

## Review

# *Candida* species: current epidemiology, pathogenicity, biofilm formation, natural antifungal products and new therapeutic options

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The incidence of fungal infections has increased significantly, so contributing to morbidity and mortality. This is caused by an increase in antimicrobial resistance and the restricted number of antifungal drugs, which retain many side effects. *Candida* species are major human fungal pathogens that cause both mucosal and deep tissue infections. Recent evidence suggests that the majority of infections produced by this pathogen are associated with biofilm growth. Biofilms are biological communities with a high degree of organization, in which micro-organisms form structured, coordinated and functional communities. These biological communities are embedded in a self-created extracellular matrix. Biofilm production is also associated with a high level of antimicrobial resistance of the associated organisms. The ability of *Candida* species to form drug-resistant biofilms is an important factor in their contribution to human disease. The study of plants as an alternative to other forms of drug discovery has attracted great attention because, according to the World Health Organization, these would be the best sources for obtaining a wide variety of drugs and could benefit a large population. Furthermore, silver nanoparticles, antibodies and photodynamic inactivation have also been used with good results. This article presents a brief review of the literature regarding the epidemiology of *Candida* species, as well as their pathogenicity and ability to form biofilms, the antifungal activity of natural products and other therapeutic options.

## Introduction

The incidence and prevalence of invasive fungal infections have increased since the 1980s, especially in the large population of immunocompromised patients and/or those hospitalized with serious underlying diseases (Arendrup *et al.*, 2005; Espinel-Ingroff *et al.*, 2009). *Candida* species belong to the normal microbiota of an individual's mucosal oral cavity, gastrointestinal tract and vagina (Shao *et al.*, 2007), and are responsible for various clinical manifestations from mucocutaneous overgrowth to blood-stream infections (Eggimann *et al.*, 2003). These yeasts are commensal in healthy humans and may cause systemic infection in immunocompromised situations due to their great adaptability to different host niches. The genus is composed of a heterogeneous group of organisms, and more than 17 different *Candida* species are known to be aetiological agents of human infection; however, more than 90% of invasive infections are caused by *Candida albicans*, *Candida glabrata*, *Candida parapsilosis*, *Candida tropicalis* and *Candida krusei* (Pfaller *et al.*, 2007). The expanding population of immunocompromised patients that use intravenous catheters, total parenteral nutrition, invasive

procedures and the increasing use of broad-spectrum antibiotics, cytotoxic chemotherapies and transplantation are factors that contribute to the increase of these infections (Ortega *et al.*, 2011). The pathogenicity of *Candida* species is attributed to certain virulence factors, such as the ability to evade host defences, adherence, biofilm formation (on host tissue and on medical devices) and the production of tissue-damaging hydrolytic enzymes such as proteases, phospholipases and haemolysin (Silva *et al.*, 2011b).

Currently, an increase in the number of yeasts that are resistant to antifungal drugs is recognized worldwide; therefore, the use of *in vitro* laboratory tests may aid the doctor in choosing an appropriate therapy (Ingham *et al.*, 2012). The ability of *Candida* species to form drug-resistant biofilms is an important factor in their contribution to human disease. As in the vast majority of microbial biofilms (Rajendran *et al.*, 2010), sessile cells within *C. albicans* biofilms are less susceptible to antimicrobial agents than are planktonic cells (Kuhn & Ghannoum, 2004). The progression of drug resistance within *Candida* biofilms has been associated with a parallel increase in the maturation process

(Sardi *et al.*, 2011). Furthermore, some studies have also shown that biofilms of *Candida* develop statically in the presence of a minimal matrix and exhibit the same level of resistance to drugs (fluconazole and amphotericin B) as cells grown in a shaker and exhibiting large amounts of matrix (Seneviratne *et al.*, 2008; Sardi *et al.*, 2011). The increase in resistant strains necessitates a search for new targets for new antifungal agents (J. M. White *et al.*, 1998; Sardi *et al.*, 2011). In the present report, the literature on the current epidemiology, pathogenicity, biofilm formation and resistance of *Candida* species, as well as new therapies, including natural antifungal agents and nanoparticles, is reviewed.

## Epidemiology

Several *Candida* species are commensal and colonize the skin and mucosal surfaces of humans. Critically ill or otherwise immunocompromised patients are more prone to develop both superficial and life-threatening *Candida* infections (Hasan *et al.*, 2009). *Candida* infections also constitute the most common fungal infections in AIDS patients (Fidel, 2006; Hasan *et al.*, 2009). These patients predominantly develop oropharyngeal candidiasis, which can lead to malnutrition and interfere with the absorption of medication.

*C. albicans* is the predominant cause of invasive fungal infections (Horn *et al.*, 2009) and represents a serious public health challenge with increasing medical and economic importance due to the high mortality rates and increased costs of care and duration of hospitalization (Almirante *et al.*, 2005; Lai *et al.*, 2012).

Although *C. albicans* is the most prevalent species involved in invasive fungal infections, the incidence of infections due to non-*albicans* species is increasing. In a study with 2019 patients at major North American medical centres, a predominance of non-*albicans* species was observed; although *C. albicans* was the most frequently isolated species, it was followed by *C. glabrata* and other non-*C. albicans* species. This change in epidemiology could be associated with severe immunosuppression or illness, prematurity, exposure to broad-spectrum antibiotics and older patients (Horn *et al.*, 2009). In European countries, an analysis showed that more than half of the cases of candidaemia were caused by *C. albicans*, and the incidence rates for non-*albicans* candidaemia infections were 14 % each for *C. glabrata* and *C. parapsilosis*, 7 % for *C. tropicalis* and 2 % for *C. krusei* (Tortorano *et al.*, 2006). Changes in the epidemiology have also been observed in Latin American countries. In Chile, the prevalence of *C. albicans* has changed, and a progressive increase of non-*albicans* infection has been observed; *C. parapsilosis* was the most frequent species, followed by *C. tropicalis* and *C. glabrata*. All isolates were susceptible to amphotericin B; however, 50 % of the *C. glabrata* isolates were resistant to fluconazole (Ajenjo H *et al.*, 2011). According to the Brazilian Network Candidaemia Study, *C. albicans* accounted for 40.9 % of cases in Brazil, followed by *C. tropicalis* (20.9 %), *C.*

*parapsilosis* (20.5 %) and *C. glabrata* (4.9 %) (Colombo *et al.*, 2006; Nucci *et al.*, 2010).

Other species have been isolated in healthy people and patients. *Candida dubliniensis* was usually found in combination with other yeast species, especially *C. albicans* (Sullivan *et al.*, 2004). A high prevalence of *C. dubliniensis* in the oral cavities of HIV-infected and AIDS patients has also been reported (Tintelnot *et al.*, 2000; Lasker *et al.*, 2001). Since the first description of *C. dubliniensis* from the oral cavities of HIV-positive patients from Ireland (Z. Khan *et al.*, 2012), subsequent epidemiological studies have revealed that this species is prevalent globally in association with human (Loreto *et al.*, 2010; Z. Khan *et al.*, 2012) and non-human habitats with a possibility of inter-host transmission (Nunn *et al.*, 2007). The species has now been reported from other body sites/specimens, such as the vagina, urine, skin and gastrointestinal tract of both HIV-positive and HIV-negative patients (Loreto *et al.*, 2010; Mokaddas *et al.*, 2011; Z. Khan *et al.*, 2012). Rarely do patients colonized with this species develop candidaemia (Loreto *et al.*, 2010). The reasons for this limited ability of *C. dubliniensis* to cause invasive disease have been the focus of recent studies (Jackson *et al.*, 2009). It has been shown that the *C. dubliniensis* genome lacks important hypha-related virulence genes and that it has a limited ability to undergo yeast-to-hyphal transformation (Moran *et al.*, 2012), which in turn may decrease its potential to invade deeper tissue.

*C. parapsilosis* has emerged as a significant nosocomial pathogen with clinical manifestations that include endophthalmitis, endocarditis, septic arthritis, peritonitis and fungaemia, usually associated with invasive procedures or prosthetic devices (Cantón *et al.*, 2011), and with neonatal infections in the northern hemisphere, although this species is found in patients of all ages in Latin America (Almirante *et al.*, 2005; Nucci *et al.*, 2010). Pires *et al.* (2011a) isolated 100 strains of *C. parapsilosis* from a haemodialysis unit; using molecular analysis, 53 % were found to be *C. parapsilosis* and 47 % corresponded to *Candida orthopsilosis*. Tavanti *et al.* (2005) suggested that the *C. parapsilosis* complex could be replaced by three different but related species named *C. parapsilosis sensu stricto*, *C. orthopsilosis* and *Candida metapsilosis* on the basis of differences observed for randomly amplified polymorphic DNA and DNA sequencing of different genes. In the FUNGEMYCA study realized by Cantón *et al.* (2011), 400 out of 1356 isolates were identified as *C. parapsilosis sensu lato* (29.5 %), and this species was isolated the second most frequently from blood after *C. albicans* in Spain. Of these 400 isolates, 364 were identified by molecular methods; *C. parapsilosis sensu stricto* represented 90.7 % of isolates, *C. orthopsilosis* 8.2 % and *C. metapsilosis* 1.1 %. Candidaemia due to *C. tropicalis* has been associated with cancer, especially in patients with leukaemia or neutropenia (Colombo *et al.*, 2007). Candidaemia due to *C. glabrata* has been reported to be related to the use of fluconazole (Nucci *et al.*, 2010). *Candida guilliermondii* and *Candida rugosa* were previously uncommon agents; however, the incidence of these is increasing (Pfaller *et al.*, 2009;

Nucci *et al.*, 2010). *C. rugosa* (1.1 %) has been described in the oral cavity of diabetic patients (Pires-Gonçalves *et al.*, 2007).

Concern is rising about the high incidence of infections caused by non-*albicans* species and the emergence of antifungal resistance (Pereira *et al.*, 2010). Among the non-*albicans* species, *C. tropicalis* and *C. parapsilosis* are both generally susceptible to azoles; however, *C. tropicalis* is less susceptible to fluconazole than is *C. albicans*. *C. glabrata* is intrinsically more resistant to antifungal agents, particularly to fluconazole. *C. krusei* is intrinsically resistant to fluconazole, and infections caused by this species are strongly associated with prior fluconazole prophylaxis and neutropenia. *Candida lusitanae*, which accounts for 1–2% of all candidaemias, is susceptible to azoles but has a higher intrinsic resistance to amphotericin B (Cruciani & Serpelloni, 2008).

There has been a gradual increase in the number of antifungal compounds and classes discovered since the 1990s; these include polyenes, azoles, echinocandins and purine analogues. Due to the increased availability of antifungal drugs, selection has occurred with consequent resistance of these micro-organisms. Doctors retain the option of giving the drugs for prophylaxis, empiric therapy, preventive treatment or while waiting for the disease to be diagnosed, so there is a degree of excessive exposure to these agents (Rodríguez-Tudela *et al.*, 2007).

### Pathogenicity of *Candida* species

An infection caused by *Candida* is termed candidiasis or candidosis. Mycoses caused by these fungi show a wide spectrum of clinical presentations and can be classified as superficial, as with cutaneous and mucosal infections, to deep, widespread and of high severity, as is the case with invasive candidiasis. According to Colombo & Guimarães (2003), the main transmission mechanism is through endogenous candidaemia, in which *Candida* species that constitute the microbiota of various anatomical sites under conditions of host weakness behave as opportunistic pathogens. Another mechanism for transmission is exogenous, and this occurs mainly through the hands of health professionals who care for patients. Also indicated in the spread of infection are health-care materials, such as contaminated catheters and intravenous solutions (Ingham *et al.*, 2012). *Candida* species are considered important pathogens due to their versatility and ability to survive in various anatomical sites (Calderone & Fonzi, 2001). It was believed decades ago that yeasts passively participated in the process of pathogenesis in the establishment of fungal infection. Thus, organic weakness or an immunocompromised host was considered the only mechanism responsible for the establishment of opportunistic infection. Today, this concept has been modified. The current consensus is that these organisms actively participate in the pathophysiology of the disease process using mechanisms of aggression called virulence factors (Tamura *et al.*, 2007).

*Candida* species are eukaryotic opportunistic pathogens that reside on the mucosa of the gastrointestinal tract as well as the mouth, oesophagus and vagina (Kim & Sudbery, 2011; Lim *et al.*, 2012). Although this commensal organism normally colonizes mucosal surfaces in an asymptomatic manner, it can become one of the most significant causes of a disabling and lethal infection (Wisplinghoff *et al.*, 2006; Vincent *et al.*, 2009). In the early 1980s, fungi emerged as major causes of nosocomial infections, mainly affecting immunocompromised patients or those who were hospitalized for long periods due to serious underlying diseases (Vidigal & Svidzinski, 2009). *Candida* species belonging to the microbiota of healthy individuals can be found scattered in the environment. It is believed that most people usually have a single strain of *Candida* in different places in the body for a long period. However, some individuals have more than one strain or species at the same time, and this is commonly observed among hospitalized patients (Klotz *et al.*, 2007b). Moreover, the potential for *Candida* species to become pathogenic should be appreciated. A crucial component of this versatility is the fact that these organisms survive as commensals in diverse and distinct anatomical sites, each with its particular environmental stresses (Vidigal & Svidzinski, 2009). Although *Candida* species can infect different anatomical sites of the human host, evidence exists that immune protection is site-specific for each type. Moreover, cutaneous candidiasis and vaginal infections are more likely to be associated with a phagocytic response involving neutrophils and mononuclear phagocytes (Vidigal & Svidzinski, 2009). The urinary tract is the anatomical site most conducive to the development of infections in hospitalized patients, although this remains a problem of questionable significance (Guler *et al.*, 2006; Vidigal & Svidzinski, 2009). Although most of these infections are of bacterial origin, it is estimated that at least 10% have fungi as the principal aetiological agent and that *Candida* species are the most frequently isolated (Sobel *et al.*, 2000). Data show the isolation of *Candida* species in 22% of urine samples from patients admitted to intensive care units (Alvarez-Lerma *et al.*, 2003). The colonization of the respiratory tract by *Candida* species is common in patients receiving mechanical ventilation for periods of longer than 2 days. This occurs due to haematogenous spread or pulmonary aspiration of the contents of colonies of oropharyngeal or gastric origin (Vidigal & Svidzinski, 2009). Prolonged intensive care unit stay and hospitalization are also important. Xie *et al.* (2008) evaluated the impact of invasive fungal infection in surgical patients with severe sepsis. The authors found that 28.3% of patients exhibited invasive fungal infection; *C. albicans* was most frequently isolated (58%), followed by *C. tropicalis* (17%) and *C. glabrata* (15%). In addition, the organ most affected by invasive fungal infection was the lung.

*Candida* pathogenicity is facilitated by a number of virulence factors, the most important of which are those for adherence to host tissues and medical devices, biofilm formation and secretion of hydrolytic enzymes (e.g.

proteases, phospholipases and haemolysins). Furthermore, although there has been extensive research to identify pathogenic factors in fungi, particularly in *C. albicans*, relatively little is known about non-*albicans* species (Silva *et al.*, 2011a).

Virulence in *C. albicans* and other pathogens includes host recognition, which enables the pathogen to bind to host cells and proteins. Additionally, degradative enzymes play a special role in virulence. Fungal invasion is facilitated more by the transition between yeast cells and filamentous growth than by yeast growth (Cullen & Sprague, 2012).

The primary factor in the fungal colonization of human tissues is adherence to host surfaces; this process is controlled and induced by several cell-signalling cascades in both the fungus and the environment. In addition, *Candida* species can adhere to the surfaces of medical devices and form biofilms. The initial attachment of *Candida* cells is mediated by non-specific factors (hydrophobicity and electrostatic forces) and promoted by specific adhesins present on the surface of fungal cells that recognize ligands such as proteins, fibrinogen and fibronectin (Li *et al.*, 2003). The phenomenon of adhesion is exhibited by specialized surface proteins, called adhesins, that specifically bind to amino acids and sugars on the surface of other cells or promote adherence to abiotic surfaces (Verstrepen & Klis, 2006).

As *Candida* species are part of the human microbiota, they are often found on biomaterials, implants and various types of catheters (Kojic & Darouiche, 2004). Biofilms cause clinical problems of concern because they increase resistance to antifungal therapy; the mechanism of biofilm resistance to antimicrobial agents is not fully known. One hypothesis is that the presence of the matrix restricts the penetration of drugs through the formation of a diffusion barrier (Kojic & Darouiche, 2004), and only the most superficial layers are in contact with lethal doses of antibiotics.

Extracellular hydrolytic enzymes appear to play an important role in adherence, tissue penetration, invasion and the destruction of host tissues (Silva *et al.*, 2011b).

The most important hydrolytic enzymes are proteases and phospholipases. Ten aspartic proteinase (Sap) isoenzymes are responsible for the proteinase activity of *C. albicans*. These proteins have molecular masses between 35 and 50 kDa and are encoded by the *SAP1–10* genes. The role of the *SAP1–6* genes is related to adherence, tissue damage and changes in the immune response. The functionality of *SAP7* has not been fully discovered, but reports exist that *Sap9* and *Sap10* are not secreted proteinases; instead, they are used in preserving the regulatory surface integrity of the yeast cells (Naglik *et al.*, 2003). *Sap* proteins have also been described in *C. tropicalis*, *C. parapsilosis* and *C. guilliermondii* (Zaugg *et al.*, 2001). Several studies have demonstrated a relationship between an increase in the synthesis and activity of extracellular hydrolytic enzymes and an increase in the pathogenic potential of the yeasts, leading to

clinical signs of severe candidiasis (Bramono *et al.*, 2006; Ingham *et al.*, 2012).

Putative roles of microbial extracellular lipases include the digestion of lipids for nutrient acquisition, adhesion to host cells and tissues, unspecific initiation of inflammatory processes by affecting immune cells and self-defence by lysing the competing microflora (Stehr *et al.*, 2004; Gácsi *et al.*, 2007). Gácsi *et al.* (2007) have shown that lipase inhibitors significantly reduce tissue damage during infection of reconstituted human tissues. Furthermore, biofilm formation was inhibited in lipase-negative *C. parapsilosis* mutants, and their growth was significantly reduced in lipid-rich media. Additionally, lipase-negative mutants were significantly less virulent in infection models.

Additionally, the production of haemolysin plays an important role in virulence. This protein is essential for survival and is related to the acquisition of iron (Vaughn & Weinberg, 1978). Haemolysins are proteins produced by micro-organisms to destroy red blood cells. Iron, an inorganic element, is essential for the development of micro-organisms, including yeasts, and the ability to obtain this element is essential for the establishment of an infectious process (Manns *et al.*, 1994).

Seven phospholipase genes have been identified (*PLA*, *PLB1*, *PLB2*, *PLC1*, *PLC2*, *PLC3* and *PLD1*); however, the role of the enzymes encoded by these remains unclear (Samaranayake *et al.*, 2006). *PLB1* has been described as having a virulence role in animal models of candidiasis. *Plb1p* is an 84 kDa glycoprotein present at hyphal tips during tissue invasion and has hydrolase and lysophospholipase-transacylase activity (Ghannoum, 2000).

*C. albicans* reversibly converts from unicellular yeast cells to either pseudohyphal or hyphal growth, a morphogenesis phenomenon (creating a transition between unicellular yeast cells and a filamentous growth form). *C. albicans* and *C. dubliniensis* form both types of filamentous growth, indicating that these yeasts are capable of growing isotropically (yeast) or apically (hyphal and pseudohyphal). The growth of hyphae, a virulence mechanism, plays an important function in tissue invasion and resistance to phagocytosis (Jayatilake *et al.*, 2006).

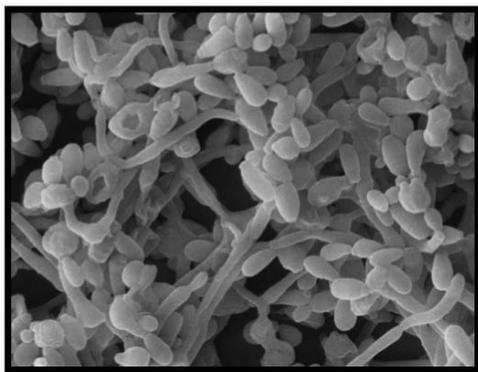
### **Candida biofilm and disease**

Biofilm formation by fungi may play an important role in pathogenesis (Martinez & Fries, 2010). Recent evidence suggests that the majority of disease produced by *C. albicans* is associated with biofilm growth (Ramage & López-Ribot, 2005). Studies have shown that the micro-organisms are almost non-existent in their planktonic free form in the tissues of the host, but are grouped together, forming a multicellular community, both in tissues and on prostheses, catheters and other surfaces (Soll, 2008). Adhesion between *Candida* species cells and materials or host cells has been implicated as an early step in biofilm formation. Attachment of *Candida* species cells to materials is mediated by

non-specific interactions, such as hydrophobic and electrostatic forces, as well as by specific adhesin-ligand bonds (Ramage & López-Ribot, 2005; Chaffin *et al.*, 1998; Chandra *et al.*, 2005). Cell hydrophobicity and charge also depend on cell growth morphology and cell surface structure (Kriznik *et al.*, 2005).

Biofilms are specific and organized communities of cells under the control of signalling molecules, rather than random accumulations of cells resulting from cell division (Fig. 1). These biological communities can be embedded in an extracellular matrix that is self-produced. Silva *et al.* (2009) showed that *C. parapsilosis*, *C. tropicalis* and *C. glabrata* are also capable of producing biofilms. In addition, the extracellular matrix contains different amounts of protein and carbohydrate for different species. Villar-Vidal *et al.* (2011), comparing biofilm formation by *C. albicans* and *C. dubliniensis*, found that biofilm formation by *C. albicans* isolates was higher than that by *C. dubliniensis*. In general, the biofilm matrix comprises carbohydrates, proteins, phosphorus and hexosamines; however, environmental conditions such as medium composition, pH and oxygen as well as the fungal strain and species affect biofilm formation and matrix composition. For example, *C. parapsilosis* biofilms contain large amounts of carbohydrates, and the protein content is low when compared with biofilms of *C. glabrata* and *C. tropicalis* (Silva *et al.*, 2009, 2011b; Baillie & Douglas, 1999).

Biofilms can grow on any biotic or abiotic moist surface. The association of organisms in biofilms is a form of protection for their development, encouraging symbiotic relationships and allowing survival in hostile environments (Davey & O'toole, 2000). Biofilms may help maintain the role of fungi as pathogenic by evading host immune mechanisms, resisting antifungal treatment and withstanding competitive pressure from other organisms. Consequently, biofilm-related infections are difficult to treat. Biofilm production is also associated with a high level of antimicrobial resistance of the associated organisms (Ozkan *et al.*, 2005). The first crucial event in the process of



**Fig. 1.** Scanning electron microscopy of a *C. albicans* biofilm.

biofilm formation is microbial attachment, which is followed by the maturation process of the biofilm (Ramage *et al.*, 2001). After the yeast associates with the substrate, many physical and chemical events enable initial adhesion of the micro-organism to the surface. After this accession stage, the biofilm goes through a maturation process. Hawser & Douglas (1994) reported that isolates of *C. parapsilosis* and *C. glabrata* were significantly less likely to produce biofilms than the more pathogenic *C. albicans*. Most infections result from pathogenic biofilms; therefore, the biomedical significance of biofilms is substantial (Ganguly & Mitchell, 2011; Harriott *et al.*, 2010). Transplantation procedures, immunosuppression, the use of indwelling devices and prolonged intensive care unit stays have increased the prevalence of fungal disease (Mukherjee & Chandra, 2004). Availability to the inside of medical devices, such as central and peripheral catheters, haemodialysis and peritoneal dialysis units and intracardiac prosthetic valves, facilitates biofilm formation (Mukherjee & Chandra, 2004; Chandra *et al.*, 2001; Ramage *et al.*, 2005). Although *C. albicans* is the fungal species isolated most often, the incidence of other species has recently increased (Medrano *et al.*, 2006), and these have also been described as the aetiological agent in nosocomial infections (Bruder-Nascimento *et al.*, 2010). The ability of *C. albicans* to form biofilms on medical devices has a profound effect on its capacity to cause human disease (Hawser & Douglas, 1994). Catheter-related infections are the major cause of morbidity and mortality among hospitalized patients, and catheter microbial biofilms are associated with 90% of these infections. Catheter-associated *Candida* biofilms can lead to bloodstream infections with an approximate incidence of one episode per 100 hospital admissions (DiDone *et al.*, 2011). *C. albicans* abiotic-surface biofilms consist of two general types of cells: yeast cells and filamentous cells (Finkel & Mitchell, 2011; Ramage *et al.*, 2006). *In vitro*, the basal biofilm layer is composed of yeast cells from which filamentous cells emanate. These yeast and filamentous cells are embedded in a dense layer of extracellular matrix. The primary component of biofilm matrix is  $\beta$ -glucan (Finkel & Mitchell, 2011; Nett *et al.*, 2007).

Device-associated *Candida* species infections cause mortality rates as high as 30% (Viudes *et al.*, 2002; Finkel & Mitchell, 2011), and the annual cost of antifungal therapies in the United States alone is estimated at US\$2.6 billion (Finkel & Mitchell, 2011). Like the biofilms formed by bacterial pathogens, *Candida* species biofilms are resistant to many antimicrobial agents; therefore, treatment can require surgical removal and later replacement of the infected device (Finkel & Mitchell, 2011). *C. albicans* biofilms consist of two main types of cells: small oval yeast-form cells (blastospores) and long tubular hyphal cells. *C. albicans* biofilms grown *in vitro* often have a foundation of yeast cells from which a hyphal layer emanates (Douglas, 2003). Extracellular matrix material is also clearly evident and is bound to both yeast and hyphal cells. The matrix is typically interspersed throughout the biofilm.

Genetic analyses indicate that both yeast cells and hyphae are crucial for biofilm formation, which suggests that each cell type has a unique role in the process (Finkel & Mitchell, 2011; Al-Fattani & Douglas, 2006; Douglas, 2003). *C. albicans* has the capacity to switch from yeast morphology to hyphal morphology, one of its major virulence determinants (Lo *et al.*, 1997; Ramage *et al.*, 2002a). In *Candida*, the dimorphic transition from the yeast to the hyphal phase is a crucial step in the formation of biofilms, and mutant strains that are defective in the ability to germinate are unable to form dense and homogeneous biofilms (Baillie & Douglas, 1999; Ramage *et al.*, 2002a; Kruppa *et al.*, 2004; Jabra-Rizk *et al.*, 2006; Brown *et al.*, 1999). The morphological transition from the yeast to the mycelial form (dimorphic switching) is induced by many environmental factors, such as serum, high temperatures (<37 °C) and neutral pH (Brown *et al.*, 1999). Farnesol, which is associated with mycelial development, may be an important regulatory (quorum-sensing) molecule in *C. albicans* biofilm formation. Ramage *et al.* (2002c) found that *C. albicans* biofilm density and morphology were drastically altered by high concentrations of farnesol, most likely as a direct consequence of the inhibitory effect of farnesol on the morphogenetic process. As the concentration of farnesol was decreased, the morphology of the biofilms changed in a dose-dependent manner to a typical hyphal morphology. Microarray analysis of biofilms exposed to farnesol revealed that genes related to drug resistance, cell wall maintenance, cell surface hydrophilicity, iron transport and heat-shock proteins were influenced in addition to genes associated with hyphae formation (Cao *et al.*, 2005; Albuquerque & Casadevall, 2012).

Other studies have shown the same result with other *Candida* species (*C. dubliniensis* and *C. tropicalis*) (Kruppa *et al.*, 2004).

Yi *et al.* (2011) observed that although a majority (approx. 90 %) of *C. albicans* strains form traditional biofilms that are impermeable to molecules of low and high molecular mass and impenetrable to white blood cells, a minority (approx. 10 %) form biofilms that are both permeable and penetrable. The formation of minority-type alternative biofilms is dictated by a change at a single genetic locus, the mating type locus. Homozygous **a/a** or  $\alpha/\alpha$  cells are mating-competent, whereas the heterozygous **a/α** cells are mating-incompetent. Cells of the mating-incompetent **a/α** genotype form impermeable, traditional biofilms whereas cells of the mating-competent **a/a** or  $\alpha/\alpha$  genotype form permeable biofilms. The characteristics of **a/a** and  $\alpha/\alpha$  biofilms are consistent with a suggested role in mating by facilitating the transfer of hormone signals through the permeable biofilm. The two types of biofilm are also regulated by different signal transduction pathways: the **a/α** form is regulated by the Ras1/cAMP pathway, and the **a/a** or  $\alpha/\alpha$  form is regulated by the MAP kinase pathway.

Nobile *et al.* (2012) combined 'classical' genetics, genome-wide approaches, RNA deep sequencing technology and

two *in vivo* animal models to comprehensively map the transcriptional circuitry controlling biofilm formation in *C. albicans*. The elucidation of this circuit has led to many new predictions about the genes involved in biofilm formation, and a set of these predictions has been validated by confirming the roles of several of these genes in biofilm development.

Many studies have focused on the formation of *C. albicans* biofilms on implanted vascular catheters because this is a major source of infection (Finkel & Mitchell, 2011). However, biofilms can also be formed on many other devices. A rat denture biofilm model that recapitulates features of denture stomatitis has been described (Nett *et al.*, 2010). Microscopic and microbiological analyses showed the signature features of biofilm development: adherent cells, the presence of extracellular matrix material and high-level drug resistance. The biofilms in another recently described rat model involving subcutaneously implanted catheters also contained characteristic matrix material and an abundant hyphal population (Ricicová *et al.*, 2010). The closely related cell wall proteins Als1 and Als3 might also function in biofilm surface attachment (Finkel & Mitchell, 2011). The expression of Als1 or Als3 in *S. cerevisiae* promotes binding to several different protein-coated substrates that may resemble the conditioned surface of an implanted device. In addition, a *C. albicans* mutant lacking both *ALS1* and *ALS3* is defective in biofilm formation *in vitro* and *in vivo* (Nobile *et al.*, 2006). One or more genes in the *ALS* family are upregulated in biofilm-associated cells when compared with planktonic cells (Chandra *et al.*, 2001). *ALS3* expression was shown to be necessary for biofilm formation on silicone substrates *in vitro* (Zhao *et al.*, 2006), whereas *ALS3* expression was not required for biofilm formation *in vivo*. Raad *et al.* (2008) demonstrated that Eap1p is a glucan-cross-linked, cell wall-localized protein. In addition, Eap1 mutants exhibited reduced adhesion to plastic surfaces and epithelial cells whereas Eap1p was able to mediate adhesion to yeast cells. *EAP1* expression was also required for biofilm formation under shear flow in both *in vitro* and *in vivo* central venous catheter biofilm models. Biofilms are the most common form of microbial growth in nature, and the National Institutes of Health estimate that biofilms cause the majority of infections in humans (Douglas, 2003; Nett *et al.*, 2010). Frequently, an implanted device, such as an intravascular catheter, is associated with biofilm infections (Ramage *et al.*, 2002b). *C. albicans* is the fourth and third leading cause of hospital-acquired bloodstream and urinary tract infections, respectively. Up to 70–80 % of *Candida* bloodstream infections are associated with central venous catheters, and the majority of *Candida* urinary tract infections are associated with bladder catheters (Nett *et al.*, 2007).

There are estimated to be more than 45 million medical devices implanted every year in the United States. Infection of these devices occurs in 60 % of patients, and *Candida* species are responsible for up to 20 % of these infections

(Kojic & Darouiche, 2004). *C. albicans* can form biofilms on almost any medical device. The most commonly involved systemic devices include vascular and urinary catheters, joint prostheses, cardiac valves, artificial vascular bypass devices, pacemakers, ventricular assist devices and central nervous system shunts (Byers *et al.*, 1992). *Candida* endocarditis was previously considered a rare disease. However, its incidence is increasing, partly because of the increased use of prosthetic intravascular devices. In patients with prosthetic valve endocarditis, a *Candida* infection may occur via a two-step process; post-operative transitory candidaemia occurs during the intensive care unit stay, leading to colonization of the prosthetic valve and subsequent biofilm formation with reduced susceptibility to antifungal agents. This theory lends support for pre-emptive antifungal therapy using agents that display activity against biofilm-associated *Candida* in patients with prosthetic heart valves at risk of candidaemia. Recently, biofilm formation on biotic surfaces has been reported, including both oral and vaginal tissues (Ozkan *et al.*, 2005; Dongari-Bagtzoglou *et al.*, 2009).

Biofilms can also be polymicrobial, formed from members of the endogenous microbiota in addition to nosocomial pathogens. These biofilms are difficult to diagnose and treat, and have the potential to serve as an infectious reservoir for a variety of micro-organisms, including bacteria and fungi. These biofilms can be found in systemic infections, where it is possible to find *C. albicans* growing with *Staphylococcus epidermidis*, *Enterococcus* species and *Staphylococcus aureus* (Klotz *et al.*, 2007a; Harriott & Noverr, 2011), and in vaginal infections by *Candida* species and *Gardnerella vaginalis*. Mixed species biofilms of fungal pathogens have also been found in skin infections, with the main agents being *Candida*, *Curvularia*, *Malessezia*, *Aureobasidium*, *Cladosporium*, *Ulocladium*, *Engodontium* and *Trichophyton* (Harriott & Noverr, 2011). In lung infections, the association between *C. albicans* and *Pseudomonas aeruginosa* is an example of an antagonistic interaction between bacteria and fungi, where *P. aeruginosa* kills yeast hyphae and biofilms of *C. albicans* (Morales *et al.*, 2010). Thus far, biofilms have not been demonstrated in the gastrointestinal tract (Harriott & Noverr, 2011). The study of the function of polymicrobial biofilms in immune evasion and manipulation of immune responses will most likely aid in the search for novel therapies that are directed at a multiplicity of species regarding immunity and drug resistance.

### ***Candida* biofilms and conventional antifungals**

Each of the antifungal classes utilizes a different means to kill or inhibit the growth of fungal pathogens (Pfaller, 2012; Kanafani & Perfect, 2008). The mechanisms of antifungal resistance are classified as either primary or secondary and are related to intrinsic or acquired characteristics of the fungal pathogen, involving either interference with the antifungal mechanism of the respective drug/drug class or a decrease in target drug levels. Resistance can also occur when

environmental factors lead to colonization or replacement of a susceptible species with a resistant species. The antifungal effects of polyene and azole antifungals are due to their actions on the fungal cell membrane, whereas echinocandins act by disrupting the fungal cell wall (Pfaller, 2012; Pemán *et al.*, 2009; T. C. White *et al.*, 1998).

The ability of *Candida* to form drug-resistant biofilms is an important factor in its contribution to human disease. Like the vast majority of microbial biofilms (Rajendran *et al.*, 2010), sessile cells within a *C. albicans* biofilm are less susceptible to antimicrobial agents than are planktonic cells (Kuhn & Ghannoum, 2004; Rautemaa & Ramage, 2011). The formation of biofilms causes clinical problems of concern because they increase resistance to antifungal therapies, and the mechanism of biofilm resistance to antimicrobial agents is not fully known. One hypothesis for this resistance is the presence of the matrix, which restricts the penetration of drugs by formation of a diffusion barrier (Nett *et al.*, 2011); hence, only the most superficial layers are in contact with lethal doses of antibiotics. Planktonic cells generally rely on irreversible genetic changes to maintain a resistant phenotype, whereas biofilms are able to persist due to their physical presence and the density of the population, which provides an almost inducible resistant phenotype irrespective of defined genetic alterations (Ramage *et al.*, 2012).

Several molecular mechanisms of resistance to antifungal agents in *C. albicans* have been described. In particular, these include the increased efflux of antifungal agents due to the overexpression of efflux genes, *CDR1*, *CDR2* (the family of ABC membrane transport proteins – the ATP-binding cassette) (Sardi *et al.*, 2011) and *MDR1* (the Major Facilitator protein family) as well as amino acid substitutions in the enzyme Erg11p (lanosterol 14- $\alpha$ -demethylase), encoded by the gene *ERG11*. *CDR1*, *CDR2* and other genes are often coregulated and are overexpressed simultaneously; therefore, it is believed that genes regulating this expression can be mutated (Staib *et al.*, 2000).

Given the increase in fungal infections, two triazoles (voriconazole and posaconazole) and three echinocandins (anidulafungin, caspofungin and micafungin) have been approved to treat and prevent these infections (Mattiuzzi & Giles, 2005) (Table 1). Of the three classes of antifungal agents currently in clinical use, only amphotericin B and the echinocandins, e.g. caspofungin, have demonstrated consistent *in vitro* activity against *C. albicans* biofilms (Kuhn *et al.*, 2002). Even with these two agents, *Candida* biofilm-related infections are extremely difficult, if not impossible, to eradicate, and infected medical devices typically must be removed (Montejo, 2011). One of the barriers to the development of new antifungal agents that are active against biofilms is that very few assays are available for either the characterization or identification of new molecules with activity towards biofilms (Ramage *et al.*, 2009). *Candida* is the fourth most common cause of bloodstream infections in hospitalized patients (Nucci *et al.*, 1998). Up to

**Table 1.** Conventional antifungal drugs and their cellular targetsAdapted source: Martinez-Rossi *et al.* (2008).

Chemical class	Drug	Target
Azoles	Miconazole	Ergosterol synthesis
	Ketoconazole	
	Fluconazole	
	Itraconazole	
	Terconazole	
	Voriconazole	
Polyenes	Posaconazole	Ergosterol (membrane function)
	Amphotericin B	
	Nistatin	
Pyrimidine	Flucytosine	DNA and RNA synthesis
Echinocandins	Caspofungin	Glucan synthesis
	Micafungin	
	Anidulafungin	

40 % of patients with intravenous catheters from which *Candida* has been isolated have underlying fungaemia (Kuhn *et al.*, 2002), and the mortality rate for patients with catheter-related candidaemia approaches 40 % (Kuhn *et al.*, 2002).

Although *C. albicans* is the most commonly isolated fungal species, other species are being isolated with increasing frequency (Kuhn *et al.*, 2002). *C. parapsilosis* has become the second most commonly isolated fungal organism in several studies (Kuhn *et al.*, 2002). Pires *et al.* (2011a) isolated 17 environmental isolates of *C. parapsilosis* from the hydraulic circuit of a haemodialysis centre. This species is of particular concern in critically ill neonates, where it is known to be associated with central lines and parenteral nutrition (Kuhn *et al.*, 2002). Antifungal therapy alone is insufficient for curing these infections, and affected devices generally need to be removed (Nucci *et al.*, 1998; Rex *et al.*, 2000). Removal of these devices has serious implications in the case of infected heart valves, joint prostheses and central nervous system shunts. The reason why device removal is necessary has been, until recently, a mystery. However, several studies have reported a near-total resistance to antifungal agents exhibited by biofilm-associated *Candida* (Chandra *et al.*, 2001; Nucci *et al.*, 1998). *C. albicans* biofilms *in vivo* (Martinez & Fries, 2010; Mukherjee & Chandra, 2004) and *in vitro* demonstrated higher susceptibility when treated with a combination of amphotericin B and posaconazole than when treated with single drugs (Martinez & Fries, 2010; Tobudic *et al.*, 2010). Combining the use of echinocandins with other drugs that have antifungal activity is becoming an important alternative form of therapy in mycoses caused by fungi that are resistant to standard antifungal monotherapy or in certain biofilm-associated diseases.

Persister cells are an important mechanism of resistance in chronic infections (Fauvert *et al.*, 2011; Ramage *et al.*,

2012), and these have gathered some attention recently in the context of fungal biofilms (Lewis, 2008; Bink *et al.*, 2011). In *C. albicans* biofilms, a small subset of yeast cells have been described that are highly resistant to amphotericin B following adhesion, which is independent of the upregulation of efflux pumps and cell membrane composition (LaFleur *et al.*, 2006). The first study described persister cells in fungi as a subpopulation of highly tolerant cells. In this study, *C. albicans* persisters were detected only in biofilms and not in various planktonic populations (LaFleur *et al.*, 2006; Ramage *et al.*, 2012). When a biofilm was killed with amphotericin B and reinoculated with cells that survived, a new biofilm was produced with a new subpopulation of persisters; this suggested that these cells were not mutants but phenotypic variants of the wild-type. In this clinically relevant scenario, prolonged and ineffectual antifungal treatment may be beneficial to the biofilm population, and this effect may be responsible for antimicrobial drug failure and relapsing infections.

### **Candida biofilm and new antifungal strategies**

The increasing incidence of drug-resistant pathogens and the toxicity of existing antifungal compounds have drawn attention towards the antimicrobial activity of natural products. The small number of drugs available for fungal treatment, most of which are fungistatic, and the emerging resistance to antifungal agents encourage the search for alternative treatments (de Oliveira *et al.*, 2006). Plants are good options for obtaining a wide variety of drugs and have been used in medicine for a long time; in particular, they are extensively used in folk medicine because they represent an economic alternative, are easily accessible and can be applicable to various pathologies (Sardi *et al.*, 2011; Cruz *et al.*, 2007). Plants therefore constitute an excellent source for substances that can be used in the formulation of new antifungal agents (Holetz *et al.*, 2002).

Many studies have focused on biofilms because they are more resistant to antimicrobials and host defences. Rossignol *et al.* (2011) studied the 18-amino acid cationic, tryptophan-rich, ApoEdpL-W peptide, derived from human ApoE apolipoprotein, which was shown to have antifungal activity against pathogenic yeasts of the *Candida* genus (except *C. glabrata*). ApoEdpL-W was active against planktonic cells and early-stage biofilms but less active against mature biofilms, possibly because of its affinity for extracellular matrix  $\beta$ -glucans. Moreover, ApoEdpL-W partially prevented the formation of biofilms on medical devices. Another study by Mandal *et al.* (2011) demonstrated *in vitro* growth inhibition of *C. tropicalis* and disrupted biofilm formation in a concentration-dependent manner with purified Tn-AFP1 (the peptide derived from *Trapa natans*). That study also demonstrated downregulation of *MDR1* and *ERG11* gene expression using real-time PCR analysis. However, usnic acid had inhibitory and fungicidal activity against biofilms of *C. parapsilosis* and *C. orthoparapsilosis* in an isolated environment (Pires *et al.*,

2012). Stanciuc *et al.* (2011), with the objective to assess the antibacterial and antifungal potential of some Romanian medicinal plants such as *Arnica montana*, *Artemisia absinthium* and *Urtica dioica*, conducted an antimicrobial screening by obtaining plant extracts and testing them on a series of Gram-positive and Gram-negative bacteria and two fungal strains. The extracts presented antimicrobial activity preferentially directed against planktonic fungal and bacterial growth; an effect on biofilm formation and development was shown only against *S. aureus* and *C. albicans*. *In vitro* assays indicated that the studied plant extracts were a significant source of natural alternatives to antimicrobial therapy, thus avoiding antibiotic therapy, the use of which has become excessive in recent years. Several studies have been conducted using natural products to evaluate interference in *C. albicans* biofilms (Furletti *et al.*, 2011; Tawechaisupapong *et al.*, 2010; Coleman *et al.*, 2010) (Table 2). Pires *et al.* (2011b) investigated the anticandidal activity of 16 essential oils on planktonic and biofilm cultures of the *C. parapsilosis* complex. The most active essential oil was cinnamon oil, which showed anticandidal activity against *C. orthopsilosis* and *C. parapsilosis* in both suspension (MIC=250 and 500 g l<sup>-1</sup>, respectively) and biofilm [minimum biofilm reduction concentration (MBRC)=1000 and 2000 g l<sup>-1</sup>, respectively] cultures. Cinnamon oil also inhibited biofilm formation (MBIC) at concentrations above 250 g l<sup>-1</sup> for both species tested. Further research is required to evaluate the antifungal potential of various plants against microbial biofilms because the total elimination of biofilms is difficult.

Another promising antifungal strategy is the use of silver nanoparticles (AgNPs). Silver is a well-known antimicrobial agent and has a long history of application in medicine with a well-tolerated tissue response and a low toxicity profile. Silver is more toxic than many other metals against a broad spectrum of sessile bacteria and fungi that colonize plastic surfaces (Nam, 2011).

More recently, the use of AgNPs has been suggested for coating medical titanium implants (Alviano *et al.*, 2005; Huang *et al.*, 2012; Lu *et al.*, 2011; García-Contreras *et al.*, 2011) in the hope of inhibiting biofilm formation and thereby reducing the incidence of microbial infections and rejection.

Silver has been described as being 'oligo-dynamic', meaning it has a toxic (bactericidal/fungicidal) effect on living cells even at low concentrations (Dastjerdi & Montazer, 2010). Silver has been shown to interfere with microbial DNA replication within bacteria and fungi. Metal ions such as silver inhibit the multiplication of micro-organisms. Silver ions can also lead to protein denaturation and cell death because of their reaction with nucleophilic amino acid residues in proteins and their attachment to thiol, amino, imidazole, phosphate and carboxyl groups of membrane proteins or enzymes (Percival *et al.*, 2005). AgNPs have also frequently been shown to inhibit yeast growth (Kim *et al.*, 2007; Mastrolorenzo *et al.*, 2000), and their antifungal activity against certain species such as *Trichophyton* species and *Candida* species (Kim *et al.*, 2008) is well documented. These studies show evidence for the molecular mechanism of AgNP activity, whereby AgNPs act on and inhibit a number of oxidative enzymes such as yeast alcohol dehydrogenase through the generation of reactive oxygen species (Kim *et al.*, 2007). This leads to protein inactivation and induces apoptosis via mitochondrial pathways (Hsin *et al.*, 2008). AgNPs have been demonstrated to exhibit a desirable and promising antifungal effect in several studies, with no serious side effects to the host.

Anti-*Candida* antibodies can reduce the binding of *Candida* to various surfaces (Rodier *et al.*, 2003; Elguezabal *et al.*, 2004; López-Ribot *et al.*, 2004; Bujdaková *et al.*, 2008). Studies with antibodies have been performed by various authors to test their effects on various fungal and bacterial organisms.

**Table 2.** Antifungal activity of natural products against a variety of biofilms

Natural products	Biofilm	Reference
Peptide (18-amino acid cationic ApoEdpL-W)	All species of <i>Candida</i> except <i>C. glabrata</i>	Rosignol <i>et al.</i> (2011)
Peptide ( <i>Trapa natans</i> )	<i>C. tropicalis</i>	Mandal <i>et al.</i> (2011)
Romanian plant extracts	<i>C. albicans</i>	Stanciuc <i>et al.</i> (2011)
<i>Coriandrum sativum</i> L. (oil)	<i>C. albicans</i>	Furletti <i>et al.</i> (2011)
Cinnamon oil	<i>C. parapsilosis</i>	Pires <i>et al.</i> (2011b)
Usnic acid	<i>C. parapsilosis</i> and <i>C. orthoparapsilosis</i>	Pires <i>et al.</i> (2012)
<i>Boesenbergia pandurata</i> and <i>Piper sarmentosum</i>	<i>C. albicans</i>	Tawechaisupapong <i>et al.</i> (2010)
Saponins	<i>C. albicans</i>	Coleman <i>et al.</i> (2010)
Polyphenols (green tea)	<i>C. albicans</i>	Evensen & Braun (2009)
<i>Ocimum americanum</i> L. essential oil	<i>C. albicans</i>	Thaweboon & Thaweboon (2009)
<i>Caesalpinia ferrea</i> Martius fruits	<i>C. albicans</i>	Sampaio <i>et al.</i> (2009)
<i>Cassia spectabilis</i> extract	<i>C. albicans</i>	Sangetha <i>et al.</i> (2009)
<i>Croton cajucara</i> Benth linalool-rich oil	<i>C. albicans</i>	Alviano <i>et al.</i> (2005)
Garlic extract	<i>C. albicans</i>	Shuford <i>et al.</i> (2005)

Fujibayashi *et al.* (2009) prepared a polyclonal anti-*C. albicans* antibody in chicken egg yolk (anti-*C. albicans* IgY) and investigated its *in vitro* effectiveness in preventing *C. albicans* adherence and biofilm formation. The results demonstrated a significant reduction in the adherence of *C. albicans* SC 5314 to human oral epithelial cells in a dose-dependent manner. The same effect was also observed in other *Candida* species including serotypes A and B. Furthermore, IgY inhibited *C. albicans* biofilm formation in serum-free medium, but the inhibition was slightly restored in medium conditioned with 10% serum. This demonstrates that anti-*C. albicans* IgY cross-reacts with various *Candida* species and may have a protective effect against oral candidiasis and reduce the dissemination of *Candida* species. This effect may be due to the blocking of the binding of *Candida* species to host cells. However, this blocking did not play a role when *Candida* formed a germ tube in the presence of serum. Therefore, anti-*C. albicans* IgY could be considered for prophylactic immunotherapy under limited conditions.

The application of photodynamic therapy has been investigated regarding its inactivation of micro-organisms that are pathogenic to the human host. Several authors have associated light emitting diodes (LEDs) with other substances (Ribeiro *et al.*, 2012; Chen *et al.*, 2012; Junqueira *et al.*, 2012). Coleman *et al.* (2010) observed that saponins may preferentially bind to fungal ergosterol compared to cholesterol, and the fungus displayed increased susceptibility to photodynamic inactivation when used in combination with photosensitizer compounds due to the ability of saponins to increase cell permeability, thereby facilitating penetration of the photosensitizers. This result suggests that it may be a suitable chemical scaffold for a new generation of antifungal compounds.

Dovigo *et al.* (2011) described the combined use of curcumin with LEDs for the inactivation of *C. albicans* in both planktonic and biofilm forms. Suspensions of *Candida* were treated with nine curcumin concentrations and exposed to LEDs at different fluences. The photodynamic effect was greatly increased in the presence of curcumin.

S. Khan *et al.* (2012) studied gold nanoparticle-enhanced photodynamic therapy including the use of methylene blue against recalcitrant pathogenic *C. albicans* biofilms. Antibiofilm assays and microscopy studies showed significant reduction of biofilm; therefore, gold nanoparticle conjugate-mediated photodynamic therapy may be used against nosocomially acquired refractory *C. albicans* biofilms.

## Conclusions

The increased incidence of systemic mycoses caused by *Candida* species in hospitalized patients is an important cause of morbidity and mortality worldwide, especially in critically ill patients. Biofilms play an important role in the perpetuation of these infections primarily with respect to

their ability to adhere to various medical devices. The interest in natural products has increased regardless of whether they are associated with other therapies. In addition, other promising strategies such as the use of nanoparticles, antibodies and, more recently, photodynamic inactivation for treating fungal infections have been studied extensively because of their promising therapeutic potential. There is a need to search for new products with effective antifungal abilities due to the adverse side effects of existing medications, increasing emergence of strains that are resistant to conventional antifungal agents, and the formation of biofilms in medical devices and tissues.

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