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Update on antifungal resistance in *Aspergillus* and *Candida*

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Abstract

Antifungal resistance in *Candida* and *Aspergillus* may be either intrinsic or acquired and may be encountered in the antifungal drug exposed but also the antifungal drug-naïve patient. Prior antifungal treatment confers a selection pressure and notoriously raises the awareness of possible resistance in patients failing therapy, thus calling for susceptibility testing. On the contrary, antifungal resistance in the drug-naïve patient is less expected and therefore more challenging. This is particularly true when it concerns pathogens with acquired resistance which cannot be predicted from the species identification itself. This scenario is particularly relevant for *A. fumigatus* infections due to the increasing prevalence of azole-resistant isolates in the environment. For *Candida,* infections resistance is most common in the context of increasing prevalence of species with intrinsic resistance. *Candida glabrata* which has intrinsically reduced susceptibility to fluconazole is increasingly common particularly among the adult and elderly population on the Northern Hemisphere where it may be responsible for as many as 30% of the blood stream infections in population-based surveillance programmes. *Candida parapsilosis* is prevalent in the paediatric setting, at centres with increasing echinocandin use and at the southern or pacific parts of the world. In the following, the prevalence and drivers of intrinsic and acquired resistance in *Aspergillus* and *Candida* will be reviewed.

Keywords: Acquired resistance, *Aspergillus*, azoles, *Candida*, echinocandins, intrinsic resistance, susceptibility testing
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Introduction

Resistance in *Aspergillus* and *Candida* has been increasingly investigated and reported because standards for susceptibility testing and associated breakpoints became available and as a consequence of the increased use of antifungal compounds [1–4].

Resistant infection can be encountered in the antifungal drug-exposed patient due to selection of intrinsically resistant species or isolates with acquired resistance belonging to species that are normally susceptible. In both cases, resistance may be expected as any antimicrobial therapy is associated with a selection pressure and therefore risk of resistance. Resistance can, however, also be encountered in the antifungal drug-naïve patient and, again, can be due either to infection with intrinsically resistant species or to isolates with acquired resistance. Whereas resistance due to intrinsically resistant species can be diagnosed through correct species identification, detection of isolates with acquired resistance is more demanding and requires appropriate and carefully performed susceptibility testing and endpoint interpretation [5].

New tools for rapid species identification of *Candida* species including the matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS), fluorescence *in situ* hybridization (FISH), etc. have improved correct species identification at clinical microbiological routine

laboratories. However, various challenges are still associated with performance and interpretation of susceptibility testing even though commercial tests are now available for most antifungal compounds and clinical breakpoints have been established [1,2,6–11]. Firstly, performance requires expertise to obtain reproducible results, second the standardization of commercial methods against the reference test is not perfect for all drug–bug combinations leading to misclassification of susceptibility test results when reference breakpoints are adopted, and finally for *Aspergillus,* such tests are not routinely performed. Hence, the greatest diagnostic challenges related to resistant infections lay with the correct and timely detection of infections due to isolates with acquired resistance and particularly so in the drug-naïve patients where, historically, resistance has not been expected.

Resistance in *Aspergillus*

From an epidemiological point of view, less is known about the true prevalence of resistant *Aspergillus* infections than about resistant infections for most other organisms. This is due to the fact that most routine laboratories do not susceptibility test their *Aspergillus* isolates and many laboratories find species (or even genus) identification of aspergilli difficult. Additionally, national surveillance programmes are lacking.

Intrinsic resistance

Aspergillus fumigatus is by far the most common species causing human infection. The wild-type isolates are susceptible to all the licensed mould active azoles (Table 1) and echinocandins [3,4,12]. However, the *A. fumigatus* species complex includes more than 30 sibling species which cannot be differentiated morphologically from one another or from *A. fumigatus*. Several of these have been isolates from humans and been shown to be intrinsically resistant to one or more antifungals (Table 2) [12–15]. Notably, however, a simple temperature tolerance test (growth at temperatures higher than 48°C) can discriminate between *A. fumigatus* and the sibling species. Intrinsic amphotericin B resistance has been recognized in *A. terreus* for many decades, but also *A. flavus* and other less common species have reduced susceptibility to amphotericin B [3]. Importantly, a number of these rare *Aspergillus* species are also resistant to azoles and in some cases also to echinocandins posing obvious challenges for patient management (Tables 1 and 2). Hence, careful species identification is mandatory for clinically important isolates.

TABLE 2. Intrinsic resistance (R) and variable (V) susceptibility in *Aspergillus*

	AMB	Azoles	Echinocandins
Aspergillus section *fumigati*			
A. fumigatiaffinis	R	R	
A. lentulus	R	R	V
N. pseudofischeri	V	R	
A. viridinutans	R	R	
N. udagawae	R	R (vor)	
A. terreus (and *A. alabamensis*)	R		
A. flavus	R	R	
A. versicolor (and *A. sydowi*)	R	V	
A. calidoustus		R	V
A. allilaceus	V		V

Data compiled from [12–15].
Vor, Voriconazole.

Acquired resistance in AF-naïve patients

Azole-resistant *A. fumigatus* infections have been reported in a number of countries. Most reports derive from Europe and involve a single molecular resistance mechanism consisting of a 34-bp tandem repeat TR_{34} in the promotor region of the azole target *CYP51A* gene and a point mutation in the target gene itself leading to an L98H amino acid substitution (TR_{34}/L98H). This resistance mechanisms has been detected in isolates deriving from Austria, Belgium, Denmark, Germany, France, the Netherlands, Norway, Spain, Sweden and the UK [16–23] and outside Europe in China, India and Iran [24,25] (Verweij, personal communication). Notably, 61% of the global market share of agricultural fungicides is used in these Western

TABLE 1. Intrinsic susceptibility pattern and epidemiological cut-off values (ECOFFs) for *Aspergillus* species and mould active azoles. Data compiled from [3,4,12]

	Aspergillus (S/I/R (ECOFF))[a]						
	calidoustus	*flavus*	*fumigatus*	*nidulans*	*niger*	*terreus*	*versicolor*
Amphotericin B	IE (ND[b])	IE (4 mg/L)	S (1 mg/L)	ND[c]	S (1 mg/L)	R (4 mg/L)	ND[b]
Itraconazole	R (ND[b])	S (1 mg/L)	S (1 mg/L)	S (1 mg/L)	IE (4 mg/L)	S (0.5 mg/L)	ND[b]
Posaconazole	R (ND[b])	IE (0.5 mg/L)	S (0.25 mg/L)	IE (0.5 mg/L)	IE (0.5 mg/L)	S (0.25 mg/L)	ND[b]
Voriconazole	R (ND[b])	IE (2 mg/L)	S (1 mg/L)	S (1 mg/L)	IE (2 mg/L)	IE (2 mg/L)	ND[c]

ECOFF: epidemiological cut-off values; IE: insufficient evidence to suggest whether this species is a good target for the compound in question. MICs are higher than for *Aspergillus fumigatus,* but there is insufficient clinical data to suggest whether this translates into poorer efficacy; ND: not done due to insufficient amount of data for ECOFF setting (low number of MIC values or data set).
[a]S/I/R: intrinsically susceptible/intermediate/resistant.
[b]MIC range higher than that for *A. fumigatus.*
[c]MIC range similar to that for *A. fumigatus.*