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Review

Antifungal drug resistance in *Candida* species

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ABSTRACT

Invasive *Candida* infections are well established infectious entities of immunocompromised or critically ill patients and are characterised by high morbidity and mortality. Owing to the common eukaryotic structure of fungi and humans, a limited number of antifungal drugs is available for therapeutic purposes. In this unsatisfactory scenario, the emergence of drug resistance represents an important health problem. Failure of antifungal treatment can be related to host factors, to the pharmacokinetic and pharmacodynamic parameters of the drug, or to morphological, reproductive modalities and biofilm production of the fungus itself. Innate or acquired antifungal resistance derives from the presence or onset of molecular mechanisms related to the toxic activity of the drug itself. The resulting resistance can thus be extended to different molecules of the same class according to a greater or lesser affinity of the molecules for the target. In addition, non-specific cellular mechanisms of extrusion of toxic substances, such as overexpression of efflux pumps, can play a role involving different antifungal classes. Here we briefly review the current antifungal susceptibility testing methods and their usefulness as predictors of antifungal resistance in *Candida* spp., focusing on assessment of the involved molecular mechanisms.

Contents

1. Introduction

Advances in medical research allow better control of neoplastic diseases and increase the survival of critically ill patients or patients with impaired function of the immune system. However, this continuous medical progress gives rise to the increase in new risk factors for the occurrence of invasive fungal infections, which are steadily increasing with a consequent increase in the use of antifungal agents both for therapeutic and prophylactic purposes. The antifungal drugs commonly used for the treatment of invasive fungal infections belong to three classes characterised by different mechanisms of action and spectrum of activity. Because of the common eukaryotic nature of fungal and human cells, it is difficult to identify specific metabolic or structural antimicrobial targets for fungi. The fundamental physiological role and the different composition of sterols (cholesterol in humans and ergosterol in fungi) render the cytoplasmic membrane of fungi a suitable target for the action of antifungals (polyenes and azoles) with a sufficient therapeutic index. Synthesis of the cell wall glucans represents an additional metabolic target, exploited by the echinocandin drugs.

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Another systemic compound (5-flucytosine) owes its antifungal activity to both the ease of entry into the fungal cell and its conversion into 5-fluorouracil (the pharmacologically active form) by fungal enzymes (Table 1).

Antimicrobial therapy or prophylaxis promotes the emergence of resistance by selecting those micro-organisms able to survive and reproduce in the presence of a given drug. Among microbes, natural competition for survival is manifested through their ability to produce and process metabolites toxic for other micro-organisms and, at the same time, to implement a number of strategies to resist the action of these substances [1,2]. Resistant mutants can pre-exist with variable frequency between the susceptible clones, and in the course of infection and therapeutic treatment they can overcome the susceptible clones, representing the main cause of the therapeutic failure [3–6].

From the point of view of the microbiologist, secondary (acquired) resistance to antimicrobials occurs when the minimum inhibitory concentration (MIC) of the causative organism increases in subsequent isolates associated with therapeutic failure [7–12]. The first observations on the emergence of drug resistance during therapy with fluconazole were in sequential strains of *Candida albicans* [13–16]. These studies demonstrated that antifungal resistance is due to multifactorial events, involving molecular modifications often related to the mechanism of action of the drug itself as well as gene overexpression. However, the definition of resistance to antifungal drugs is much more complex, and possible therapeutic failure can depend on numerous factors. According to White, failure of antifungal treatment can be related to the host (immune status, site of infection, severity of infection, presence of other materials, abscess formation, adherence to the treatment regimen), to the drug (fungistatic or fungicidal activity, dosage, pharmacokinetics, drug interactions) and, of course, to the responsible fungus (cellular organisation in yeast or hyphal morphology, 'switch' phenotype, serotype, genomic stability, fungal load, biofilm production) [17].

In vitro detection of resistance to antifungal drugs is now possible thanks to the availability of reference methods for in vitro susceptibility testing for yeasts and of clinical MIC breakpoints for some antifungals [18–22]. Resistance can be extended to different molecules of the same class according to a greater or lesser affinity of the molecules for the target. Cross-resistance to different classes of drugs can be detected in the presence of common non-specific cellular mechanisms of extrusion of toxic substances, such as efflux pumps [13–17].

The ability to form biofilms represents a further problem in the context of antifungal drug resistance. In recent years, biofilm-associated *Candida* infections have been related to a poor outcome owing to the biofilm-embedded yeast cells being resistant to antifungal treatment and because most antifungal drugs cannot penetrate the exopolymeric matrix of the biofilm [23].

In this review, we will consider the molecular mechanisms underlying resistance to antifungals in *Candida* spp.

Table 1
Drugs used for therapy of invasive fungal infections.

Class of antifungal	Mechanism of action	Spectrum of activity
5-Flucytosine	Interferes with metabolism of pyrimidine after its conversion to 5-fluorouracil, inhibiting the synthesis of nucleic acids and proteins	Yeasts and yeast-like fungi
Echinocandins (anidulafungin, caspofungin and micafungin)	Inhibit the enzyme 1,3-β-D-glucan synthase, interfering with formation of the cell wall	*Candida* and *Aspergillus* spp.
Polyenes (amphotericin B)	Bind to ergosterol and alter the integrity of the cellular membrane, with osmotic loss of electrolytes, sugars and metabolites	Yeasts and moulds
Triazoles (fluconazole*, itraconazole, posaconazole† and voriconazole)	Interfere with the synthesis of ergosterol by inhibiting the enzyme cytochrome P450 14α-sterol demethylase (lanosterol 14α-demethylase)	Yeasts and moulds (excluding *Mucorales*) *Active only on yeasts. †Sctive on some species of *Mucorales*.

2. Methods to assess in vitro susceptibility

There are two reference methods for in vitro antifungal susceptibility testing (AFST) of *Candida* spp. The methods have been developed by two scientific institutions, namely the Clinical and Laboratory Standards Institute (CLSI) and the AFST Subcommittee of the European Committee for Antimicrobial Susceptibility Testing (EUCAST), and undergo a continuous process of updating [18–22]. These reference methods show high intralaboratory and interlaboratory reproducibility and provide reliable data for developing clinical breakpoints (CBPs) to interpret in vitro results. Differences in CBPs between the two standardised methods reflect differences in the protocols (Table 2); therefore, the CBPs established by one method cannot be extended to the other [20]. CBPs can be useful in identifying drugs that are less likely to succeed in eradicating the infection and represent an important part of the clinical process that will lead to a treatment to which a given patient will respond or not [24]. The therapeutic outcome, in fact, is often influenced by several factors, including drug interactions, host and severity of *Candida* diseases [17]. A variety of commercial AFST systems have been developed as alternatives to the reliable but time-consuming reference methods recommended by the CLSI and EUCAST. Currently, two commercial methods, namely Etest and Sensititre, have been extensively evaluated with both reference systems. Both commercial methods provide very satisfactory results for reliability, reproducibility and correlation with the reference systems [25–29].

3. Echinocandins

The echinocandins (anidulafungin, caspofungin and micafungin) belong to the chemical class of lipopeptides and exert their antifungal effect through a non-competitive inhibition of 1,3-β-D-glucan synthase, a multisubunit protein complex responsible for synthesis of an essential component of the fungal cell wall, namely 1,3-β-D-glucan [30]. The characteristic mechanism of action makes this class useful for therapy of invasive infections caused by yeasts and *Aspergillus* spp. resistant to azoles, but not *Mucorales*, *Cryptococcus neoformans* and *Fusarium* spp. Echinocandins are fungicidal against yeasts and are fungistatic against *Aspergillus* spp., blocking in the latter the apical growth of the hyphae. Furthermore, they are active in vitro and in vivo against fungal biofilm [23,30,31]. Both the CLSI and EUCAST have developed CBPs for interpreting MIC values to define yeast clinical isolates as susceptible or resistant to echinocandins (Table 2). They differ essentially in the threshold values of MIC for the definition of susceptibility or resistance. EUCAST has published MIC values much lower than those of the CLSI for anidulafungin and micafungin, but has not yet established caspofungin values. These differences make it difficult to define a clinical strain as resistant in the absence of documented existence of a molecular mechanism underlying the resistance itself. Cases of therapeutic failure, in particular with caspofungin, the first echinocandin available for therapy, are reported in the literature especially for infections caused by *Candida* spp. [5,7–10,12,32–44]. High MIC values have