invasion of the underlying epithelium by anything but a virulent
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ent compare virulence among species of C. parapsilosis also differences of virulence among different strains of determine relative virulence of a pathogen, there are et al. 1999). Although both the organism and the host blood isolates were highly virulent (de Bernardis et al. (1984) used cyclophosphamide immunodepressed mice and normal mice to study pathogenicity of different Candida species and reported that C. parapsilosis was unable to cause disease in either normal or cyclophosphamide immunosuppressed mice. In contrast, Arendrup et al. (2002) using immunocompetent mice in a similar study, reported C. parapsilosis did show some virulence, with parameters such as weight loss, kidney weight, inflammation and infection and number of eyes infected. Like C. krusei and C. guilliermondii, C. parapsilosis showed less virulence compared to the species C. albicans, C. tropicalis, C. glabrata, C. kefyr, and C. lusitaniae. In other studies, Andriole and Hasenclever (1962) found mortality of diabetic mice infected with C. albicans, C. tropicalis, or C. parapsilosis.

A recent report of Nett et al. (2007) proposed β-1,3 glucan as a marker for C. albicans, C. parapsilosis, and C. glabrata biofilm using a central venous catheter (CVC) biofilm rat model. This model closely mimics a patient with CVC infection. This model has been used for diagnostic purposes, where animals with experimental CVC infection showed significantly higher serum levels of β-1,3 glucan in C. parapsilosis and C. albicans biofilm infections, compared with a model of systemic disease using tail-vein infection, representing disseminated or nonbiofilm disease.

Although in humans C. parapsilosis is associated with endophthalmitis Edwards et al. (1977) were unable to recover C. parapsilosis from the eyes in a rabbit model of haematogenous Candida endophthalmitis. They studied ocular pathogenicities of species of Candida (e.g., C. krusei, C. parapsilosis, C. guilliermondii, C. tropicalis, C. stellatoidea, and C. albicans) and found a relative resistance of ocular tissues to haematogenous Candida infections with species other than C. albicans. Thus, this model would not appear useful for the study of endophthalmitis due to C. parapsilosis.

An alternative nonmammalian model has been described by Chamilos et al. (2006b), who developed a model of candidiasis of Toll (Tl)-deficient Drosophila melanogaster. In agreement with mammalian animal model studies, C. parapsilosis was less virulent than C. albicans when injected into Tl mutant flies. This alternative model using D. melanogaster is promising for large-scale studies of virulence mechanisms of candidiasis (Chamilos et al. 2006b).

Antifungal susceptibility and therapy

Antifungal susceptibility

Widespread use of antifungal agents could be an explanation for the emergence of the more resistant non-albicans species of Candida (Pfaller et al. 1995). Low levels of azole resistance in vitro have been observed for C. parapsilosis, suggesting that the emergence of this species may be influenced by one or more confounding risk
factors in contrast to selection of species, such as C. glabrata and C. krusei, which are much less susceptible to azole drugs (Kao et al. 1999). Identification of Candida species and antifungal susceptibility provide important information for the development of recommendations for empirical antifungal therapy and can affect therapeutic choices (Fluckiger et al. 2006; Krcmery & Barnes 2002; Pfaller & Diekema 2002; Pfaller et al. 2002). In addition, antifungal resistance surveillance programs provide important information for the development of recommendations for empirical antifungal therapy and for the design of programs for the control of antifungal resistance (Pfaller & Diekema 2002).

C. parapsilosis isolates are quite susceptible to most systemic antifungal agents including polyenes (amphotericin B) and azoles (fluconazole, ketoconazole, itraconazole, voriconazole, and posaconazole) (Fluckiger et al. 2006; Mokaddas et al. 2007; Nakamura & Takahashi 2006; Ostrosky-Zeichner et al. 2003; Pappas et al. 2004; Pfaller et al. 2002; Pfaller et al. 2005c; Pfaller et al. 2000; Pfaller et al. 1995; Rex et al. 1995; Weems 1992). Bille and Glauser (1997) tested 30 isolates of C. parapsilosis, Laverdiere et al. (2007) tested 14, and Fleck et al. (2007) tested 19 isolates; all were susceptible to fluconazole and amphotericin B. However, Seidenfeld et al. (1983) reported tolerance to amphotericin B in C. parapsilosis, with minimal fungicidal concentrations more than 32-fold higher than the MIC. Resistance to amphotericin has been noted (Ostrosky-Zeichner et al. 2003).

Global surveillance studies, reported by Pfaller et al. (Pfaller & Diekema 2004), indicated that reduced susceptibility to fluconazole is uncommon among bloodstream isolates of C. parapsilosis, most frequent in surgical ICU’s (Pfaller et al. 2008b). Similarly, Sarvikivi et al. (2005) observed fluconazole resistance in only one C. parapsilosis isolate during a 10-year period of fluconazole prophylaxis and Kovacicova et al. (2000) reported resistance in only two strains of C. parapsilosis, over a period of 3 years. However, in addition to fluconazole resistance, resistance to itraconazole and voriconazole has been noted (Arias et al. 1994; Ostrosky-Zeichner et al. 2003; Pfaller et al. 2007).

In an experimental study, Segal et al. (1975) described the development of resistance to fluconosine by 23% of strains of C. parapsilosis. However, in the European Confederation of Medical Microbiology (ECMM) survey of candidemia in Italy, only one of the 99 isolates of C. parapsilosis tested showed resistance to fluconosine (Tortorano et al. 2003). Lin et al. (1995) showed a difference in susceptibility to fluconosine between group I and II isolates of C. parapsilosis, with a tendency for increased resistance to fluconosine among the group II isolates.

The emergence of yeast species with decreased susceptibility to contemporary antifungal regimens demonstrates the need for new antifungal agents (Capoor et al. 2005, Nguyen et al. 1996). The echinocandin class of antifungals offers an alternative to the standard regimens of azole or polynene agents and provides additional advantages of reduced toxicity (Messer et al. 2004). Caspofungin, micafungin and anidulafungin, echinocandin antifungal agents, compromise cell wall structural integrity through non-competitive inhibition of the synthesis of 1,3-β-D-glucan (Cappelletty & Eisenstein-McKittrick 2007; Denning 2003; Pfaller et al. 2006b). Compared to other Candida species, C. parapsilosis tends to be associated with a higher MIC of echinocandins (Cappelletty & Eisenstein-McKittrick 2007; Fleck et al. 2007; Laverdiere et al. 2007; Marco et al. 1998; Messer et al. 2004; Nakamura & Takahashi 2006; Ostrosky-Zeichner et al. 2003; Pfaller 2004; Pfaller et al. 2005a; Pfaller et al. 2006a; Pfaller et al. 2006b; Reboli et al. 2007). The meaning of these higher MICs is still being investigated, but changes in the glucan synthase subunit Fks1 might explain the reduced activity of echinocandins against C. parapsilosis (Park et al. 2005; Reboli et al. 2007). A recent report indicated the C. parapsilosis complex has a naturally occurring polymorphism resulting in the substitution of alanine at position 660 for a conserved proline, which is present in other fungal Fks1 (Garcia-Effron et al. 2008). The unique mitochondrial respiratory network of C. parapsilosis might play an important role for its decreased susceptibility to the echinocandins (Chamilos et al. 2006a). Chamilos et al. (2006a) observed a decrease in caspofungin MICs after simultaneous inhibition of all respiratory pathways.

From early studies, anidulafungin was demonstrated to have a minimal inhibitory concentration range for 90% of C. parapsilosis strains [MIC90] of <2-4 mg/L (Arevalo et al. 2003; Marco et al. 2003; Marco et al. 1998; Ostrosky-Zeichner et al. 2003; Pfaller et al. 1997). Marco, et al. (1998) reported a discrepancy between caspofungin and anidulafungin susceptibility for C. parapsilosis. Anidulafungin showed less activity against C. parapsilosis than did caspofungin (MIC90, ≥2 versus 1 μg/mL). Messer, et al. (2004) reported only 36% of 106 C. parapsilosis strains tested were inhibited by anidulafungin at <1 mg/L; however, nearly all isolates were inhibited at ≤4 mg/L. Reboli et al. (2007) directly compared the efficacy of anidulafungin with that of fluconazole for the treatment of candidemia and other forms of invasive candidiasis. In that study, anidulafungin showed higher MICs for C. parapsilosis compared to other species of Candida, and patients with a C. parapsilosis infection treated with fluconazole showed a somewhat better response rate compared to anidulafungin. However, the absolute difference in rate of successful response against C. parapsilosis was not significant. Recently, a
prospective sentinel surveillance determined the in vitro activity of all three echinocandins against 5346 invasive isolates of Candida, 14% consisting of C. parapsilosis from 90 medical centers worldwide from 2001 to 2003. C. parapsilosis showed less susceptibility to all three agents compared to C. albicans, C. glabrata, C. tropicalis, C. krusei and C. kefyr (Pfaller et al. 2008a).

For antifungal susceptibility testing it is important to identify the organism to species level, so that appropriate comparisons can be made. Differences in antifungal susceptibility might be explained by comparison of traits and mechanistic pathways across species lines rather than within a single species. Recently, our laboratory determined the susceptibility patterns of the strongly related species C. parapsilosis sensu stricto (formerly C. parapsilosis group I), C. orthopsilosis (formerly C. parapsilosis group II), and C. metapsilosis (formerly C. parapsilosis group III) to fluconazole, caspofungin and anidulafungin (van Asbeck et al. 2008b). C. parapsilosis sensu stricto had significantly higher caspofungin and anidulafungin MICs than C. orthopsilosis or C. metapsilosis. C. metapsilosis was the least susceptible of the species to fluconazole. This observation showed us that significant differences in drug susceptibility occur among the sibling species. In addition, C. parapsilosis sensu stricto was significantly more susceptible to caspofungin than anidulafungin. Gomez-Lopez et al. (2008) studied the susceptibility profile of C. orthopsilosis and C. metapsilosis, which were highly susceptible to all antifungals tested in this study (amphotericin B, flucytosine, fluconazole, voriconazole, ravuconazole, posaconazole, caspofungin, micafungin, and anidulafungin). C. orthopsilosis and C. metapsilosis were more susceptible to amphotericin B and echinocandins, although no significant conclusions could be made because few isolates were tested. There was a trend for C. parapsilosis sensu stricto and C. orthopsilosis to be more susceptible to caspofungin than the other two echinocandins, consistent with our significant differences for the former (van Asbeck et al. 2008b), and prior studies of the C. parapsilosis family (Marco et al. 1998; Pfaller et al. 2006a; Pfaller et al. 2006b). Others (Lockhart et al. 2008a) have also reported echinocandin MICs higher for C. parapsilosis sensu stricto than the sister species, also true for amphotericin B. Tavanti et al. (2007) determined susceptibility patterns of amphotericin B, ketoconazole, voriconazole and caspofungin for C. orthopsilosis. Only one strain showed resistance to flucytosine and was dose-dependently susceptible to itraconazole. No resistance to caspofungin was found. In biofilms, C. parapsilosis MICs to azoles rise, whereas echinocandin susceptibility is similar to that for planktonic growth (Katragkou et al. 2008).

Paradoxical growth of some C. albicans isolates was observed in high concentrations of caspofungin (i.e., those above the minimal inhibitory concentration) (Stevens et al. 2004, Stevens et al. 2005). The effect was very reproducible, but re-test of cells growing at high concentrations showed the parental phenotype (paradoxical effect again), not resistance development. Chamilos et al. (2007) recently reported this phenomenon among the other echinocandins, anidulafungin, and micafungin in Candida species. Paradoxical growth was seen in 90% of isolates (n = 10) of C. parapsilosis tested against caspofungin. However, no paradoxical growth of C. parapsilosis was observed with anidulafungin or micafungin. We (van Asbeck et al. 2008b) studied paradoxical growth among the closely related species C. parapsilosis sensu stricto, C. orthopsilosis, and C. metapsilosis. Thirty-seven percent of the C. parapsilosis sensu stricto isolates tested displayed paradoxical growth in caspofungin. Despite close similarities among sibling species, only C. parapsilosis sensu stricto seemed to have a high capability to avoid caspofungin inhibition. Melo et al. (2007) indicated paradoxical growth was more common in Candida cells grown as biofilms, compared to planktonic growth. All 3 species in the C. parapsilosis family demonstrated paradoxical growth in biofilms, and the paradoxical growth was more prominent in biofilms. Those cells demonstrating the paradoxical effect appear to be more resistant to host defenses (van Asbeck et al. 2009), possibly related to cell wall changes (Stevens et al. 2006), and thus this phenomenon may prove to be a factor in poor therapeutic response to echinocandins in some infections with this organism. Animal model studies with Candida have not shown a consistent relationship of the paradoxical effect in vitro with in vivo resistance, though a “flattening” of the in vivo clearance despite increasing doses could be an in vivo expression of the effect (Clemons, et al. 2006).

Unconventional drug development pathways have included inhibitors of aspartic proteinases, as several known antiretroviral protease inhibitors have this property, and congeners may be developed that could be useful in arresting fungal pathogenesis.

Preclinical antifungal drug trials

Animal models provide a rapid way to test experimental therapies or examine approved drugs for new indications (Capilla et al. 2007). Few preclinical trial data for infections due to C. parapsilosis have been published. Longman et al. (1990) described the efficacy of fluconazole for therapy, as well for prophylaxis, of endocarditis due to C. albicans and C. parapsilosis in a rabbit model. Therapeutically, 14 doses of fluconazole eradicated cardiac vegetations of C. parapsilosis and a two-dose prophylactic regimen prevented experimental endocarditis
by *C. parapsilosis* and *C. albicans*. Similarly, Witt et al. (1993) used a rabbit model of *Candida* endocarditis (both *C. parapsilosis* and *C. tropicalis*) and reported amphotericin B or fluconazole effective therapeutically; prophylactic efficacy of fluconazole and amphotericin B has also been demonstrated in the same model (Bayer et al. 1996).

Barchiesi et al. (2006) observed in a neutropenic murine model of systemic candidiasis the efficacy of caspofungin against different *Candida* species. Depending on the isolate tested, mice infected with *C. parapsilosis* required relatively high drug doses of caspofungin, 1 and/or 5 mg/kg/day to show efficacy. More recently, Barchiesi et al. (2007) described the efficacy of combination therapy and obtained synergistic interaction of caspofungin and amphotericin B against *C. parapsilosis* tested in the same murine model. Other combination studies using amphotericin B and the monoclonal antibody to heat shock protein 90, Mycobrag, have been performed in a systemic murine model and demonstrated no enhanced efficacy of the combination in reduction of fungal burden due to *C. parapsilosis*, in contrast to significant enhancement of efficacy against *C. albicans*, *C. krusei*, or *C. glabrata* (Matthews et al. 2003). Additional studies of the efficacy of various antifungal drugs alone or in combination will be required in the future.

As a result of the data with the echinocandins and in particular caspofungin, it is not clear consequently that these should be the drugs of first choice for treating infections with this organism. Whether the higher MICs for caspofungin will affect clinical outcomes is unknown. In vivo human data do not clearly yet correlate with the in vitro data for the echinocandins for *C. parapsilosis*, and still support the efficacy of echinocandins for invasive candidemia (Safdar et al. 2002).

### Treatment

Mora-Duarte et al. (2002) performed a double-blind trial to compare caspofungin with amphotericin B deoxycholate for the primary treatment of invasive candidiasis. *C. parapsilosis* caused only 19% of cases of fungemia in the caspofungin-treated group in this study, but was associated with 42% of cases in the subset of patients with persistent fungemia. Conversely, the species distribution in cases of persistent fungemia in the amphotericin B-treated group more closely paralleled the overall distribution of infecting species in all types of fungemia. The overall rate of favorable response for *C. parapsilosis* was similar for caspofungin versus amphotericin B, 70% versus 65% (2002). Treatment failures for caspofungin were most commonly from blood. This suggested that caspofungin may be used successfully for treatment of *C. parapsilosis* fungemia, but physicians should be aware of the possibility that this species might respond less readily to this antifungal drug.

Candidemia and acute hematogenously disseminated candidiasis caused by *C. parapsilosis* may be treated with amphotericin B deoxycholate (0.6 mg/kg per day), fluconazole (6 mg/kg per day) or caspofungin (70 mg loading dose followed by 50 mg/day) (Pappas et al. 2004). Therapy for candidemia should be continue for 2 weeks after the last positive blood culture results and resolution of symptoms and signs (Pappas et al. 2004). In situations involving slime producing organisms, such as vascular catheter related infection, the organism is difficult to eradicate using antifungal therapy alone, but several studies recommend removal of the infecting device to prevent invasive mycoses and their complications (Nakamura & Takahashi 2006; Pfaller et al. 1995). When feasible, it may prove useful in initial management to remove indwelling catheters, which is likely to particularly affect the prevalence of secondary infections caused by *C. parapsilosis* (Nakamura & Takahashi 2006). This maneuver needs to be studied in randomized trials.

Very low birth weight neonates and premature neonates with disseminated cutaneous neonatal candidiasis who are at risk for developing acute disseminated candidiasis should be considered for systemic therapy, as is the approach in those with disseminated visceral candidiasis. Little information on pharmacokinetics and response rates is available for this group of patients. However, amphotericin B deoxycholate appears to be first choice in treatment, primarily due to the lack of experience with other antifungal agents (Pappas et al. 2004). Fluconazole may be used as a second line agent for very low birth weight neonates and premature neonates with disseminated cutaneous neonatal candidiasis who are at risk for developing acute disseminated candidiasis (Pappas et al. 2004). Although prevention of nosocomial fungal infections in premature infants is desirable, long-term use of fluconazole may lead to the emergence of resistance in *C. parapsilosis*. Thus, fluconazole prophylaxis should be undertaken with caution (Chapman 2007; Sarvikivi et al. 2005; Yoder et al. 2004). Very low birth weight children, during the first six weeks of life, have an increased risk for candidemia caused by *C. parapsilosis* (Kaufman et al. 2001). Prevention of fungal colonization and invasive infections by fluconazole prophylaxis has been shown effective in these infants (Kaufman et al. 2001; Kickligter et al. 2001). In neonates, Krcmery et al. (2001) reported a better outcome and less mortality for breakthrough fungemias (fungemias which occurred during fluconazole therapy), compared to non-breakthrough fungemias. Although not much is described about caspofungin treatment in neonates, Odio et al. (2004) showed successful treatment with
caspofungin of invasive candidiasis due to *C. albicans*, *C. parapsilosis*, *C. tropicalis*, and *C. glabrata*, in neonates who were resistant to or intolerant of amphotericin B. Caspofungin might be an appropriate alternative for treatment of invasive candidiasis in premature neonates when there is decreased response to the other antifungals such as amphotericin B or fluconazole (Odio et al. 2004).

No treatment for *Candida* endocarditis has been formally tested in prospective randomized controlled studies (Garzoni et al. 2007). Combined medical and surgical therapy appears to be the best treatment for *Candida* endocarditis. The Infectious Diseases Society of America (IDSA) and another study recommend surgical valve replacement of either native valve or prosthetic valve infections (Garzoni et al. 2007; Pappas et al. 2004). Amphotericin B deoxycholate was the most frequently prescribed antifungal drug in previous studies, then flucytosine and fluconazole (Garzoni et al. 2007). Rapid and efficient treatment is important, because this may improve the outcome of fungal endocarditis (Garzoni et al. 2007).

Endophthalmitis caused by *Candida* species can be treated with amphotericin B (Pappas et al. 2004; Stern et al. 1985). A good alternative choice of treatment is fluconazole and it is particularly useful for follow-up therapy (Pappas et al. 2004; Torres Perez et al. 2004). In addition, fluconazole may be effective for the treatment associated with an intraocular lens implant, however, removal of the implant is required (Kauffman et al. 1993). Since treatment can save vision, evidence of intraocular infection should be sought as soon as possible (Feman et al. 2002). Maragon et al. (2004) showed sensitivity to amphotericin B, fluconazole, itraconazole, ketoconazole, flucytosine, and voriconazole of *Candida* isolates, including *C. albicans*, *C. parapsilosis*, and *C. tropicalis* recovered from patients with endophthalmitis. Voriconazole may play a role in the therapeutic management of endophthalmitis caused by *Candida* species, because of the highest potency of this drug against *Candida* species (Maragon et al. 2004). Vitrectomy appears to be an important therapeutic maneuver in treatment of endophthalmitis (Stern et al. 1985; Stevens 1983). All patients with candidemia should undergo an ophthalmological examination to exclude the possibility of endophthalmitis. Therapy for endophthalmitis should be continued until complete resolution of visible disease (Pappas et al. 2004).

Peritonitis caused by *C. parapsilosis* should be treated more aggressively than other *Candida* spp, since *C. parapsilosis* peritonitis has a higher complication rate (complications such as abscess formation and persistent peritonitis after catheter removal) than other *Candida* spp. (Chen et al. 2006). Intravenous amphotericin B, oral or intravenous fluconazole remain the drugs of choice for peritoneal candidiasis and are recommended therapy by the IDSA (Pappas et al. 2004). Both agents are also effective for peritoneal candidiasis caused by *C. parapsilosis* (Chen et al. 2004, Kaitwatcharachai 2002). Chen et al. (2004) observed in a retrospective study comparable efficacy of treatment with fluconazole alone to intraperitoneal amphotericin B alone or intraperitoneal amphotericin B combined with intravenous fluconazole. However, combination of fluconazole and amphotericin B should be used cautiously, because of the possibility of antagonism (Kaitwatcharachai 2002). Recently Chen et al. (2006) showed that when fluconazole is used as initial therapy, complications are significantly higher in patients with *C. parapsilosis* peritonitis than patients with peritonitis caused by other species. In disease associated with catheters used for peritoneal dialysis, early catheter removal is often required for successful therapy for peritoneal candidiasis (Bren 1998; Kaitwatcharachai 2002; Pappas et al. 2004).

In general, management of *Candida* arthritis depends on site of infection (Pappas et al. 2004). Intravenous amphotericin B and fluconazole have been used for medical therapy of *Candida* arthritis (Brooks & Pupparo 1998; Pappas et al. 2004). Arthritis caused by *Candida* species that involves prosthetic joints requires removal of the primary prosthesis to eradicate the infection (Brooks & Pupparo 1998).

Vaginal candidiasis due to *C. parapsilosis* is cleared from subsequent culture relatively easily (Nyirjesy et al. 2005). Short-courses of treatment such as topical boric acid (600 mg/day for 14 days), oral fluconazole 200 mg twice weekly for 1 month or topical antimycotic therapy, such as topical flucytosine, successfully clear the vaginitis infections caused by *C. parapsilosis* (Nyirjesy et al. 2005; Pappas et al. 2004). Other choices in treatment are “over the counter” clotrimazole, buconazole, or miconazole as vaginal applications (Nyirjesy et al. 2005, Pappas et al. 2004).

Not much about treatment of candiduria due to *C. parapsilosis* has been described. Following the IDSA guidelines (Pappas et al. 2004), candiduria should be treated in symptomatic patients, neutropenic patients, very low birth weight infants, patients with renal allografts and patients undergoing urologic manipulation. Therapy with fluconazole (oral or intravenous), amphotericin B (intravenous), or flucytosine (oral) are effective and require 7–14 day courses to be successful. When possible, removal of a urinary catheter might be helpful.

Itraconazole, fluconazole, and terbinafine are effective in treating onychomycosis due to *Candida* species (Gupta & Shear 2000). The IDSA indicated itraconazole to be the most appropriate treatment for onychomycosis caused by *Candida* species (Pappas et al. 2004). Terbinafine may be more effective against *C. parapsilosis* compared to *C. albicans* (Gupta & Shear 2000). Higher doses of drugs and longer duration of therapy are
required for onychomycosis caused by *Candida* species than is the case for nail infections caused by dermatophytes (Gupta & Shear 2000).

Treatment of otomycosis involves local debridement and topical antifungal therapy for up to 14 weeks, depending on the depth and extent of infection.

**Summary and conclusions**

In this review we have discussed various aspects of epidemiology, clinical manifestations, pathogenicity, and antifungal susceptibility of *C. parapsilosis*. *C. parapsilosis* has emerged as a major pathogen in the past two decades. Earlier studies on different aspects of *C. parapsilosis* may have been made more difficult because *C. parapsilosis* appears to be a group of three sibling species. Thus, comparisons of traits may have been across species lines rather than within a single species. Since the pathogen itself is emerging. Therefore, comprehensive studies of their epidemiology, pathogenesis and resistance must be performed in the future.

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