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Antifungal resistance in *Aspergillus*

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There are currently few data on drug resistance in *Aspergillus*, but the recent development of susceptibility tests have made it possible, to some extent, to study resistance in azoles and polyenes. This paper reviews the limited data available. The increased incidence of aspergillosis and the introduction of newer drugs make it important to continue to monitor closely trends in antifungal drug resistance.

Keywords antifungal, *Aspergillus*, resistance

Introduction

There is universal agreement that the outcome of invasive aspergillosis (IA) is dictated by the host immune status. This is illustrated by the fact that compromised host defenses from persistent neutropenia or impaired monocyte/macrophage function adversely affect the prognosis despite the use of potent antifungal drugs. Nonetheless, the role played by anti-*Aspergillus* drugs during therapy of IA should not be minimized as incorrect dose, poor absorption, distribution of metabolism, drug interactions, and drug toxicities clearly contribute to a poor outcome. Clinical resistance has long been thought to be the result of failure of therapy or relapse of *Aspergillus* infection, not due to *Aspergillus* exhibiting resistance to drug. Antifungal drug resistance in *Aspergillus*, unlike with *Candida*, has received little attention.

Clinically relevant data on *Aspergillus* showing resistance to available drugs, namely polyenes, azoles and echinocandins, are scarce. In the past, IA did not elicit much clinical interest as the incidence of infection was low and the pathogen was not readily recovered from patients. Importantly, the lack of a standardized *in vitro* susceptibility test has led to difficulties correlating clinical outcome to *in vitro* findings. This paper reviews the available data on antifungal drug resistance in *Aspergillus* that may contribute to treatment failure.

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In vitro susceptibility test of *Aspergillus*

Developing and standardizing an *in vitro* susceptibility test method for *Aspergillus* has posed enormous problems. Although the hyphal form is routinely recovered in clinical samples, most *in vitro* tests have examined the conidia, since the latter are easily quantified and thus standardization of inoculum size is straightforward; however, the clinical relevance of conidial susceptibility is questionable. Technical difficulties have concerned the ideal test medium (composition/pH), the size of fungal inoculum, the appropriate incubation temperature, and the duration of incubation. Slow growth rate and dimorphic characteristics of *Aspergillus* under different conditions have further contributed to the difficulties in methodology. Despite such problems, the National Committee for Clinical Laboratory Standards (NCCLS) has published a standardized broth microdilution method for testing *Aspergillus* [1]. This method is reproducible and appears reliable, although appears more consistent for azoles than polyenes.

Arbitrary breakpoints for susceptibility have not been established; however, isolates for which minimal inhibitory concentrations (MICs) of azoles and polyenes exceed 1 μg/ml are generally considered resistant. With echinocandins, the endpoints on the microtiter plates are not clear-cut since these agents do not have fungicidal activity against *Aspergillus*. To accommodate this the minimal effective concentration (MEC) has been proposed to indicate when there is morphologic damage. Currently, there are no acceptable methods to evaluate the *in vitro* activity and resistance of echinocandins against *Aspergillus*. In the laboratory, echinocandin-resistant *Aspergillus* isolates have been recovered using cell wall digests [2]. Literature

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examining *in vitro* or *in vivo* resistance of *Aspergillus* to azoles or polyenes is limited.

Resistance to azoles

Resistance can be described as primary (fungal pathogen intrinsically resistant to antifungal drug) or secondary (development of resistance during drug exposure). Primary or acquired resistance of *Aspergillus* to azoles has been noted, but is uncommon. A prevalence of 2%–3% itraconazole resistance (MIC $\geq$ 16 μg/ml) was noted in a study of 156 clinical isolates [3]. In a collection of *A. fumigatus* clinical isolates taken from over 100 patients between 1945 and 1998, three isolates (recovered from a lung transplant patient) were itraconazole resistant; all three were voriconazole susceptible [4]. The investigators found the range of MICs of itraconazole during the 53-year study period had remained the same before and after the introduction of itraconazole. However, Balajee *et al*. [5] found rising MICs of itraconazole among *Aspergillus* isolates recovered from patients in a stem cell transplant center between 1991 and 2000 [5]. Attempting to establish a correlation between *in vitro* findings and *in vivo* observations, Dannaoui *et al*. [6] introduced itraconazole-resistant and itraconazole-susceptible *Aspergillus* isolates in a murine model. These isolates were of the same strain and had been recovered from a patient's sputum before and after exposure to itraconazole. These investigators and others did demonstrate *in vitro*/*in vivo* correlation using the animal model [6,7].

Cross-resistance between azoles has also been shown [8,9]. *Aspergillus* isolates with high MICs to itraconazole (MIC $>$ 1 μg/ml) also had relatively high MICs for the structurally-similar azole, posaconazole, but low MICs of the structurally-dissimilar azoles, voriconazole and ravuconazole. This phenomenon of cross-resistance was confirmed in an animal model infected with itraconazole-resistant *A. fumigatus*, and treated with itraconazole or posaconazole. Itraconazole-treated animals died, while those that received higher doses of posaconazole had improved survival.

In the clinical setting of invasive aspergillosis, itraconazole or voriconazole resistance in *Aspergillus* resulting in treatment failure has not been demonstrated. A single case report of IA occurring during prophylaxis with itraconazole for six years in a patient with chronic gramulomatous disease has been documented; the pathogen was found to be resistant to all azoles *in vitro*. The infection was treated successfully with a high dose of voriconazole [10]. Recently, multiple reports have documented cases of serious voriconazole-resistant fungal infections largely infections due to Zygomyces, during prophylaxis or empiric therapy with voriconazole among stem cell transplant recipients [11–13]. With increased use of azoles among severely compromised hosts, breakthrough infections due to azole-resistant pathogens are to be anticipated.

Resistance to polyenes

Resistance to polyenes is extremely rare in *Aspergillus* spp., except among *A. terreus. In vitro*, most *A. terreus* isolates are resistant to amphotericin B (AmB), but susceptible to itraconazole or voriconazole [14]. Walsh *et al*. [15] demonstrated improved outcome with posaconazole or itraconazole, as compared with amphotericin B, in the treatment of neutropenic rabbits infected with *A.* terreus. Azole-treated animals had rapid clearance of *Aspergillus* galactomannan antigenemia, reduced fungal burden and improved survival. Low ergosterol content in the cell membrane of *A. terreus* was the postulated mechanism for the poor activity of amphotericin B. This extends to humans, as there is poor outcome with amphotericin B (72% mortality) in *A. terreus*-infected patients. Such patients, when treated with voriconazole, had improved survival ($P = 0.02$ vs. AmB) [16].

No good data exist for polyene resistance in non-*terreus Aspergillus* spp. Using the NCCLS susceptibility method, a longitudinal survey of over 500 *Aspergillus* clinical isolates did not find any change in susceptibility to azoles or polyenes over the ten-year study period [17]. No resistant *Aspergillus* isolates were encountered. A preliminary report has documented a steady increase in AmB-resistance *in vitro* among *Aspergillus* isolates recovered since 2001 [18]. About 20% of *A. fumigatus* and *A. flavus* isolates recovered in 2004 had minimum lethal concentrations (MLCs) of amphotericin B $\geq$ 16 μg/ml compared to 0% in 2001. In the laboratory, mutagenized strains of AmB-resistant *A. fumigatus* have been recovered using ultraviolet light. Such isolates appear to be biologically fit and exhibit resistance to amphotericin B in the animal model [19]. Interestingly, following azole exposure, *Aspergillus* has been shown to become resistant to AmB [20]; presumably, the azole makes the organism deficient in ergosterol thus rendering amphotericin B ineffective. Such antagonism with sequential exposure to azole followed by polyene has not been seen in clinical practice; however, the laboratory observation merits caution.

In a retrospective study of 29 immunocompromised hosts with IA and treated with AmB, *in vitro* susceptibility to the drug predicted clinical outcome [21]. Remarkably, 22 of 23 patients infected with *Aspergillus* resistant to AmB (MIC > 2 μg/ml) died, while none of the remaining six infected with susceptible *Aspergillus* isolates died. The study did not, however, elaborate on other clinical features and had utilized older non-standardized methods of susceptibility testing.

Since AmB has been the only drug used against IA in past decades, the emergence of resistance during therapy is of interest. Whether such acquired resistance to AmB emerges during therapy of IA is unclear, since most clinical failures have generally been attributed to poor host defenses and sequential isolates during an infection are seldom available. The limited data available suggest that the emergence of resistance to azoles or polyenes during therapy is uncommon [22,23].

Conclusion

Unlike with *Candida*, available clinical data on drug resistance in *Aspergillus* are scarce at present. Although imperfect, the recent standardization of susceptibility testing for filamentous fungi has now made it possible to study the phenomenon of resistance of *Aspergillus* to azoles and polyenes. Reliable tests to evaluate the *in vitro* activity of echinocandins are not available as yet. The exact prevalence of azole-resistant (primary or secondary) *Aspergillus* is not known, but limited *in vitro* and *in vivo* data suggest the existence of azole resistance and its clinical relevance. Against polyenes, *A. terreus* must be considered to be resistant and such infections are preferably treated with azoles. However, polyene resistance among non-terreus *Aspergillus* spp. appears rare. With the increasing incidence of aspergillosis and the widespread use of newer drugs for prolonged periods, particularly in immunocompromised hosts, the emergence of resistance is only a matter of time. It is therefore prudent to prospectively follow trends of antifungal drug susceptibility in *Aspergillus*.

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