Epidemiology and Antifungal Resistance in Invasive Aspergillosis according to Primary Disease - Review of the Literature

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Abstract

Aspergilli, less susceptible to antifungals emerge and resistance to azoles have been found mainly in *Aspergillus fumigatus*; this has launched a new phase in handling aspergillosis. Resistant strains have currently been reported from Belgium, Canada, China, Denmark, France, Norway, Spain, Sweden, The Netherlands, UK and the USA. Centres in the UK (Manchester) and The Netherlands (Nijmegen) have described particularly high frequencies (15 and 10% respectively), and a significant increase in azole resistance in recent years. The reason of this high incidence may be due to long term azole therapy in patients with chronic aspergillosis in Manchester, and due to high use of agricultural azoles in Nijmegen. The primary underlying mechanism of resistance is as a result of alterations in the *cyp51A* target gene, with a variety of mutations found in clinical isolates and one genotype identified in the environmental (LH98). Reports on well documented in vitro and in vivo resistance to echinocandins are rare for *Aspergillus* species and resistance may be under-diagnosed as susceptibility testing is less frequently performed due to technical reasons.

Key words: Epidemiology, antifungal resistance, *Aspergillus*, azoles, candins

Introduction

Invasive fungal infections (IFD) are increasingly recognized and represent a primary cause of morbidity and mortality in critically ill patients [1-4]. A variety of factors, including immunosuppressive agents, broad-spectrum antibiotics, and antineoplastic agents influence the incidence and severity of IFDs [1]. Transplant and haematopoietic stem cell transplant recipients, intensive care unit and surgical patients display the population at risk [1, 4-7]. *Aspergillus* species have become the most important pathogens [8]. The introduction of voriconazole, posaconazole and echinocandins (caspofungin, micafungin and anidulafungin) improved the therapeutic option for treatment of invasive aspergillosis (IA) [9]. Although the outcome of IA is largely influenced by the state of immunosuppression, factors related to the fungus also play a role. Until recently, species identification was sufficient to guide antifungal therapy, but the emergence of acquired resistance limits the use of species identification for predicting activity of antifungal agents. Aspergilli, less susceptible to antifungals emerged and acquired resistance to azoles have been found mainly in *Aspergillus fumigatus* [10]; this has launched a new era in handling aspergillosis. This article reviews the epidemiology and antifungal resistance against azoles and candins with particular emphasis on *Aspergillus* species.

Epidemiology of Invasive Aspergillosis

In a 4-year-study Pagano et al. [11] showed that 64% of IFDs in patients with haematological malignancies were caused by moulds and among them 90% were due to *Aspergillus* species. Overall, the incidence of IA varies according to underlying diseases, pathogen [lf] and geographic location [10, 12, 13]; rates of up to 7% are reported in Europe [11]. Mortality rates for IA are high and vary according to patient population, ranging from 38% in patients with acute myelogenous leukaemia, from 50-60% in patients with organ transplantation and from 70-85% in other immunosuppressed patients [11-16].

Although *A. fumigatus* still represents the leading cause of IA, species like *Aspergillus terreus* and *Aspergillus flavus* become more frequent [15, 17]. These non-*A. fumigatus* may be intrinsically resistant to antifungal agents (eg *A. ustus*) [18] and the clinical presentation and evolution of IA may differ from commonly observed *A. fumigatus* infections [18-23]. Maximizing the efforts to culture the causative pathogen appears necessary to allow identification and susceptibility testing.

Epidemiology of Azole and Candine Resistance in Clinical A. fumigatus

Antifungal drug resistance is normally quantified using the minimum inhibitory concentration (MIC). The MIC represents the lowest drug concentration that results in a notably reduction or complete lack of fungal growth. In vitro resistance can be primary (intrinsic) or secondary (acquired). Primary resistance occurs

Table 1. Azole resistance in *A. fumigatus.*

Phenotypes	Description
Pan-azole resistant	MICs are in the resistant range for all available active azoles
Multi azole resistance	MICs are in the resistant range for more than one, but not all azoles
Itraconazole, voriconazole or posaconazole resistant	MICs are in the resistant range for a single azole

MICs= minimal inhibitory concentrations

Table 2. Clinical overview of azole resistance in *A. fumigatus.*

Location	Clinical presentation	Epidemiology	Pathomechanism
Nijmegen	Invasive pulmonary aspergillosis CNS aspergillosis Osteomyelitis	Mainly azole naive Previous azole exposure	Dominant resistance mechanism: TR/L98H
Manchester	Chronic aspergillosis Aspergilloma ABPA CNS aspergillosis	Previous azole exposure	Multiple resistance mechanisms: G448S, M220, P216L, TR/L98H

ABPA=allergic bronchopulmonary aspergillosis

naturally, without prior exposure to the drug. Secondary resistance is generated following exposure to an antifungal and may be associated with an altered gene expression [10, 24]. Clinical resistance is defined as the failure to eradicate an infection despite the administration of an adequate antifungal [24]. Such failures can be attributed to a combination of the host, the pathogen and the drug [24].

Using the European Committee for Antibiotic Susceptibility Testing (EUCAST) methodology breakpoints were recently proposed for *A. fumigatus* and itraconazole, voriconazole and posaconazole [25]; for itraconazole and voriconazole, <2 mg/L (susceptible), 2 mg/L (intermediate) and >2 mg/L (resistant); for posaconazole, <0.25, 0.5 and >0.5 mg/L respectively. It is suggested to differentiate between single-azole, pan-azole and multi-azole resistance (see Table 1), the majority of infections is associated with clinical failure when treated with the ascertained agent [26].

It was thought that acquired resistance of *Aspergillus* species to triazoles is unfrequently [27, 28]. Yet reports from the Netherlands and Manchester display an alarming increase of azole resistance in *A. fumigatus* since 1998 [26, 29, 30]. The first published case of itraconazole-resistance in *A. fumigatus* appeared in 1997 [31]; in 2000, a survey testing over 900 isolates showed a 2% prevalence of ITC resistance in Manchester [32]. In 2007 the percentage of patients with an azole-resistant *A. fumigatus* increased up to 15% [25, 26]. In the Netherlands azole resistance increased dramatically from 2.5% in 2000, to 4.9% in 2002, to 6.6% in 2004 and to 10% in 2009 [25]. This represents an increasing frequency of 6% per year and is an issue due to the limited number of antifungals. Overall, azole-resistance differs from country to country and occurred sporadically in Belgium, Denmark, France, Sweden, Spain and Norway [33-39]. In Spain, the prevalence is about 2% among clinical *A. fumigatus* and in Austria about 0%. In the USA, species with

MICs of voriconazole and posaconazole > 2 mg/L remain rare, less than 1% [27].

The clinical presentation and disease evolution may be related to the underlying genotypes in *A. fumigatus* (Table 2). Isolates have been collected from patients suffering from chronic aspergillosis [29] and invasive diseases [26, 33].

Azole drug resistance in *A. fumigatus* has been reported both, before and after drug exposure; acquired resistance appears to develop through treatment of patients or through exposure of isolates to azole fungicides in the environment [40].

These findings have major implications for clinical practice especially as fungal drug resistance is an acute issue due to the limited number of antifungal compounds. Alternative strategies utilising combination therapy will become more attractive. Experts expect that triazole resistance in this haploid, sparingly sexual worldwide airborne fungus will increase [8]. Key elements in the management of patients will be an accurate speciation of *Aspergillus* species and the performance of in vitro susceptibility testing for an approbiate antifungal treatment. Presently we do not have exact data on the prevalence of azole-resistance Aspergilli in Germany, but seems to be rather low than high. Anyway, a shift has occurred in the epidemiology of invasive infections in Europe [8]. Where invasive candidiasis was once the predominant type of invasive fungal infections, invasive mould infections have become increasingly important, including those caused by unusual pathogens. Moulds have become the leading cause of IFD in some populations. *Aspergillus* species are the most frequent mould pathogens, but the number of infections caused by previously rare pathogens, such as the Zygomycetes and Fusarium species, is increasing. The reasons for the shift in the epidemiology are multifactorial, but are a result, at least in part, of the increased use of extensive voriconazole and echinocandins as prophylaxis/treat-

ment [8]. As a result of growing numbers of immuno-suppressed patients with risk factors, the patient populations for IA will expand and will include patients with haematological malignancy, ICU intervention, pulmonary disease [e.g.chronic obstructive pulmonary disease (COPD) and asthma], SOT recipients and patients with solid tumours.

Much less is currently known about echinocandin resistance in *Aspergillus*, in part because susceptibility testing is not routinely performed and because the methods suffer from technical difficulties and suboptimal reproducibility [41, 42]; breakthrough infections with *A. fumigatus* showing high minimum effective concentrations have been reported sporadically [43]. So far, the selection pressure of candins has risen and the development of resistance is presumed to be inevitable.

MECHANISM OF AZOLE-RESISTANCE IN *A. FUMIGATUS*

The triazoles block the ergosterol biosynthetic pathway at the C14-α-demethylation stage [44]. These antifungals bind to lanosterol 14-α-demethylase (14-α-DM, or *Cyp51p*) which is encoded by the *Erg11* genes. Such step leads to depletion of ergosterol and an accumulation of lanosterol and other toxic 14-α-methylated sterols.

Several pathomechanisms account for azole resistance in *A. fumigatus*; these include a modification of target enzymes, an increased expression of drug efflux mechanisms, an overexpression of target enzymes, an incorporation of exogenous cholesterol, an overexpression of HSP90 and of a sterole-regulatory element binding protein [45, 46]. The resistance phenotype depends on the amino-acid substitution and more than one azole can be affected. Azole-resistant isolates have been reported as multidrug resistant [47], multiazole resistant [48], azole cross-resistant [27] and multiple-triazole resistant [26, 33] isolates. In most cases azole resistance has been associated with point mutations in *cyp51A*, which represents the target enzyme of the azoles [25]; hot spots at codons 54, 98 and 220 are most frequently characterized [26, 33, 35, 48-50]. Interestingly, other mutations have been found in azole susceptible strains and so are unlikely to be associated with resistance [51].

The resistance mechanisms differ between the Dutch and British azole-resistant isolates; in the Netherlands, the presence of a single resistance mechanism (denoted by TR/L98H, a point mutation at codon 98 accompanied by a tandem repeat in the promoter region), was found in over 90% of clinical *A. fumigatus* isolates. By contrast, several *CYP51A* mutations are present in the UK strains [25, 33] and no prevalence of any one alteration. The reasons for this might be due to differences in the patient population from which the isolates originate [25]. For the Dutch isolates, an environmental source is very likely. Azole-resistance may develop due to exposure of *A. fumigatus* to azole fungicides for plant protection [30, 33]. Howard et al. suggest that the reasons of the widespread increase of azole-resistance in the UK may be part of long-term azole drug exposures in patients [29].

MECHANISM OF ECHINOCANDIN-RESISTANCE IN *ASPERGILLUS* SPEZIES

The β-(1,3)-D-glucan is an integral part of the fungal cell wall. Echinocandins are a unique class which block the β-(1,3)-D-glucan synthesis by inhibiting β-(1,3)-D-glucan synthase [52]. This process leads to abnormal hyphal growth in moulds [24]. Much less is currently known about echinocandin resistance in *Aspergillus* and only few clinical isolates associated with treatment failures have been investigated. In such isolate mutation in the *FKS1* target gene was not detected, but expression of the *FKS1* gene was found to be upregulated [37]. Manipulated or laboratory-selected strains with various degrees of caspofungin resistance have been described [42, 53, 54]. Some of these strains have been found to have mutations in the ECM33 gene (*AfuEcm33*), encoding cell wall proteins. Strains with mutations in the *FKS1* gene encoding a subunit of the ß -1,3-D-glucan synthase enzyme have been generated [42]. In other resistant *Aspergillus* mutants the glucan synthase exhibited a wild-type *AfFKS1* gene sequence, where the function, level, and the enzyme itself were susceptible to caspofungin [42, 45].

Differences in the susceptibility to the echinocandins exist among the *Aspergillus* spezies. For example, *Aspergillus niger* is much more susceptible to echinocandins than other species probably in charge of its different cell-wall composition [55]. *Aspergillus lentulus* isolates are less susceptible to caspofungin, although they maintain susceptibility to anidulafungin and micafungin. The analysis of the A. lentulus fks sequence did not reveal a polymorphism at any of the known hot-spot regions of the gene [56].

CROSS RESISTANCE AMONG AZOLES AND ECHINOCANDINS

Cross-resistance patterns are closely linked with the position of the mutation in the *cyp51A* gene [29, 57]. Isolates with alterations at eg codons 98 demonstrate a pan-azole resistance phenotype. Isolates with mutations at codon 54 remain voriconazole susceptible although cross-resistant to posaconazole.

Cross-resistance patterns in isolates with M220 alterations appear to be unpredictable, particularly with respect to voriconazole. The risk of cross-resistance between the azole compounds is high, in one report 74% and 65% of itraconazole resistant isolates were cross-resistant to posaconazole and voriconazole respectively [29].

Between itraconazole, voriconazole and ravuconazole cross-resistance was demonstrated in 10 clinical isolates of *A. fumigatus* obtained from patients with long-term exposure to itraconazole or voriconazole [50]. Also, broad-spectrum cross-resistance among all the azoles has been shown in *A. fumigatus* in a patient receiving prolonged itraconazole prophylaxis [47]. Overall, there is a limited number of reported cases that help us to understand the clinical impact of azole resistance on clinical outcome. For example, in a small case series of patients with IA and no respond to voriconazole, treatment with posaconazole was successful in 50% of infections [58]. On the other hand

in animal model of IA caused by an itraconazole-resistant *A. fumigatus* strain, posaconazole, which is structurally similar to itraconazole, was active in high doses [59].

The potential frequency of cross-resistance amongst echinocandins in *Aspergillus* species is still unclear and has not been investigated in detail [60]. At present, there is no evidence that the activity and efficacy of other antifungal compounds, such as the polyenes and echinocandins, is attenuated in azole-resistant isolates [26, 33].

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