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Azole-resistant aspergillosis

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Summary Azole-resistance in *Aspergillus fumigatus* is emerging and is becoming an increasing problem in the management of aspergillosis. Two types of development of resistance have been described; resistance acquired during azole treatment in an individual patient and through environmental exposure to fungicides. The main molecular mechanism of azole resistance in *A. fumigatus* is explained by mutations in the *cyp51A*-gene. The environmental route of resistance development is particularly worrying and may affect all patients whether azole exposed or naïve, and whether suffering from acute or chronic aspergillosis. No management guidelines to assist clinicians confronted with azole-resistant aspergillosis are available and pre-clinical and clinical evidence supporting treatment choices is scarce.

Introduction

The use of mould-active azoles has clearly improved the survival and quality of life of patients with invasive aspergillosis[1] so it is not surprising that they have become the mainstay of therapy for the treatment and prevention of this condition.[2,3] Azoles differ from the other 2 classes of antifungals, the polyenes and echinocandins, in the availability of oral formulations facilitating treatment particularly outside the hospital setting. Oral treatment with either itraconazole or voriconazole is commonly used in patients with chronic forms of aspergillosis, including chronic pulmonary aspergillosis,[4] allergic bronchopulmonary aspergillosis (ABPA),[5,6] asthma with fungal sensitization (SAFS)[7] and persistent infection of the airways with *Aspergillus* species in patients with cystic fibrosis.[8] The oral formulation of the newest triazole, posaconazole, is licensed for the prevention of invasive fungal infections in adult patients with acute leukaemia or myelodysplastic syndrome, and in human stem cell transplant recipients treated for graft-versus-host disease.[9,10] Prevention of invasive fungal infection using either itraconazole or posaconazole is standard of care in patients with chronic granulomatous disease (CGD).[11,12] The azole antifungal drugs display their activity by targeting lanosterol 14α-demethylase which is involved in ergosterol biosynthesis and is encoded by the *cyp51A* gene.[13] The resulting depletion of ergosterol leads to altered permeability of the fungal membrane and defective fungal cell wall synthesis.[14]

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Susceptibility testing

The term resistance generally relates to an *in vitro* susceptibility test indicating that the minimal inhibitory concentration (MIC) of a particular drug that is necessary to kill or inhibit the pathogen of interest is higher than a defined breakpoint. Resistance often corresponds to a higher probability of treatment failure. Interpretative breakpoints for *Aspergillus fumigatus* have been published to differentiate azole-susceptible and − resistant isolates (Table 1).[15–17] The wild type antifungal susceptibility patterns of other *Aspergillus* species do differ from *A. fumigatus*, and are referred to as 'intrinsic' resistance if elevated MIC values are found as compared to *A. fumigatus*.[18] Interpretative breakpoints are not available for non-*fumigatus Aspergillus* species and it is not possible to classify those species based on their MICs. Recent changes in the taxonomy of *Aspergillus* species have had major implications for our understanding of antifungal drug susceptibility profiles. Sequence-based molecular tools have extended the number of species within specific *Aspergillus* sections, previously indistinguishable by macroscopic and microscopic characteristics. These taxonomic changes have prompted the establishment of MIC distributions within each *Aspergillus* section and their sibling species. Within the *Aspergillus* section *fumigati*, *A. fumigatus* is intrinsically susceptible to these mould-active azoles (Table 1), but several sibling species exhibit reduced susceptibility to these drugs (e.g. *Neosartoria udagawae*, *Neosartorya pseudofischeri*, *Aspergillus lentulus*).[19] However, the focus of this review will be on *A. fumigatus sensu strictu* and the emergence of azole-resistant aspergillosis caused by *A. fumigatus*.

Resistance mechanisms

Medical

The first two documented cases of *A. fumigatus* resistance to itraconazole were described in 1997.[20] One patient was shown to have been primarily infected by an itraconazole resistant isolate, while the 2nd patient developed resistance during itraconazole treatment for acute invasive aspergillosis. In 2002, we reported a boy with CGD suffering from invasive aspergillosis caused by a multiple-azole resistant *A. fumigatus*[21] and proposed that acquired resistance induced by prolonged itraconazole prophylaxis had developed. When this occurs it is commonly due to several highly diverse point mutations in the *cyp51A* gene which confer resistance to one or multiple azoles in isolates obtained from an individual patient.[22,23]

Environmental

In 2007, a case series of 9 patients with multiple-azole resistant invasive aspergillosis was reported by a single centre.[24] Five patients suffered from breakthrough invasive aspergillosis while either on itraconazole prophylaxis (n = 4) or voriconazole treatment (n = 1). Four patients did not have any previous azole exposure at the time of developing azole-resistant invasive aspergillosis. Remarkably, 12 of the 13 *A. fumigatus* isolates carried the same TR_{34}/L98H resistance mechanism in the *cyp51A* gene, suggesting the possible emergence of resistance by the organism while it was in the environment. In a subsequent study by Snelders et al., azole-resistant *A. fumigatus* isolates prospectively collected over a period of 14 years were also found to be carrying the same resistance mechanism.[25] The frequency of azole-resistant *A. fumigatus* isolates increased from 0% before the year 1997 to 10.1% by 2010. Since then, this resistance mechanism has increasingly been reported from other centres in Europe, as well as in China, India and the Middle East.[26–30]

The predominance of such a single resistance mechanism is suggestive of acquisition from a common environmental source and contrasts with the diversity of resistance-causing mutations commonly observed in individual patients exposed to azoles. Spread of *A. fumigatus*, azole-resistant or otherwise, by person-to-person transmission is extremely rare and only occurs if aerosols containing infectious conidia are exhaled. Only in patients with an aspergilloma, a cavity in which sporulation may occur, conidia be transmitted by coughing and infection may develop upon re-inhalation by a susceptible host although this is extremely rare.[31] In the search for an environmental route of development of resistance, it has rapidly become clear that there is a widespread agricultural use of azole fungicides for crop protection and manufacturing use for preservation of materials such as paint, coatings and mattresses.[31,32] A relationship between the TR_{34}/L98H resistance mechanism and the non-medical use of azole fungicides (DMIs, 14α-demethylase inhibitors) was determined in a study performed in the Netherlands.[33] Five triazole DMIs were identified with molecular similarities to medical triazoles and which were able to induce the TR_{34}/L98H resistance mechanism in a laboratory setting. The authorisation and use of those 5 DMIs preceded the first clinical report of an *A. fumigatus* isolate harbouring the TR34/L98H resistance mechanism. The authors of this paper hypothesized that the continuous environmental exposure to DMIs and the lack of an apparent fitness cost of this azole-resistance in isolates has facilitated emerge of this particular resistance mechanism.[33] Even more recent

Table 1 Wild-type MIC distribution for *A. fumigatus* and clinical breakpoints (adapted from 15, 19).

	Epidemiological cut-off values	Clinical breakpoints	
		Susceptible	Resistant
Itraconazole	1 mg/L	<2 mg/L	>2 mg/L
Voriconazole	1 mg/L	<2 mg/L	>2 mg/L
Posaconazole	0.5 mg/L	<0.5 mg/L	>0.5 mg/L