Voriconazole Resistance in *Aspergillus fumigatus*: Should We Be Concerned?

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(See the Major Article by van der Linden et al on pages 513–20.)

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The triazole class of antifungal agents provides the backbone of human antifungal therapy. Resistance is therefore problematic. The clinical impact depends on resistance frequency and which pathogens are affected. Triazole resistance in *Aspergillus fumigatus* was first described in 1997 (in isolates from California in 1989) and has increased in frequency over the last decade. Itraconazole resistance has now been described from most developed countries (including Canada, India, China, and the United States), and a large proportion of these isolates are cross-resistant to voriconazole and posaconazole [1]. To add to this evolving scenario, in this issue of *Clinical Infectious Diseases*, van der Linden and colleagues in Nijmegen, the Netherlands, have now described voriconazole resistance, often without accompanying itraconazole or posaconazole resistance, and a new explanatory mechanism (TR_{46}/Y121F/T289A) [2]. The isolates were picked up using drug-containing agar for primary culture of clinical specimens from all over the Netherlands and from many different patient types. The Nijmegen group has been particularly interested in environmental spread of triazole-resistant *A. fumigatus*, and has previously documented widespread dissemination of another specific-resistance mechanism, TR_{34}/L98H, which confers voriconazole and itraconazole resistance [3]. Isolates containing the new resistant mechanism have been isolated from patients' homes and backyards, at a lower frequency than the TR_{34}/L98H-containing strains. In Nijmegen, the hospital pediatric department yielded both strains from the air. Among 140 resistant strains identified in the environment, 14 (10%) contained the new resistant mutation TR_{46}/Y121F/T289A [2]. One such strain has been identified in neighboring Belgium, but not elsewhere, yet.

So what are the implications of these findings? Patients with invasive aspergillosis caused by a TR_{34}/L98H multiazole-resistant isolate have an 88% mortality rate, compared with 30%–50% in those infected with a susceptible isolates. In our series, among 12 evaluable patients with a triazole-resistant strain, 7 failed therapy (58%) and 5 failed to improve [4]. *Aspergillus fumigatus* is not only an invasive opportunistic pathogen, but is also responsible for chronic pulmonary and rhinosinus disease, airway infection (*Aspergillus* bronchitis, recently rediscovered [5]), and allergic bronchopulmonary and rhinosinus disease. Worldwide, approximately 300 000 people are estimated to develop invasive aspergillosis annually, 1.5%–10% of the millions of highly immunocompromised patients at risk worldwide [6]. Voriconazole resistance is particularly problematic for these patients, as it is accepted first-line therapy. Chronic pulmonary aspergillosis (CPA) after pulmonary tuberculosis is estimated to affect 1.2 million people, approximately 40% of the total estimated 3 million with CPA, with approximately 450 000 deaths each year [6, 7]. Long-term oral antifungal therapy is partially effective, but azole resistance greatly compromises outcomes. Allergic bronchopulmonary aspergillosis (ABPA) and severe asthma with sensitization both improve with oral antifungal therapy, including voriconazole. As ABPA affects approximately 2.5% of the 200 million adults with asthma worldwide [8], dissemination of resistant strains will greatly compromise clinical outcomes for the most problematic patients.

Identifying resistant *Aspergillus* isolates is clearly important for all patients requiring antifungal therapy. Furthermore, prediction of some resistance patterns is possible from knowing the resistance mutation(s). Culture yields are currently low even from good specimens (and essentially zero from blood) but could be enhanced

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with higher-volume cultures and/or supplemented with polymerase chain reaction. Direct detection of resistant mutations is possible now, even in the absence of a positive culture [9]. An outstanding question is whether a single colony should be picked for minimum inhibitory concentrations, or multiple colonies, or whether direct use of drug-containing agar is the best approach to screen for resistance, with a subsequent susceptibility test. These issues should be prospectively evaluated.

Should our guidelines for first-line antifungal therapy change? Or, more precisely, at what frequency of resistance is a change appropriate? In addition, with substantial variation in geographical frequency of different resistance mechanisms, local guidelines may be required, rather than national or international guidelines, as is routine for antibacterial therapy. Options include a switch to amphotericin B or an echinocandin, or the addition of one of these agents to voriconazole, with step-down to itraconazole or posaconazole for a minority of the infecting strains. Although this would seem immediately attractive for covering for the possibility of resistant *A. fumigatus*, step-down therapy from intravenous to oral therapy allowing discharge home becomes problematic, unless a susceptible isolate is cultured. Given that voriconazole yields response and survival rates 15%–20% higher than nonazole regimens (6 postregistration studies in addition to the original registration study) [10], only resistant rates exceeding 10% should trigger a wholescale shift in first-line therapy. Rates of 3% ampicillin resistance in *Haemophilus influenzae* prompted a shift in empirical therapy for meningitis, but this assumes equal efficacy of an alternate regimen, which is not the case for invasive aspergillosis. A better option would be initial combination therapy, with strenuous efforts to obtain a positive culture and susceptibility test, or careful monitoring of response with quantitative biomarkers on therapy, especially after a switch to oral therapy.

Can the emergence of voriconazole resistance be prevented or minimized? There are multiple lines of strong circumstantial evidence pointing to a link between triazole fungicide use and the emergence of environmental strains of *A. fumigatus* resistant to triazoles. These issues were addressed by a committee convened by the European Centre for Disease Control (including the authors of this editorial), which made several recommendations in February 2013 [11]. The global crop protection market was worth $48 billion in 2011 and is growing at about 5% annually. Crop loss due to fungi is estimated to total approximately 10% of all crops, with further losses caused by spoilage of stored foodstuffs. Of all pesticides sprayed on crops, 40% are fungicides. More than 25% of total fungicide sales are azoles and most are triazoles. Triazoles are important fungicides for controlling rusts and mildews on arable crops, *Septoria* leaf blotch on wheat, barley, and rye scald, and light leaf spot of oilseed rape, as well as controlling fruit rot, and many applications annually are required on hops, soft fruit, and fruit trees to prevent such conditions as apple scab. Triazoles are the major seed treatments for wheat and barley. Wood preservation is a major use for certain triazoles combined with copper-based preservatives. Some triazoles are used as fungicides in building and decorating materials. The fungicide triazoles, notably difenoconazole, propiconazole, epoxiconazole, bromuconazole, and tebuconazole, are active against *A. fumigatus* and structurally very similar to the medical triazoles in terms of their binding to the target protein lanosterol 14-α demethylase [12]. The extensive use of these agents is postulated to have led to the development of TR_{34}/L98H and TR_{46}/Y121F/T289A resistance in *A. fumigatus*. Azole resistance is found in many plant pathogenic fungi, including, for example, *Mycosphaerella graminicola* causing leaf blotch in wheat, *Botrytis cinerea* causing fruit rot (gray mold) in strawberries, and *Monilinia fructicola* causing brown rot of many stone fruits, such as peaches and apricots.

Withdrawal of those fungicide triazoles implicated in triazole resistance in *A. fumigatus* would reduce food yields from certain crops. In Europe, where the problem appears to be centered, loss of fungicide azoles is predicted to result in loss of food self-sufficiency for many European Union countries, with potentially profound food supply and economic consequences [13].

However, a gradual increase in azole resistance in *A. fumigatus* will have equally profound impacts on morbidity and mortality with a consequent increase in healthcare costs. *Aspergillus fumigatus*, with its extraordinary ability to disseminate in the environment, will not respect borders, mountain ranges, or oceans.

Conclusive evidence linking the implicated triazole fungicides to the emergence of TR_{34}/L98H and TR_{46}/Y121F/T289A is required in controlled field experiments. Detailed tracking epidemiologic studies in the environment and among clinical isolates are also required. Reduction of key implicated triazole fungicide use in settings where it is not essential would be a sensible precautionary approach, such as in wood preservation and in decorating materials. Registration and launch of new fungicides should require an experimentally verified risk assessment to exclude the possibility of inducing resistance in *A. fumigatus* in the field. Countries that have not yet approved the implicated triazole fungicides might choose to pause before approval, pending further studies. Given the problems of azole resistance in crops, a strong user and science base is longstanding; the US Environmental Protection Agency (www.epa.gov), the Fungicide Resistance Action Committee (www.frac.info/frac/index.htm), and ADAS (www.adas.co.uk) have addressed phytopathogen fungicide resistance rapidly with the introduction of strategies to reduce resistance, keeping the azoles as effective fungicides for many years despite the continuous identification

of azole-resistant plant pathogens [14]. Given the slim pipeline of new antifungal agents, complacency and inaction with respect to this topic is a poor option, given the history of antimicrobial resistance and the remarkable dispersal potential of *A. fumigatus*.

Note

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