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Antifungal drug resistance mechanisms in pathogenic fungi: from bench to bedside

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Abstract

The phenotypic methods for identification of antifungal resistance are reliable procedures, and MIC determination by reference techniques is the gold standard to detect resistant clinical isolates. In recent years, progress has been made towards the description of resistance mechanisms at molecular level. There are methods of detection that can be useful for clinical laboratories, but lack of standardization precludes their full and effective integration in the routine daily practice. The molecular detection of *Candida* resistance to azoles and to echinocandins and of *Aspergillus* resistance to triazoles can be clinically relevant and could help to design more efficient prevention and control strategies. This text reviews the present state of the detection of mechanisms of resistance at the molecular level in *Candida* spp. and *Aspergillus* spp. and its relevance to clinical practice.

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Introduction

The standardization of antimicrobial susceptibility testing (AST) is a long process that must comply with several requirements. To this end, it is essential to develop reliable reference procedures. Antifungal susceptibility testing has been standardized in last two decades, and it is still under development for some compounds and fungal species [1,2].

There are two standards for antifungal susceptibility testing both based largely on broth microdilution methods: One created by the European Committee on Antibiotic Susceptibility Testing (EUCAST) and the other one created by the Clinical Laboratory Standard Institute (CLSI, former NCCLS). Both organisations have led standardization processes, have performed reproducibility studies and recommended methods for quality control assurance [2–5].

In addition, the EUCAST and the CLSI have developed breakpoints and epidemiological cut-off values (ECVs) that are now established for *Candida* spp. and *Aspergillus* spp. [6–9]. The procedure to develop interpretative breakpoints is a multistep process based on the analysis of the MIC distributions and the clinical relationship between MIC values and efficacy. There are currently available breakpoints to interpret AST results of amphotericin B, azoles and echinocandins for *Candida* and amphotericin B and azoles for *Aspergillus*.

After standardization of AST and setting breakpoints, reliable but more practical techniques of AST should be validated for use in clinical laboratories as the dilution standard reference procedures are rather complex methods for routine susceptibility testing [1,10]. There are several commercial and disc diffusion techniques that exhibit a high correlation with results of reference procedures and that are already used in many clinical laboratories. The extended use of AST methods has defined the prevalence of strains with high MIC values and some resistance mechanisms at the molecular level [11,12]. A number of molecular studies are currently in progress to ascertain the frequency of these mutants among wild-type populations [13,14]. The results of those surveys could be very useful to design strategies of prevention and control of emergence of resistance.

This text reviews the present situation of the detection of mechanisms of resistance at the molecular level in *Candida* spp. and *Aspergillus* spp. and their relevance to the clinical practice. The reliability of molecular tools for the detection of resistance is also analysed as well as their performance to monitor the incidence of mutants with resistance to antifungal agents.

Detection of Molecular Resistance to Amphotericin B

Table 1 shows stages of the standardization process of AST by antifungal agent and by fungal species. In the case of amphotericin B, the system for MIC determination is able to detect strains exhibiting high MIC values [15]. Several studies reported, however, that reference procedures are not completely reliable to classify correctly some amphotericin B-resistant strains [16]. The reference techniques yield a range of amphotericin B MIC values that spans only three of four twofold serial dilutions precluding reliable discrimination between susceptible and resistant populations. Nevertheless, as some rare isolates were described as intrinsically resistant to the polyenes and they exhibit very high amphotericin B MIC values, breakpoints were proposed and set for *Candida* and *Aspergillus* some years ago [5,17].

Resistance to amphotericin B is an uncommon phenomenon in *Candida* (1–3%) and *Aspergillus*, although a proportion of *A. terreus* and *A. flavus* has higher MIC values [13,15,18]. Because the shortage of clinical strains with resistance to amphotericin B, not many studies on the molecular description of resistance mechanisms have been reported. It has been published that resistance is associated with mutants with low levels of ergosterol and disturbances of levels and composition of phospholipids in the membrane. Some of these changes have been associated with mutations in genes *ERG2*, *ERG3* and *ERG11*. In addition, the polyenes induce oxidative stress in fungal cells, and resistant isolates can have higher levels of antioxidative enzymes and/or alterations in the production of free radicals [19–22].

Table 2 shows a summary of the current state of the molecular detection of resistance mechanisms by antifungal compound and by *Candida* and *Aspergillus* species, from the point of view of clinical laboratories. Determining resistance mechanisms of amphotericin B at the molecular level is clinically irrelevant. The number of strains exhibiting resistance *in vitro* is low, and mutants harbouring DNA changes related to rises in the MIC value of amphotericin B are hardly ever found in clinical samples [11,15]. There are no reliable molecular tools to detect amphotericin B resistance. Some reports have included ergosterol quantification or determinations of catalase activity and production of free radicals, but these procedures are phenotypic techniques that have not been standardized so far [23]. It can be concluded that currently molecular determination of amphotericin B resistance is not more useful than MIC determination for susceptibility testing and for the management of patients. Molecular detection is not

TABLE 2. Current situation of the molecular detection of resistance mechanisms by antifungal compound and by species from the point of view of clinical laboratories

Antifungal agents	Fungal species	Clinical relevance of molecular testing	Availability of reliable molecular tools	Integration in routine daily practice
Amphotericin B	*Candida* spp.	No	No	No
	Aspergillus spp.	No[a]	No	No
Azoles	*Candida* spp.	Yes	Yes	Possible
	Aspergillus spp.	Yes	Yes	Possible
Echinocandins	*Candida* spp.	Yes	Yes	Possible[b]
	Aspergillus spp.	No	No	No

[a]Some strains of *A. terreus* exhibit high MIC values of amphotericin B, and it could have clinical relevance.
[b]A significant number of resistance mechanisms are still unknown, and number of resistant strains is low.

TABLE 1. Current situation of the antifungal susceptibility testing field by antifungal agent, by species and by stages of standardization process

Antifungal agents	Fungal species	Availability of reference procedures	Breakpoint setting	Integration of AST in clinical laboratories in routine daily practice	Molecular description of resistance	Prevention and control strategies to avoid emergence of resistance
Amphotericin B	*Candida* spp.	Yes	Yes	Yes	No[e]	No
	Aspergillus spp	Yes	Yes[b]	Yes	No	No
Azoles	*Candida* spp.	Yes	Yes[c]	Yes	In progress	No
	Aspergillus spp	Yes	Yes[b]	Yes	In progress	No
Echinocandins	*Candida* spp.	Yes	Yes[d]	Yes	In Progress	No
	Aspergillus spp	Yes[a]	No	In Progress	No	No

[a]No totally standardized yet for testing filamentous fungi.
[b]The Clinical Laboratory Standard Institute (CLSI) has not proposed yet breakpoints for *Aspergillus*.
[c]The CLSI has not proposed posaconazole breakpoints for *Candida*.
[d]The European Committee on Antibiotic Susceptibility Testing is in process of setting caspofungin breakpoints for *Candida*.
[e]Irrelevant as number of resistant mutants is very low.