Multidrug resistance in pathogenic yeasts: emphasis on the role of ABC and MFS multidrug transporters

Catarina Costa[1,2], **Pedro Pais**[1,2] and **Miguel C. Teixeira**[1,2]

[1] Department of Bioengineering, Instituto Superior Técnico, Universidade de Lisboa, Av Rovisco Pais 1049-001 Lisbon, Portugal

[2] Biological Sciences Research Group, Institute for Bioengineering and Biosciences, Instituto Superior Técnico, Universidade de Lisboa, Av Rovisco Pais 1049-001 Lisbon, Portugal

In the past decades, infections caused by opportunistic pathogens have increased dramatically due to the growing number of immunocompromised patients. Also, a significant increase in the prevalence of resistance to antifungal agents was registered, resulting in high levels of morbidity, mortality and in an increase in health care costs. Therefore, a understanding the mechanisms of antifungal drug resistance is crucial to improve the methods to diagnose resistance, develop new antifungal agents for the treatment of infections caused by resistant fungi and prevent the emergence of resistance in the first place.

The most clinically relevant pathogenic yeasts belong to the *Candida* and *Cryptococcus* genera, and are represented mainly by *Candida albicans*, *Candida glabrata*, *Candida parapsilosis*, *Candida tropicalis* and *Candida krusei*, which altogether cause approximately 99% of all known human cases of candidiasis, and by *Cryptococcus neoformans*.

To treat infections caused by pathogenic fungi, four major classes of antifungal drugs (azoles, polyenes, fluoropyrimidines and echinocandins), with different targets and modes of action, are currently used in clinical practice. However, simultaneous resistance to one or more of these antifungals has been registered, compromising therapeutic effectiveness. The limited number of antifungal drugs available and their use as prophylactic agents has further increased the prevalence of fungal pathogens, which are considered innately resistant to some of the antifungal classes, such as *C. glabrata* and *C. krusei*. The understanding of the complex mechanisms underlying the acquisition of multidrug resistance (MDR) is, thus, crucial to devise new strategies to diagnose, treat and eradicate fungal infections.

Since multidrug resistance is often attributed to the action of multidrug efflux pumps from both the ATP-Binding Cassette (ABC) and the Major Facilitator Superfamily (MFS), this chapter is dedicated to the mechanisms by which pathogenic yeasts are able to develop resistance towards several antifungal drugs used in clinical practice, with particular emphasis on the role of the multidrug efflux pumps belonging to these superfamilies.

Keywords: multidrug resistance; multidrug transporters; antifungal drugs; *Candida* species; *Cryptococcus neoformans*

1. Epidemiology

Pathogenic yeasts, such as those belonging to the *Candida* genus (*Candida* spp.), are able to cause infections in immunocompromised individuals, that can range from superficial infections (in the skin, epithelium, etc.) to disseminated infections (invasive penetration of vital organs), which can ultimately result in death. In the United States of America, about 11.5% of the 80.000 bloodstream infections that are detected every year are caused by yeasts of the *Candida* genus and the mortality rate associated reaches over 30% of the cases [1].

The most relevant pathogenic *Candida* species are *Candida albicans*, *Candida dubliniensis*, *Candida glabrata*, *Candida krusei*, *Candida parapsilosis* and *Candida tropicalis* [2]. Pathogenic *Candida* species are commonly isolated from the oral cavity and the vulvovaginal and urinary tract. Currently, infections caused by *C. albicans* are still the most frequent, but its incidence has dropped to give rise to those caused by *C. glabrata*, *C. tropicalis* and *C. parapsilosis*, a fact that seems to be related with a decreased susceptibility of these species towards the drugs used in clinical practice [3, 4]. Infections caused by these four *Candida* species are responsible for around 95% of all identified *Candida* infections.

The incidence of infections caused by *Candida* species varies according to the geographic region and the infection site. For instance, the incidence of *C. glabrata* is higher in adults than in children and even lower in newborns [5]. On the other hand, infections caused by *C. parapsilosis* have their highest incidence in newborns, transplanted individuals and patients receiving parenteral nutrition [6]. *C. tropicalis* is often isolated from neutropenic patients or individuals suffering from malignant diseases [7].

Infections caused by *C. glabrata* are the second most frequent in North America and, of all caused by non-*albicans* *Candida* species, are the ones with the highest mortality rate [8]. It is also important to refer that *C. albicans* and *C. glabrata* have been isolated together from around 70% of the patients suffering from oral candidiasis, suggesting that these two species might have a synergistic effect on infections occurring in this niche [9].

C. parapsilosis is the second most frequently isolated *Candida* species in bloodstream infections, right after *C. albicans* [10], in both Europe and Latin America [11].

Patients infected by *C. tropicalis* often require longer periods of hospitalization than those infected with *C. albicans*. This could be related with a higher level of virulence exhibited by this species and to the fact that it is less susceptible to the antifungal drugs used in clinical practice. Also, in a study performed in 12 Brazilian hospitals, *C. tropicalis* was the second most frequently isolated *Candida* species, present in 33-48% of the infected patients [7, 12].

Outside the *Candida* genus, one of the most common pathogenic yeast is *Cryptococcus neoformans*. Unlike what is known for *Candida* species, *C. neoformans* can cause disease both in immunocompromised and immunocompetent individuals. Its basidiospores can be inhaled and disseminate within the organism, through the respiratory tract, causing pulmonary infections and subsequently, due to this organism preference for the central nervous system, cause life-threatening meningoencephalitis [13]. *C. neoformans* is found worldwide, while a related species, *Cryptococcus gattii*, which is endemic in tropical regions, was found mainly in Australia, Papua New Guinea and in an outbreak in Canada [14]. The clinical prevalence of *C. neoformans* has continuously increased in the past 2 decades in HIV-infected and organ transplanted patients as well as in individuals undergoing chemotherapy. *C. neoformans* is the most common fungal pathogen in the central nervous system and the third most common cause of central nervous system complications in AIDS patients. It is estimated that approximately 1 million new cases of cryptococcal meningitis are reported each year and the mortality rate attributable to *C. neoformans* in AIDS patients is approaching alarming proportions, reaching almost 30% [14], and representing a serious threat to public health and especially immunocompromised patients.

Cryptococcus infections also represent a relevant threat in organ-transplant patients, as they occur in up to 5% of these patients with an overall mortality rate reaching 42% [15]. As in the case of *Candida* infections, azoles can be used in the fight against cryptococcosis, but most cases of central nervous system infections are treated with a combination of amphotericin B and flucytosine as a first line treatment. However, some researchers consider cryptococcal infections to be virtually incurable, given the ability of these yeasts to remain dormant within host cells [14].

2. **Resistance against antifungal therapy**

Infections caused by *Candida* and *Cryptococcus* spp. are often hard to treat, and the high mortality rates associated to these fungal pathogens result from the late diagnosis and from the acquisition of antifungal drug resistance..

Antifungal drugs commonly used to treat infections caused by these pathogenic yeasts include azoles, often used as the first line therapy and in prophylaxis, which are subdivided in imidazoles (e.g. clotrimazole, ketoconazole) and triazoles (e.g. fluconazole, posaconazole); as well as the newest family of antifungal drugs, the echinocandins (e.g. caspofungin, anidulafungin and micafungin). Drugs such as polyenes (e.g. amphotericin B) and pyrimidine analogs (e.g. flucytosine) are more commonly used for combined therapy, often as last resource. These four families of antifungal drugs were developed in the past decades in an attempt to respond efficiently to the growing number of clinical isolates resistant to the previously used antifungals. However, this development of resistance occurs much more rapidly than the development and implementation of new drugs and, therefore, it is of the utmost importance to understand the mechanisms behind this phenomenon.

There are mainly four ways by which fungal pathogens can acquire resistance to antifungal drugs: i) drug inactivation; ii) drug target alteration; iii) lower drug uptake and iv) active drug extrusion [16, 17]. In clinical practice it would be of paramount importance to identify which resistance mechanism is taking place in a certain fungal isolate, as well as identify resistance patterns to different drugs across species in order to adopt a personalized and efficient treatment in each case. For instance, azoles have been widely used in the treatment of *Candida* spp; however, resistance episodes to these drugs have been increasingly reported, especially for fluconazole, widely used in the treatment of systemic infections. Additionally, despite azole resistant strains of *C. neoformans* are more rarely encountered in comparison with *Candida* spp., several clinical isolates, collected from cerebrospinal fluid, were found to exhibit moderate to high resistance to fluconazole [18]. Echinocandins have rose in popularity as a first line treatment of *Candida* infections, due to their relatively lower resistance values when compared with other antifungals used during a more extendend period of time, such as azoles. Unfortunately, they are clinically ineffective against *Cryptococcus* spp. [19], as *C. neoformans* is intrinsically resistance to these drugs. Although the mechanism behind this observation is still unknown, it is postulated that it can be derived from drug efflux from the cells or degradation of the drug both intra or extracellularly. From all antifungal drugs used in clinical practice, amphotericin B is extensively used either alone or in combination with other antifungals as a first line treatment for cryptococcal infections. However, amphotericin B resistant *C. neoformans* clinical isolates have been reported, further increasing the need to understand how antifungal drug resistance by fungal pathogens develops. The number of cases describing resistance to previously effective drugs is growing each day, further impairing effective treatment in clinical practice. Close monitoring of resistance patterns across both fungal species and drug families is gaining increasing importance in an effort to keep track of multiple resistance profiles worldwide. Table 1 shows the results of a compilation of studies addressing the percentage of clinical isolates, from several geographic regions, found to be resistant to four antifungal drugs used in the treatment of systemic infections by pathogenic yeasts, demonstrating the worldwide abundance of antifungal resistance across several pathogenic yeast species.

Resistance to several antifungal drugs from the same family (e.g. several azoles) has been widely described and it is now a major concern in terms of resistance to multiple drugs. However, acquisition of resistance to drugs from distinct families represents another relevant concern. In fact, a study by Joseph-Horne and colleagues [20] found *C. neoformans* isolates that displayed cross-resistance against amphotericin B (polyene) and fluconazole (azole) and reduced levels of intracellular drug accumulation, proposing a possible role for a common multidrug transporter. Similarly, cross-resistance between drugs from distinct families has also been reported in *Candida* spp. Clinical isolates from *C. tropicalis* and *C. glabrata* were found to be resistance against both azoles and amphotericin B [21-23]. Interestingly, a case was also reported where an agricultural antifungal, prochloraz, induced cross-resistance to azole drugs. The underlying mechanism responsible for this phenomenon was found to be the upregulation of multidrug transporters [24]. Some concern is also growing related to the use of echinocandins and development of cross-resistance. Signs of multidrug resistance among more traditional antifungals, such as azoles, and the more recent echinocandins have already been reported [25-27]. Not only multiple antifungal resistance has been reported in isolated episodes, but also cross-resistance between more than one family of antifungal drugs is increasing among clinical isolates obtained from patients in several epidemiological studies, especially concerning *Candida* spp. Multiple resistance across *C. albicans*, *C. glabrata*, *C. tropicalis* and *C. parapsilosis* have been found in 22% of the isolates collected [28]. Accordingly, 11.1-58.3% of *C. glabrata* isolates resistant to echinocandins were found to have cross-resistance against fluconazole or other azoles as well [25, 26, 29]. Also for *C. tropicalis*, 6.3% of the isolates found in one study were resistant to azoles and echinocandins [26]. Not only have cross resistance between echinocandins and fluconazole been reported, but also between echinocandins and amphotericin B [30].

Table 1 Estimate on the percentage (%) of non-susceptible or resistant clinical isolates against four antifungal drugs used in clinical practice, across several geographic regions [28, 30-56].

Species	% resistance			
	Fluconazole	**Amphotericin B**	**Caspofungin**	**5-Flucytosine**
C. albicans	0.5-33.3	3.2-6.8	3.8-6	4.4-45
C. glabrata	3.4-70	2.5-60	1.3-16.2	0.8-35
C. tropicalis	1.3-37.7	6.7-18.1	0.7-13	6.7-25
C. parapsilosis	21-29.7	4.5-20	0.4-1.5	2-7.7
C. neoformans	4.8-33	3.2-17.4	≤5	≤1.6

3. Multidrug resistance: the role of multidrug transporters

Antifungal drug resistance is particularly more serious when it develops not only against the administered drug, but also to other non-related chemical compounds. This multidrug resistance phenomenon relies, in most cases, on the activity of multidrug efflux pumps that belong in fungi to two superfamilies: the ATP-binding cassette superfamily (ABC) – whose transporters are energized by ATP hydrolysis – and the Major Facilitator Superfamily (MFS) – whose transporters are energized by the proton gradient across the membrane.

In all pathogenic *Candida* species, including *C. glabrata* and *C. krusei*, that have a decreased susceptibility towards azoles when compared to *C. albicans*, the action of these transporters is known to be closely related to the acquisition of drug resistance, in particular to azoles. Similarly to the case of *Candida* spp., the expression of multidrug resistance transporters is one of the major drug resistance mechanisms displayed by *C. neoformans*.

3.1 ABC Superfamily Efflux Pumps

The ATP-binding cassette transporters have a ubiquitous distribution in living organisms, going from bacteria to man [57, 58]. These transporters have been widely characterized and some of them have been found to have clinical significance in what concerns antifungal drug resistance [57]. In most cases, these transporters were found to be intrinsically related to multidrug resistance, and particularly to antifungal drug resistance. For example, in *C. albicans*, *CaCDR1* – the first ABC transporter described in a fungal pathogen [59] - and *CaCDR2*, two homologs of *ScPDR5*, were found to be upregulated in fluconazole-resistant isolates [60], the disruption of the former turning this species much more susceptible to azoles [61]. Both these transporters were found to be regulated by the transcription factor CaTac1, the most important transcription regulator of multidrug resistance in *C. albicans*, and gain of function mutations in Tac1 were found to lead to the constitutive expression of *CaCDR1* and *CaCDR2* [62]. In fluconazole-resistant *C. dubliniensis* isolates the overexpression of *CdCDR1* and *CdCDR2* was also observed [63].

C. glabrata expresses three well studied ABC transporters, CgCdr1, CgPdh1 (or CgCdr2) and CgSnq2. The deletion of the first in an azole-resistant strain, leads to the intracellular accumulation of fluconazole and hypersusceptibility to other azoles, and further CgPdh1 deletion aggravates this phenotype, similarly to what occurs in *C. albicans* [64, 65]. Deletion of CgSnq2 was also found to lead to an increased susceptibility towards different azoles and 4-nitroquinoline-*N*-oxide, in an azole-resistant strain [66]. Interestingly, all these transporters have been found to be regulated by the transcription factor CgPdr1, known to be the major regulator of multidrug resistance in *C. glabrata* [67].

A homolog of *CDR1* was also found in *C. tropicalis* and shown to be overexpressed in a strain that developed resistance against fluconazole [68]. In *C. krusei*, which is intrinsically resistant to fluconazole, two partial genes encoding for ABC transporters, *CkABC1* and *CkABC2*, were cloned [69] and *CkABC1* expression was later shown to be related with increased azole resistance in this species, indicating its potential role as a multidrug resistance transporter [70, 71].

The most well characterized ABC transporter of *C. neoformans* is Afr1 [19]. The disruption of this gene was found to lead to a higher susceptibility to azoles, while the expression of this gene, from a plasmid, was able to rescue the susceptibility phenotype of the deletion mutant [72]. Additionally, CnAfr1 appears to be not only required for azole drug resistance in *C. neoformans*, but also for virulence, as its overexpression was also found to increase strain virulence in mice [73] and to modulate the host's immune system [74]. A second ABC transporter in *C. neoformans*, cnMdr1, has also been characterized. Its expression in a susceptible *Saccharomyces cerevisiae* strain resulted in increased resistance to fluconazole and itraconazole, but not to echinocandins or polyenes [75].

3.2 Major Facilitator Superfamily Efflux Pumps

When compared with the ABC transporters, a lot less is known about the MFS transporters that are involved in drug efflux. These transporters belong to two families: the DHA1 (drug:H^+ antiporter family 1), with 12 transmembrane domains, and the DHA2 (drug:H^+ antiporter family 2), with 14 transmembrane domains.

The first MFS drug efflux pump ever to be identified in pathogenic fungi was *Candida albicans* Mdr1. This transporter has homologs in several other *Candida* species and was shown to be regulated by the transcription factor CaMrr1, appearing to be specifically involved in fluconazole resistance by decreasing its accumulation inside the cell [61]. In *C. dubliniensis*, the homolog of this transporter was found to be overexpressed in most fluconazole resistant isolates, while upon elimination, resistance to this antifungal drug was completely abolished [63].A *MDR1* homolog was also found in *C. tropicalis* and shown to be overexpressed in a strain that had acquired resistance towards fluconazole [66].

In *C. glabrata*, CgFlr1 was shown to be involved in benomyl resistance, a pesticide used in agriculture, but no connection was found between this transporter and antifungal resistance [76]. Meanwhile, other transporters, such as CgAqr1, CgQdr2 and CgTpo3, have also been studied. CgAqr1 was found to be involved in flucytosine resistance mediating, directly or indirectly, the accumulation of this drug. It also confers resistance to acetic acid, which is frequently found in the vaginal mucosa, probably contributing to the persistence in this niche [77]. CgQdr2 was identified as a determinant of resistance to imidazoles such as clotrimazole, miconazole, tioconazole and ketoconazole. Also, this transporter was shown to play an active part in the efflux of these drugs, and its expression was found to be activated in clotrimazole-stressed cells, under the control of the transcription factor CgPdr1 [78] , the major regulator of multidrug resistance in *C. glabrata*. CgTpo3 was found to be involved in the resistance to both imidazoles and triazoles, such as fluconazole, and to the polyamine spermine, found in high concentrations in the urogenital tract. Moreover, *CgTPO3* was upregulated in *C. glabrata* cells exposed to spermine, in a CgPdr1-dependent manner. Also, this transporter seems to be involved in the efflux of azoles and spermine, and the control of the intracellular concentration of this polyamine seems to be important for azole resistance [79].

It is also important to refer that in a recent exhaustive compilation of the number of MFS transporters present in eight species of pathogenic fungi clinically relevant (*C. albicans*, *C. glabrata*, *C. guilliermondii*, *C. parapsilosis*, *C. lusitaniae*, *C. tropicalis*, *C. neoformans* and *Aspergillus fumigatus*) it was shown that their genomes encode about 300 putative DHA transporters and that only 14 of them have already been characterized, corresponding to approximately 5% of the total number of transporters [80].

Also, from BLAST analysis using *S. cerevisiae* PDR genes as queries, 32 PDR transporters were identified in four medically important human fungal pathogens: *C. albicans*, *C. glabrata*, *Aspergillus fumigatus* and *Cryptococcus neoformans* [57]. In most cases, these PDR transporters were found to be intrinsically related not only with MDR, but particularly with antifungal drug resistance.

These observations highlight the importance of further studying these transporters to fully understand the mechanisms of antifungal drug resistance acquisition in pathogenic fungi.

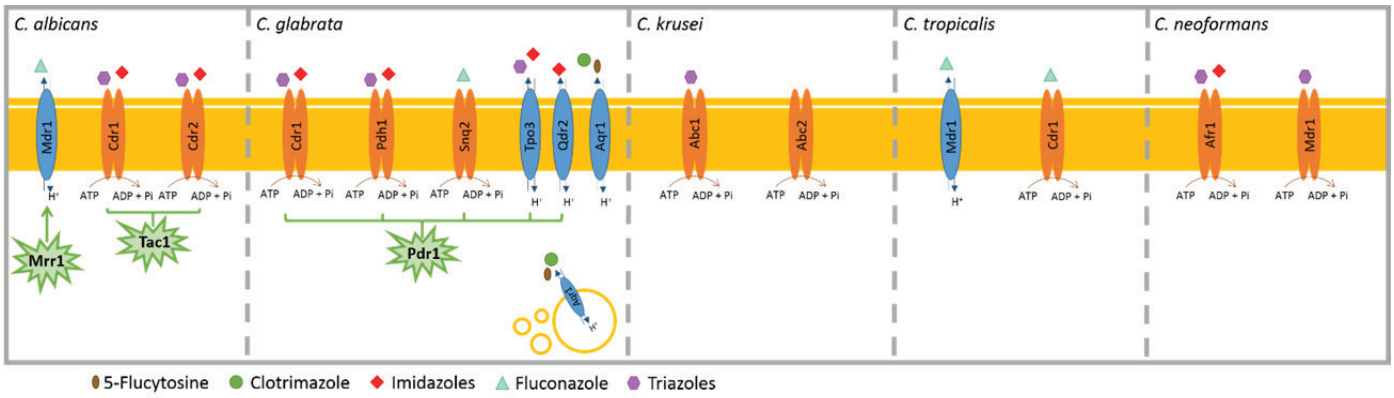

Fig. 1 ABC (orange) and MFS (blue) transporters present in *C. albicans*, *C. glabrata*, *C. krusei*, *C. tropicalis* and *C. neoformans* described as being involved in the acquisition of multidrug resistance. In green are represented the transcription factors known to be involved in the regulation of these transporters.

4. How to overcome multidrug resistance?

The currently used antifungal therapies are becoming limited in their ability to treat infections caused by pathogenic yeasts. The development of new antifungals appears, thus, imperative. Researchers all over the world have been testing new compounds, many of which of natural origin, or several drugs already available in the market for their antifungal ability, but the advance has been slow.

It is also important to refer that the increasing number of fluconazole resistant strains has been suggested to derive from an extensive use of this antifungal as a prophylactic agent. New forms of prophylaxis that do not result in a direct contact of the fungal pathogens with the therapeutic drugs that are ultimately used to treat infections caused by them are, thus, required. This could be a valuable lesson for a more careful usage of new antifungal drugs.

Moreover, the understanding of the mechanisms that lead these organisms to acquire resistance is essential to allow the identification of new targets for the development of new drugs. Genome-wide studies could help provide a global comprehension of multidrug resistance, which is known to be a multifactorial phenomenon.

Given that the action of multidrug efflux pumps plays a tricky part in this equation, finding and developing drugs that block them could also be an effective way to overcome this problem. One of the possible strategies to be used in overcoming multidrug resistance phenomena is combination therapy. Other than the combined use of amphotericin B and flucytosine in the treatment of cryptococcal meningitis, this approach is usually not applied with other antifungal drugs or other pathogenic yeasts. The combined treatment with two different drugs targeting two steps of the same pathway (e.g. allylamines and azoles, both targeting membranar ergosterol content) or the use of two drugs targeting two distinct pathways (e.g. echinocandins and polyenes, targeting the cell wall and cell membrane components, respectively) could yield better results in overcoming multidrug resistance, as well as helping decrease the dosage of antifungals required when compared to single-agent therapies.

Once multidrug resistance is commonly associated with the expression of multidrug resistant transporters, some strategies have been developed to inhibit their action and chemosensitize antifungal resistant cells. Inhibitors are designed to influence the cellular ATP level, this way affecting the power supply for ABC transporters; influence membrane phospholipids to increase membrane permeability for ions that decrease the activity of ABC transporters; or establish specific interactions with proteins, such as the transporter itself [81]. More recently, a derivative from a D-octapeptide, named RC21v3, was used to inhibit *C. albicans* Cdr1 multidrug transporter. RC21v3 is a high-affinity inhibitor that interacts with the extracellular surface of the transporter protein, resulting in chemosensitization of azole-resistant clinical isolates to fluconazole and other azole drugs [82]. Following a similar approach, a set of transmembrane peptide mimics were develop in order to disrupt the activity of the same Cdr1 multidrug transporter in *C. albicans* [83]. These synthesized peptides are able to target the transmembrane helixes of the target protein, interfering with specific interactions in the protein structure. This way, the peptide mimics are able to prevent proper assembly and folding of the multidrug transporter, effectively acting as antagonists to block drug efflux. These strategies represent a potential way of overcoming drug resistance by directly disrupting the activity of multidrug transporters, hopefully shutting down a major mechanism for multiple antifungal resistance. Alternatively, approaches using natural inhibitors have also been pursued. Tanabe and colleagues [84] described two natural cyclodepsipetides (unnarmicin A and C) obtained from marine-derived fungi and bacteria extracts that were able to sensitize *C. albicans* cells overexpressing multidrug transporters, acting as efflux pump inhibitors. Another study described the use of jatrophane diterpenoids from herbaceous origin that strongly inhibited drug-efflux activity of the *C. albicans* ABC transporter Cdr1 and the MFS transporter Mdr1, by effectively competing with the antifungal for efflux by these multidrug transporters [85].Furthermore, it is important to develop new methods of diagnosis, that are more rapid and

efficient, and that allow unequivocal identification of which strain(s) are causing the infection and to which compounds do they are resistant to, or at least prone to develop resistance to. An overall better understanding of the patient's clinical history and of the strain that is causing the infection is determinant to choose the most efficient therapeutic option.

Acknowledgments The work carried out by the authors in this field was supported by "Fundação para a Ciência e a Tecnologia" (FCT) (Contracts PTDC/EBB-BIO/119356/2010 and UID/BIO/04565/2013, and post-doctoral and doctoral grants to CC and PP, respectively).

References

[1] Schulze, J. and U. Sonnenborn, *Yeasts in the gut: from commensals to infectious agents.* Dtsch Arztebl Int, 2009. 106(51-52): p. 837-42.
[2] Hube, B., *From commensal to pathogen: stage- and tissue-specific gene expression of Candida albicans.* Curr Opin Microbiol, 2004. 7(4): p. 336-41.
[3] Butler, G., et al., *Evolution of pathogenicity and sexual reproduction in eight Candida genomes.* Nature, 2009. 459(7247): p. 657-62.
[4] Pfaller, M.A. and D.J. Diekema, *Epidemiology of invasive candidiasis: a persistent public health problem.* Clin Microbiol Rev, 2007. 20(1): p. 133-63.
[5] Krcmery, V. and A.J. Barnes, *Non-albicans Candida spp. causing fungaemia: pathogenicity and antifungal resistance.* J Hosp Infect, 2002. 50(4): p. 243-60.
[6] Trofa, D., A. Gacser, and J.D. Nosanchuk, *Candida parapsilosis, an emerging fungal pathogen.* Clin Microbiol Rev, 2008. 21(4): p. 606-25.
[7] Colombo, A.L., et al., *Prospective observational study of candidemia in Sao Paulo, Brazil: incidence rate, epidemiology, and predictors of mortality.* Infect Control Hosp Epidemiol, 2007. 28(5): p. 570-6.
[8] Messer, S.A., R.N. Jones, and T.R. Fritsche, *International surveillance of Candida spp. and Aspergillus spp.: report from the SENTRY Antimicrobial Surveillance Program (2003).* J Clin Microbiol, 2006. 44(5): p. 1782-7.
[9] Redding, S.W., et al., *Candida glabrata oropharyngeal candidiasis in patients receiving radiation treatment for head and neck cancer.* J Clin Microbiol, 2002. 40(5): p. 1879-81.
[10] Costa-de-Oliveira, S., et al., *A first Portuguese epidemiological survey of fungaemia in a university hospital.* Eur J Clin Microbiol Infect Dis, 2008. 27(5): p. 365-74.
[11] Silva, S., et al., *Candida glabrata, Candida parapsilosis and Candida tropicalis: biology, epidemiology, pathogenicity and antifungal resistance.* FEMS Microbiol Rev, 2012. 36(2): p. 288-305.
[12] Miranda, L.N., et al., *Candida colonisation as a source for candidaemia.* J Hosp Infect, 2009. 72(1): p. 9-16.
[13] Karkowska-Kuleta, J., M. Rapala-Kozik, and A. Kozik, *Fungi pathogenic to humans: molecular bases of virulence of Candida albicans, Cryptococcus neoformans and Aspergillus fumigatus.* Acta Biochim Pol, 2009. 56(2): p. 211-24.
[14] Lin, X. and J. Heitman, *The biology of the Cryptococcus neoformans species complex.* Annu Rev Microbiol, 2006. 60: p. 69-105.
[15] Silveira, F.P. and S. Husain, *Fungal infections in solid organ transplantation.* Med Mycol, 2007. 45(4): p. 305-20.
[16] Anderson, J.B., *Evolution of antifungal-drug resistance: mechanisms and pathogen fitness.* Nat Rev Microbiol, 2005. 3(7): p. 547-56.
[17] Ernst, R., et al., *Multidrug efflux pumps: substrate selection in ATP-binding cassette multidrug efflux pumps--first come, first served?* FEBS J, 2010. 277(3): p. 540-9.
[18] Bii, C.C., et al., *Antifungal drug susceptibility of Cryptococcus neoformans from clinical sources in Nairobi, Kenya.* Mycoses, 2007. 50(1): p. 25-30.
[19] Shapiro, R.S., N. Robbins, and L.E. Cowen, *Regulatory circuitry governing fungal development, drug resistance, and disease.* Microbiol Mol Biol Rev, 2011. 75(2): p. 213-67.
[20] Joseph-Horne, T., et al., *Cross-resistance to polyene and azole drugs in Cryptococcus neoformans.* Antimicrob Agents Chemother, 1995. 39(7): p. 1526-9.
[21] Forastiero, A., et al., *Candida tropicalis antifungal cross-resistance is related to different azole target (Erg11p) modifications.* Antimicrob Agents Chemother, 2013. 57(10): p. 4769-81.
[22] Hull, C.M., et al., *Facultative sterol uptake in an ergosterol-deficient clinical isolate of Candida glabrata harboring a missense mutation in ERG11 and exhibiting cross-resistance to azoles and amphotericin B.* Antimicrob Agents Chemother, 2012. 56(8): p. 4223-32.
[23] Eddouzi, J., et al., *Molecular mechanisms of drug resistance in clinical Candida species isolated from Tunisian hospitals.* Antimicrob Agents Chemother, 2013. 57(7): p. 3182-93.
[24] Faria-Ramos, I., et al., *Environmental azole fungicide, prochloraz, can induce cross-resistance to medical triazoles in Candida glabrata.* FEMS Yeast Res, 2014. 14(7): p. 1119-23.
[25] Pfaller, M.A., et al., *Frequency of decreased susceptibility and resistance to echinocandins among fluconazole-resistant bloodstream isolates of Candida glabrata.* J Clin Microbiol, 2012. 50(4): p. 1199-203.
[26] Wang, E., et al., *The ever-evolving landscape of candidaemia in patients with acute leukaemia: non-susceptibility to caspofungin and multidrug resistance are associated with increased mortality.* J Antimicrob Chemother, 2015.
[27] Cleveland, A.A., et al., *Declining incidence of candidemia and the shifting epidemiology of Candida resistance in two US metropolitan areas, 2008-2013: results from population-based surveillance.* PLoS One, 2015. 10(3): p. e0120452.

[28] Razzaghi-Abyaneh, M., et al., *Species distribution and antifungal susceptibility of Candida spp. isolated from superficial candidiasis in outpatients in Iran.* J Mycol Med, 2014. 24(2): p. e43-50.
[29] Sanguinetti, M., B. Posteraro, and C. Lass-Florl, *Antifungal drug resistance among Candida species: mechanisms and clinical impact.* Mycoses, 2015. 58 Suppl 2: p. 2-13.
[30] Farmakiotis, D., J.J. Tarrand, and D.P. Kontoyiannis, *Drug-resistant Candida glabrata infection in cancer patients.* Emerg Infect Dis, 2014. 20(11): p. 1833-40.
[31] Mohamadi, J., et al., *Anti-fungal resistance in candida isolated from oral and diaper rash candidiasis in neonates.* Bioinformation, 2014. 10(11): p. 667-70.
[32] Nieto, M.C., O. Telleria, and R. Cisterna, *Sentinel surveillance of invasive candidiasis in Spain: epidemiology and antifungal susceptibility.* Diagn Microbiol Infect Dis, 2015. 81(1): p. 34-40.
[33] Pfaller, M.A., et al., *Isavuconazole, micafungin, and 8 comparator antifungal agents' susceptibility profiles for common and uncommon opportunistic fungi collected in 2013: temporal analysis of antifungal drug resistance using CLSI species-specific clinical breakpoints and proposed epidemiological cutoff values.* Diagn Microbiol Infect Dis, 2015. 82(4): p. 303-13.
[34] Schmalreck, A.F., et al., *Species and susceptibility distribution of 1062 clinical yeast isolates to azoles, echinocandins, flucytosine and amphotericin B from a multi-centre study.* Mycoses, 2012. 55(3): p. e124-37.
[35] Shi, X.Y., et al., *Molecular identification and antifungal susceptibility of 186 Candida isolates from vulvovaginal candidiasis in southern China.* J Med Microbiol, 2015. 64(Pt 4): p. 390-3.
[36] Zarei Mahmoudabadi, A., A. Rezaei-Matehkolaei, and F. Ghanavati, *The susceptibility patterns of Candida species isolated from urine samples to posaconazole and caspofungin.* Jundishapur J Microbiol, 2015. 8(3): p. e24298.
[37] Posteraro, B., et al., *Antifungal Susceptibility Profiles of Bloodstream Yeast Isolates by Sensititre YeastOne over Nine Years at a Large Italian Teaching Hospital.* Antimicrob Agents Chemother, 2015. 59(7): p. 3944-55.
[38] Czaika, V., et al., *Detection of azole susceptibility patterns in clinical yeast strains isolated from 1998 to 2008.* New Microbiol, 2014. 37(4): p. 465-94.
[39] Pfaller, M.A., et al., *Geographic variation in the susceptibilities of invasive isolates of Candida glabrata to seven systemically active antifungal agents: a global assessment from the ARTEMIS Antifungal Surveillance Program conducted in 2001 and 2002.* J Clin Microbiol, 2004. 42(7): p. 3142-6.
[40] Gopinathan, S., et al., *Detection of FUR1 Gene in 5-Flucytosine Resistant Candida Isolates in Vaginal Candidiasis Patients.* J Clin Diagn Res, 2013. 7(11): p. 2452-5.
[41] Haddadi, P., et al., *Yeast colonization and drug susceptibility pattern in the pediatric patients with neutropenia.* Jundishapur J Microbiol, 2014. 7(9): p. e11858.
[42] Ibrahim, N.H., et al., *The effect of antifungal combination on transcripts of a subset of drug-resistance genes in clinical isolates of Candida species induced biofilms.* Saudi Pharm J, 2015. 23(1): p. 55-66.
[43] Andrade-Silva, L., et al., *Susceptibility profile of clinical and environmental isolates of Cryptococcus neoformans and Cryptococcus gattii in Uberaba, Minas Gerais, Brazil.* Med Mycol, 2013. 51(6): p. 635-40.
[44] Oxman, D.A., et al., *Candidaemia associated with decreased in vitro fluconazole susceptibility: is Candida speciation predictive of the susceptibility pattern?* J Antimicrob Chemother, 2010. 65(7): p. 1460-5.
[45] Pfaller, M.A., et al., *Antifungal susceptibilities of Candida, Cryptococcus neoformans and Aspergillus fumigatus from the Asia and Western Pacific region: data from the SENTRY antifungal surveillance program (2010-2012).* J Antibiot (Tokyo), 2015.
[46] Lee, C.H., et al., *Correlation of anti-fungal susceptibility with clinical outcomes in patients with cryptococcal meningitis.* BMC Infect Dis, 2012. 12: p. 361.
[47] Matos, C.S., et al., *Microbiological characteristics of clinical isolates of Cryptococcus spp. in Bahia, Brazil: molecular types and antifungal susceptibilities.* Eur J Clin Microbiol Infect Dis, 2012. 31(7): p. 1647-52.
[48] Asadzadeh, M., et al., *Antifungal susceptibility of clinical Candida parapsilosis isolates in Kuwait.* Mycoses, 2008. 51(4): p. 318-23.
[49] Hegazi, M., et al., *Characteristics and risk factors of candidemia in pediatric intensive care unit of a tertiary care children's hospital in Egypt.* J Infect Dev Ctries, 2014. 8(5): p. 624-34.
[50] Zaoutis, T.E., et al., *Antifungal susceptibility of Candida spp. isolated from pediatric patients: a survey of 4 children's hospitals.* Diagn Microbiol Infect Dis, 2005. 52(4): p. 295-8.
[51] Marti-Carrizosa, M., et al., *Fungemia in a Spanish hospital: the role of Candida parapsilosis over a 15-year period.* Scand J Infect Dis, 2014. 46(6): p. 454-61.
[52] Pfaller, M., et al., *Use of epidemiological cutoff values to examine 9-year trends in susceptibility of Candida species to anidulafungin, caspofungin, and micafungin.* J Clin Microbiol, 2011. 49(2): p. 624-9.
[53] Fernandez-Ruiz, M., et al., *Candida tropicalis bloodstream infection: Incidence, risk factors and outcome in a population-based surveillance.* J Infect, 2015.
[54] Chander, J., et al., *Epidemiology of Candida blood stream infections: experience of a tertiary care centre in North India.* J Infect Dev Ctries, 2013. 7(9): p. 670-5.
[55] Montagna, M.T., et al., *Susceptibility to echinocandins of Candida spp. strains isolated in Italy assessed by European Committee for Antimicrobial Susceptibility Testing and Clinical Laboratory Standards Institute broth microdilution methods.* BMC Microbiol, 2015. 15: p. 106.
[56] Pfaller, M.A., et al., *Echinocandin and triazole antifungal susceptibility profiles for clinical opportunistic yeast and mold isolates collected from 2010 to 2011: application of new CLSI clinical breakpoints and epidemiological cutoff values for characterization of geographic and temporal trends of antifungal resistance.* J Clin Microbiol, 2013. 51(8): p. 2571-81.
[57] Cannon, R.D., et al., *Efflux-mediated antifungal drug resistance.* Clin Microbiol Rev, 2009. 22(2): p. 291-321, Table of Contents.
[58] Kuchler, K., *The ABC of ABCs: multidrug resistance and genetic diseases.* FEBS J, 2011. 278(18): p. 3189.
[59] Morschhauser, J., *Regulation of multidrug resistance in pathogenic fungi.* Fungal Genet Biol, 2010. 47(2): p. 94-106.

[60] Liu, T.T., et al., *Genome-wide expression and location analyses of the Candida albicans Tac1p regulon.* Eukaryot Cell, 2007. 6(11): p. 2122-38.
[61] Sanglard, D., et al., *Susceptibilities of Candida albicans multidrug transporter mutants to various antifungal agents and other metabolic inhibitors.* Antimicrob Agents Chemother, 1996. 40(10): p. 2300-5.
[62] Coste, A., et al., *A mutation in Tac1p, a transcription factor regulating CDR1 and CDR2, is coupled with loss of heterozygosity at chromosome 5 to mediate antifungal resistance in Candida albicans.* Genetics, 2006. 172(4): p. 2139-56.
[63] Wirsching, S., et al., *MDR1-mediated drug resistance in Candida dubliniensis.* Antimicrob Agents Chemother, 2001. 45(12): p. 3416-21.
[64] Sanglard, D., et al., *The ATP binding cassette transporter gene CgCDR1 from Candida glabrata is involved in the resistance of clinical isolates to azole antifungal agents.* Antimicrob Agents Chemother, 1999. 43(11): p. 2753-65.
[65] Sanglard, D., F. Ischer, and J. Bille, *Role of ATP-binding-cassette transporter genes in high-frequency acquisition of resistance to azole antifungals in Candida glabrata.* Antimicrob Agents Chemother, 2001. 45(4): p. 1174-83.
[66] Torelli, R., et al., *The ATP-binding cassette transporter-encoding gene CgSNQ2 is contributing to the CgPDR1-dependent azole resistance of Candida glabrata.* Mol Microbiol, 2008. 68(1): p. 186-201.
[67] Vermitsky, J.P. and T.D. Edlind, *Azole resistance in Candida glabrata: coordinate upregulation of multidrug transporters and evidence for a Pdr1-like transcription factor.* Antimicrob Agents Chemother, 2004. 48(10): p. 3773-81.
[68] Barchiesi, F., et al., *Experimental induction of fluconazole resistance in Candida tropicalis ATCC 750.* Antimicrob Agents Chemother, 2000. 44(6): p. 1578-84.
[69] Katiyar, S.K. and T.D. Edlind, *Identification and expression of multidrug resistance-related ABC transporter genes in Candida krusei.* Med Mycol, 2001. 39(1): p. 109-16.
[70] Ricardo, E., et al., *In vivo and in vitro acquisition of resistance to voriconazole by Candida krusei.* Antimicrob Agents Chemother, 2014. 58(8): p. 4604-11.
[71] Lamping, E., et al., *Abc1p is a multidrug efflux transporter that tips the balance in favor of innate azole resistance in Candida krusei.* Antimicrob Agents Chemother, 2009. 53(2): p. 354-69.
[72] Posteraro, B., et al., *Identification and characterization of a Cryptococcus neoformans ATP binding cassette (ABC) transporter-encoding gene, CnAFR1, involved in the resistance to fluconazole.* Mol Microbiol, 2003. 47(2): p. 357-71.
[73] Sanguinetti, M., et al., *Role of AFR1, an ABC transporter-encoding gene, in the in vivo response to fluconazole and virulence of Cryptococcus neoformans.* Infect Immun, 2006. 74(2): p. 1352-9.
[74] Orsi, C.F., et al., *The ABC transporter-encoding gene AFR1 affects the resistance of Cryptococcus neoformans to microglia-mediated antifungal activity by delaying phagosomal maturation.* FEMS Yeast Res, 2009. 9(2): p. 301-10.
[75] Niimi, K., et al., *Overexpression of Candida albicans CDR1, CDR2, or MDR1 does not produce significant changes in echinocandin susceptibility.* Antimicrob Agents Chemother, 2006. 50(4): p. 1148-55.
[76] Chen, K.H., et al., *The bZip transcription factor Cgap1p is involved in multidrug resistance and required for activation of multidrug transporter gene CgFLR1 in Candida glabrata.* Gene, 2007. 386(1-2): p. 63-72.
[77] Costa, C., et al., *The dual role of candida glabrata drug:H+ antiporter CgAqr1 (ORF CAGL0J09944g) in antifungal drug and acetic acid resistance.* Front Microbiol, 2013. 4: p. 170.
[78] Costa, C., et al., *Candida glabrata drug:H+ antiporter CgQdr2 confers imidazole drug resistance, being activated by transcription factor CgPdr1.* Antimicrob Agents Chemother, 2013. 57(7): p. 3159-67.
[79] Costa, C., et al., *Candida glabrata drug:H+ antiporter CgTpo3 (ORF CAGL0I10384g): role in azole drug resistance and polyamine homeostasis.* J Antimicrob Chemother, 2014. 69(7): p. 1767-76.
[80] Costa, C., et al., *MFS multidrug transporters in pathogenic fungi: do they have real clinical impact?* Front Physiol, 2014. 5: p. 197.
[81] Bartosiewicz, D. and A. Krasowska, *Inhibitors of ABC transporters and biophysical methods to study their activity.* Z Naturforsch C, 2009. 64(5-6): p. 454-8.
[82] Niimi, K., et al., *Specific interactions between the Candida albicans ABC transporter Cdr1p ectodomain and a D-octapeptide derivative inhibitor.* Mol Microbiol, 2012. 85(4): p. 747-67.
[83] Maurya, I.K., et al., *Rationally designed transmembrane peptide mimics of the multidrug transporter protein Cdr1 act as antagonists to selectively block drug efflux and chemosensitize azole-resistant clinical isolates of Candida albicans.* J Biol Chem, 2013. 288(23): p. 16775-87.
[84] Tanabe, K., et al., *Inhibition of fungal ABC transporters by unnarmicin A and unnarmicin C, novel cyclic peptides from marine bacterium.* Biochem Biophys Res Commun, 2007. 364(4): p. 990-5.
[85] Rawal, M.K., et al., *Jatrophanes from Euphorbia squamosa as potent inhibitors of Candida albicans multidrug transporters.* J Nat Prod, 2014. 77(12): p. 2700-6.

