Azole resistance in *Aspergillus fumigatus*: a growing public health concern

Edith Vermeulen[a], *Katrien Lagrou*[a,b], *and Paul E. Verweij*[c]

Purpose of review
Reports from the end of the 2000s forced the medical community to take azole resistance in *Aspergillus fumigatus* into account. Not only patients with chronic aspergillus disease, who develop resistance during long-term azole treatment, but also azole-naive patients are at risk, owing to the presence of resistant strains in the environment. The purpose of this review is to overview the latest findings concerning the origin, evolution, and implications of azole resistance in *A. fumigatus*.

Recent findings
TR_{34}/L98H is the predominant resistance mechanism of environmental origin in *A. fumigatus*. Recent epidemiological data show that this mechanism is an expanding problem, with reports from China, Iran, and India. However, the TR_{34}/L98H strains from the Middle East are genotypically different from the European isolates; their emergence is, therefore, not due to simple geographical spread of the 'European' isolates. A new environmental resistance mechanism, TR_{46}/Y121F/T289A, was detected in the Netherlands, conferring voriconazole resistance. In patients chronically treated with triazoles, the spectrum of resistance has become more diverse, with the emergence of non-*CYP51A*-mediated mechanisms. Central registration of treatment and outcome data of patients with resistant aspergillus disease are needed.

Summary
Azole resistance in *A. fumigatus* is evolving to a global health problem.

Keywords
aspergillosis, *Aspergillus fumigatus*, CYP51A, drug resistance, fungal

INTRODUCTION

Triazoles are the mainstay of therapy in infections with the opportunistic fungus *Aspergillus fumigatus*. The emergence of resistance is, therefore, of clinical concern. The first reports of patients with azole-resistant *A. fumigatus* isolates date from 1997, from patients receiving itraconazole therapy from Sweden [1] and California (isolates obtained in 1989) [2]. The characterization of two genes (*CYP51A* and *CYP51B*) encoding the azole target enzyme in *A. fumigatus*, sterol 14-α-demethylase, greatly contributed to the understanding of azole resistance mechanisms [3]. In the first decade after the discovery of azole resistance in *A. fumigatus*, only sporadic cases of resistance were published and resistance was considered an infrequent event. Two reports since the late 2000s changed this perception. First, in 2007, a series of Dutch patients – including azole-naive patients – were described with invasive aspergillosis due to pan-azole-resistant strains and resistance was attributable to one predominant resistance mechanism, TR_{34}/L98H [4]. This mechanism consists of a tandem repeat of 34 bases (TR_{34}) in the promotor of the *CYP51A* gene, leading to enhanced expression, combined with a leucine to histidine amino acid substitution (L98H) [4,5]. In 2009 a second report, from a specialized referral center for patients with chronic and allergic aspergillosis in Manchester, described resistance to have increased dramatically [6]. This situation differed from the TR_{34}/L98H-resistance problem in the Netherlands, as a variety of different *CYP51A*-related

[a]Department of Microbiology and Immunology, Catholic University of Leuven, [b]Department of Laboratory Medicine, University Hospitals Leuven, Leuven, Belgium and [c]Department of Medical Microbiology, Radboud University Nijmegen Medical Centre, Nijmegen, the Netherlands

Correspondence to Paul E. Verweij, MD, PhD, UMC St Radboud Medical Microbiology, PO Box 9101, 6500 HB Nijmegen, the Netherlands. Tel: +31 24 361 43 56; fax: +31 24 354 02 16; e-mail: P.Verweij@mmb.umcn.nl

Curr Opin Infect Dis 2013, 26:493–500
DOI:10.1097/QCO.0000000000000005

Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.

ED_013165_00000353-00001

KEY POINTS

- Environmental resistance is a global and evolving public health problem, with TR_{34}/L98H described in India, China, and Iran and the emergence of a new resistance mechanism, TR_{46}/Y121F/T289A, in Europe.
- The spread of TR_{34}/L98H strains in Europe probably originated from a common ancestor, but they are genotypically distinct from isolates from the Middle East and India.
- Given an increasing number of reports suggesting a shift from *CYP51A*-mediated resistance to non-*CYP51A*-mediated resistance, research should be aimed at identifying new resistance mechanisms, such as the cdr1B efflux transporter, *HapE*, and *CYP51B*.
- The evidence for a fungicide-driven route of resistance development is increasing, but final proof is still lacking. Also the implication of withdrawal of certain fungicides for the dynamics of azole-resistant populations in the field is unknown.
- The revision of first-line therapy guidelines in regions with high (>10%) prevalence of resistance is still an open question; the central registration of the treatment and outcome of patients with resistant aspergillus disease is needed to guide this discussion.

resistance mechanisms were found [6]. These two reports reflect the two routes of resistance selection currently recognized. In patients with chronic aspergillus disease and long-term azole treatment, resistance develops 'in-patient' [6]. These patients were initially infected with a susceptible *A. fumigatus* strain, but this isolate evolved to a resistant phenotype under the selection pressure of azole treatment. The resistant isolate is isogenic to the initial infecting strain. Filamentous fungi are multicellular organisms, so a point mutation conferring azole resistance in one hyphal cell does not impact the phenotype immediately. However, if conidiospores (conidia) are produced from this mutated hyphal element, all conidia will harbor the mutation as will all hyphae that evolve from these spores [7]. A variety of resistance mechanisms (including *CYP51A* point mutations, especially in the hotspot regions at codon 54 or 220) in genetically unrelated strains have been described. In a follow-up study from Manchester, up to 17% of patients harbored a resistant isolate, with many isolates appearing to have a resistance mechanism that is not *CYP51A*-related [8]. This shift to non-*CYP51A*-mediated resistance mechanisms is not understood.

The epidemiology of azole resistance in the Netherlands suggested an alternative route of resistance selection. Evidence is accumulating that *A. fumigatus* becomes resistant in the environment. Patients are believed to inhale azole-resistant *A. fumigatus* spores and subsequently develop aspergillus disease that is refractory to medical triazoles. An important difference with the 'in-patient' route of resistance selection is the dominance of a single resistance mechanism, which has also been found in environmental isolates. In geographical areas with environmental azole resistance, azole-resistant invasive aspergillosis is observed. The risk of resistance selection in patients with invasive aspergillosis through azole therapy appears to be very low.

From 2007 through 2011, the TR_{34}/L98H resistance mechanism was also detected in six other European countries, including the United Kingdom [6], Spain [5], Belgium [9], Denmark [10], France [11], and Norway [12]. The geographical spread of TR_{34}/L98H is believed to be associated with the widespread use of agricultural fungicides [7]. As the selective pressure provided by these compounds in the environment continues, there is a need for strict monitoring of aspergillus resistance. The scope of this article is to overview the recent findings in this evolving field.

EPIDEMIOLOGY OF AZOLE RESISTANCE IN *ASPERGILLUS FUMIGATUS*

When comparing different studies reporting resistance epidemiology, the investigated subpopulation of patients should be taken into account, as higher resistance percentages are reported in patients under chronic azole treatment and different routes of resistance selection are involved. Another factor influencing comparability between resistance percentages is attributable to the difference between reported 'prevalence' (the percentage of patients with a resistant *A. fumigatus* among all patients with *A. fumigatus* cultured from a clinical isolate) or 'resistance rate' (the percentage of resistance among *A. fumigatus* isolates), the latter not corrected for duplicate sampling from the same patient. Table 1 gives an overview of the reported rates and prevalences of resistance.

In recent studies, the high prevalence of resistance in chronic and allergic aspergillosis reported in Manchester was also observed in other centers, with a prevalence of 4.5–8% in cystic fibrosis patients [10,18••]. In these patients, a variety of 'in-patient' resistance mechanisms were found, but also isolates harboring environmental resistance mechanisms. In France, resistance was seen more frequently in patients with previous triazole exposure, but the resistance mechanism involved was predominantly TR_{34}/L98H and the TR_{34}/L98H isolates were genotypically different from the initial infecting strain

Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.

ED_013165_00000353-00002

Table 1. Rate of resistant isolates among clinical *Aspergillus fumigatus* isolates and prevalence of resistance in colonized or infected patients

Country, ref.	Study period	Study isolates	Resistance rate	Resistance prevalence	TR_{34}/L98H rate	TR_{34}/L98H prevalence
UK, [6]	1997–2007	Clinical isolates, irrespective of relevance; referral center for chronic/allergic aspergillosis	34/519 (6.6%)	20/400 (5%)	2/519 (0.4%)	2/400 (0.5%)
UK, [8]	2008–2009	Clinical isolates sent for susceptibility testing; referral center for chronic/allergic aspergillosis	64/230 (27.8%)	28/157 (17.8%)	0/230 (0%)	0/157 (0%)
The Netherlands, [12]	1994–2007	Clinical isolates, irrespective of relevance	63/2061 (3.1%)	45/1320 (3.4%)	–	39/1320 (3.0%)
The Netherlands, [13]	2007–2009	Clinical isolates, irrespective of relevance	82/1792 (4.6%)	63/1192 (5.3%)	74/1792 (4.1%)	57/1192 (4.8%)
The Netherlands, [14▪]	2009–2011	Clinical isolates, irrespective of relevance	–	63/921 (6.8%)	–	47/921[a] (5.1%)
Spain, [15]	2010–2011	Clinical isolates, irrespective of relevance	1/156 (0.6%)	–	–	–
Spain, [16▪]	1999–2011	Clinical isolates from proven or probable invasive aspergillosis or aspergilloma	6/343 (1.8%)	6/148 (4.1%)	0/343 (0%)	0/150 (0%)
Denmark, [10]	2007–2009	Clinical isolates from cystic fibrosis patients, irrespective of relevance	–	6/133 (4.5%)	–	2/133 (1.5%)
France, [17]	2006–2009	Clinical isolates from patients with hematological malignancy, irrespective of relevance	1/118 (0.8%)	1/89 (1.1%)	0/118 (0%)	0/89 (0%)
France, [11]	2010–2011	Clinical isolates from cystic fibrosis patients, irrespective of relevance	–	6/131 (4.6%)	–	2/131 (1.5%)
France, [18▪▪]	2010–2011	Clinical isolates from cystic fibrosis patients, irrespective of relevance	9/85 (10.6%)	4/50 (8.0%)	5/85 (5.9%)	3/50 (6%)
Germany, [19▪]	2011–2012	Clinical isolates irrespective of relevance	3.2% (17/527)	–	6/527 (1.1%)	–
Japan, [20▪]	1994–2010	Clinical isolates, irrespective of relevance (obtained from Pneumology Dept.)	11.2% (22/196)	–	0/196 (0%)	–
India, [21▪]	2005–2010	Clinical isolates from patients suspected of bronchopulmonary aspergillosis	2/103 (1.9%)	2/85 (2.4%)	2/103 (1.9%)	2/85 (2.4%)
Iran, [22▪]	2003–2009	Clinical isolates obtained from patients with aspergillus diseases	3.2% (4/124)	–	3/124 (2.4%)	–
USA, [23]	2001–2006	Isolates recovered from transplant recipients with proven or probable invasive aspergillosis	1/181 (0.6%)	–	–	–

Resistance rate: resistant isolates/all isolates tested; resistance prevalence: percentage of patients with a resistant strain among patients with *Aspergillus fumigatus* from a clinical sample (corrected for repeat sampling); TR_{34}/L98H rate: isolates with *CYP51A* mutation TR_{34}/L98H/all isolates tested; TR_{34}/L98H prevalence: percentage of patients with a resistant strain due to TR_{34}/L98H among patients with *A. fumigatus* from a clinical sample (corrected for repeat sampling).
[a]Thirteen of 921 resistant strains (1.4%) were attributable to the new environmental resistance mechanism TR_{46}/Y121F/T289A.

Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.

ED_013165_00000353-00003

[18••]. If TR_{34}/L98H spores are present in the environment, patients with chronic (azole susceptible) aspergillus disease can still breathe in these spores during azole treatment and acquire 'environmental' resistance, before a phenotypically relevant mutation could develop 'in-patient'. The prevalence of an environmental resistance mechanism in patients chronically treated with azoles might be considered a pseudo-marker for the abundance of environmental resistance.

In 2012–2013, more countries reported TR_{34}/L98H, including the first reported cases from Germany [19•,24•,25], follow-up studies and cases from France [18••], the Netherlands [14•] and Spain [26•], and environmental presence of TR_{34}/L98H in Italy [27]. TR_{34}/L98H was also reported outside of Europe, in isolates from China, India, and Iran (Fig. 1), indicating that TR_{34}/L98H has become a global problem [21•,22•,28,29••,30]. As in-vitro susceptibility testing of clinical or environmental *Aspergillus* isolates is not routinely performed in many centers, azole resistance is probably still underestimated [22•].

The recovery of TR_{34}/L98H in many countries raises the issue of whether geographical (airborne) migration of resistant TR_{34}/L98H-bearing spores takes place, or there is independent local development and subsequent selection of TR_{34}/L98H in unrelated strains, or both. Recently, genetic markers were used to investigate 142 European isolates, which indicated that TR_{34}/L98H isolates showed less genetic variation than azole-susceptible isolates or those with a different genetic basis of resistance. This suggests a common ancestor of the TR_{34}/L98H mechanism that developed locally, possibly in the Netherlands, and subsequently migrated across Europe [31••]. In contrast, all Indian TR_{34}/L98H isolates (environmental and clinical) shared the same microsatellite genotype, indicating clonal spread, but this genotype was not recovered elsewhere [29••]. The authors hypothesized that the Indian genotype is an adaptive recombinant progeny derived from a cross between two strains, one from outside India, possibly azole-resistant, and a native strain from India, followed by rapid geographic migration [29••]. Clinical Iranian TR_{34}/L98H isolates genotypically also cluster separately from European isolates [22•]. A direct comparison of the Indian and Iranian isolates and more TR_{34}/L98H isolates from the Middle East would enhance our understanding of the origin and migration of TR_{34}/L98H [22•].

While TR_{34}/L98H is still spreading, a new environmental *CYP51A*-mediated resistance mechanism was described in the Netherlands, which migrated rapidly across Dutch hospitals [14•] and was also found in domestic homes. This new mechanism consists of a 46 bp tandem repeat together with substitutions (TR_{46}/Y121F/T289A) and is associated with voriconazole therapy failure. A fatal case of invasive aspergillosis due to a TR_{46}/Y121F/T289A strain was already described in neighboring country Belgium [32•]. Whereas TR_{34}/L98H leads to pan-azole resistance, with a pronounced loss of activity of itraconazole, TR_{46}/Y121F/T289A causes high-grade voriconazole resistance, with moderately attenuated (and variable) itraconazole and posaconazole minimum inhibitory concentrations

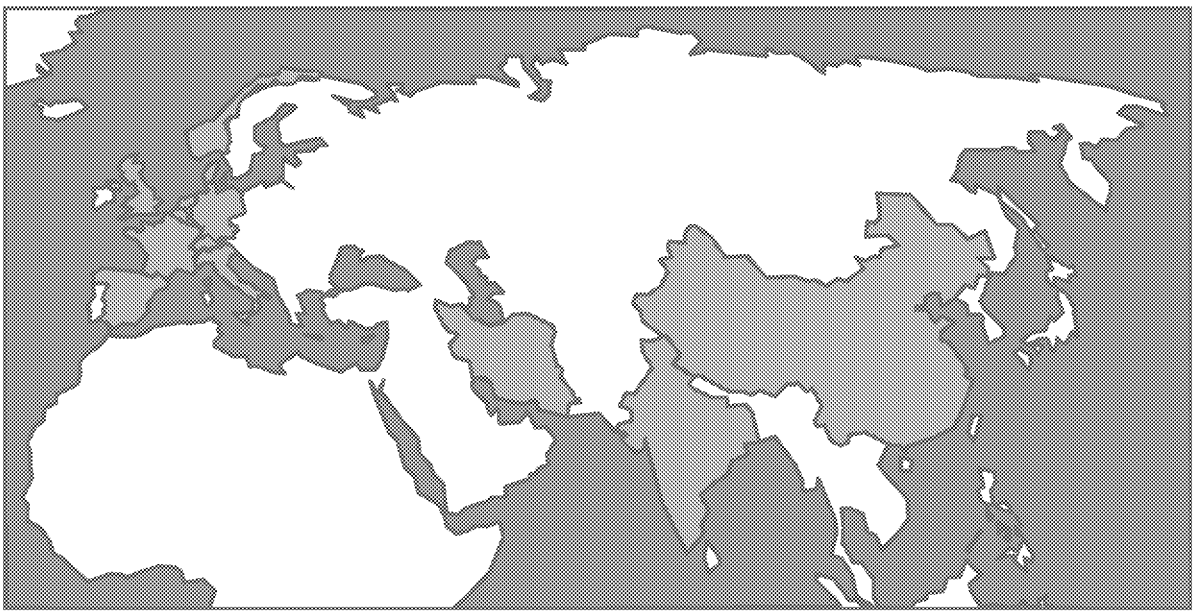

FIGURE 1. Geographical spread of the TR_{34}/L98H resistance mechanism (countries reporting TR_{34}/L98H marked in orange).

Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.

ED_013165_00000353-00004

(MICs) [14▪]. Reduced therapeutic efficacy of itraconazole and posaconazole is certainly expected with TR_{46}/Y121F/T289A, but needs further documentation. The emergence of TR_{46}/Y121F/T289A underlines the continued selection pressure of azole fungicides in the environment. Surveillance studies are needed to monitor whether this new environmental resistance mechanism will further disseminate geographically.

NEW FUNDAMENTAL INSIGHTS INTO *ASPERGILLUS FUMIGATUS* RESISTANCE

The European Centre for Disease Prevention and Control (ECDC) very recently published a report integrating all evidence for a causal role of fungicides in resistance in *A. fumigatus* [33▪▪], which is an important step in the acknowledgement of this emerging problem not only within but also beyond the medical community. This is needed, owing to the potential implications of this issue for both human health and food security [34▪]. Table 2 summarizes the factors that support the hypothesis of a fungicide-driven route of resistance selection. As long as the molecular mechanisms at the origin of aspergillus' resistance are unclear, it will not be unraveled whether fungicides only provide selective pressure and aid in the spread of resistant strains, or really play a causal role in resistance development. The circumstances under which resistance selection might take place remain unknown. Genotyping studies indicate that the genetic diversity observed in TR_{34}/L98H is too large to be solely explained by asexual reproduction. Experiments demonstrated that TR_{34}/L98H strains can undergo a sexual cycle *in vitro* and cross with isolates of different genetic backgrounds [31▪▪]. Abundant phylogenetic incompatibility, consistent with recombination, was found in samples from India, supportive of sexual mating in natural populations of *A. fumigatus* [29▪▪]. However, it is unclear whether and in what conditions this phenomenon occurs in nature. It is, therefore, unknown whether this plays a role in the origin of the tandem repeat, as the underlying molecular mechanisms leading to the creation of tandem repeats are not well understood in general. The tandem repeat consisting of 46 bases in the recently emerging environmental voriconazole-resistant strains (TR_{46}/Y121F/T289A) includes the base sequence involved in TR_{34}, but with two additional flanking fragments. Tandem repeats are inherently unstable, but the mutation rate is also affected by external factors, such as the transcription rate and indirectly also by environmental stress [38]. There is no evidence that suggests that TR_{46}/Y121F/T289A evolved from a strain with TR_{34} to date. Microsatellite genotyping showed that TR_{46}/Y121F/T289A strains cluster together, but a different clade than TR_{34}/L98H and apart from wild-type control isolates [14▪].

In different studies, *A. fumigatus* with *CYP51A*-unrelated resistance was described, and the proportion of these isolates appears to have increased [8,19▪,20▪,28]. As a consequence, different genes are now investigated to identify the resistance mechanisms involved. Recent studies described a higher basal expression of the cdr1B efflux transporter [39▪], a mutation in the CCAAT-binding transcription factor complex subunit HapE [40▪▪] and high (both basal or azole-induced) CYP51B expression [41▪] to play a role in azole resistance. The relative importance of these different elements is yet unknown. There is also evidence that the *CYP51A* promotor has regulatory element(s) negatively influencing gene transcription, located upstream of the 34-base element involved in the tandem

Table 2. Evidence for a fungicide-driven route of resistance selection

	Reference
Agricultural triazole fungicides[a] have a comparable molecule structure to medical triazoles, binding to the same active site of the target enzyme	[35▪▪]
Bioinformatic studies suggest that the presence of L98H not only hinders the docking of the medical triazoles but also of the triazole fungicides[a]	[35▪▪]
TR_{34}/L98H also leads to resistance of agricultural triazole fungicides against *A. fumigatus*, in in-vitro susceptibility testing	[35▪▪]
The authorization of five triazole fungicides[a] for use in the Netherlands (1990–1996) preceded the first TR_{34}/L98H isolate (in 1998)	[35▪▪]
TR_{34}/L98H involves two genomic changes, which is unlikely to occur in a patient receiving azole therapy. The origin of tandem repeats is not well understood, but has also been found in phytopathogenic fungi, which lost susceptibility to azole fungicides.	[36▪]
TR_{34}/L98H isolates have been reported from geographical areas that correspond with the highest usage of azole fungicides (Arendrup)	[37]

[a]Propiconazole, tebuconazole, epoxiconazole, difenoconazole, and bromuconazole.

Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.

ED_013165_00000353-00005

repeat [42**]. Full sequencing of the *CYP51A* promotor is, therefore, an interesting research option in aspergillus resistance of unknown origin.

IMPLICATIONS FOR PATIENTS WITH AZOLE-RESISTANT ASPERGILLOSIS

The increasing trend of resistance in several countries suggests the need for routine susceptibility testing of aspergilli from clinical isolates, in all centers caring for immunocompromised patients or patients with chronic or allergic aspergillosis. Early detection of resistance is important, especially in cases of invasive aspergillosis. In culture-positive patients, in-vitro susceptibility testing can take place; breakpoints have been published that enable interpretation of the MIC [43]. In culture-negative patients, detection of resistance remains problematic. Although resistance mutations have been detected directly in clinical specimens [44,45], the sensitivity of PCR is inadequate given the fact that the *CYP51A* gene is a single copy gene. Furthermore, the increasing diversity of CYP51A-mediated resistance mechanisms and the emergence of non-*CYP51A*-related resistance underscore the need for new (molecular) tools that enable the detection of a broad range of mechanisms.

A case series of patients with invasive aspergillosis caused by a TR_{34}/L98H isolate indicated a higher mortality rate compared with patients infected with a wild-type strain (88% versus 30–50%) [13]. Although some colleagues feel that revision of treatment guidelines is not yet indicated [46], in areas where resistance is widespread adjustment of the guidelines should be considered depending on the local epidemiology [47**]. Denning and Bowyer [47**] suggested that first-line therapy (with voriconazole) should remain unchanged, at least as long as the local resistance prevalence does not exceed 10%. In the setting of azole resistance, liposomal amphotericin B (L-AMB) is an important therapeutic alternative as no cross-resistance is described and L-AMB is recommended as an alternative first-line treatment in (azole susceptible) invasive aspergillosis (IDSA AI) and in salvage therapy (IDSA AII) [48]. Recent research examined the activity of different antifungal drugs and combinations against azole-resistant strains. The activity of AMB and caspofungin against resistant strains did not differ from the activity against wild-type strains in in-vitro experiments [49]. In a murine model of disseminated azole-resistant aspergillosis, L-AMB was equally effective against azole-susceptible and azole-resistant strains, independent of the underlying resistance mechanism [50*]. An alternative approach would be to start primary therapy with a combination of voriconazole and an echinocandin. Voriconazole and anidulafungin were synergistic in mice infected with voriconazole-susceptible *A. fumigatus*. However, in voriconazole-resistance (voriconazole MIC of 4 mg/l), only an additive interaction was observed [51*]. There is concern that in voriconazole-highly resistant *A. fumigatus* infection, the efficacy of the combination would rely only on that of anidulafungin [51*,52–54]. Anidulafungin monotherapy was only effective in 45% of mice infected with voriconazole-resistant strains, compared with 72% in mice infected with susceptible strains, raising questions about the value of this therapeutic alternative [53]. Anidulafungin is currently not approved for the treatment of invasive aspergillosis. At present, clinical data regarding alternative therapeutic options are very limited.

TOPICS FOR FUTURE RESEARCH

The growing problem of azole resistance in *A. fumigatus* should prompt research into the origin and management of resistance selection in the environment and on management strategies of patients with azole-resistant disease.

The origin and patterns of migration of TR_{34}/L98H and other environmental resistance mechanisms should be investigated by genotyping of resistant isolates from different areas of the world. This will help to understand the dynamics of migration. Field experiments are warranted that investigate the impact of withdrawal of certain fungicides on the population of resistant isolates within a wild-type population. The relation between azole fungicide exposure and the emergence of resistance mechanisms in *A. fumigatus* has not yet been proven.

For patient management, international surveillance networks are critical to determine the local epidemiology of azole resistance and to detect the emergence of new resistance mechanisms early. Diagnostic tools are urgently warranted that allow rapid detection of resistance mechanisms in culture-positive and, especially, in culture-negative patients. For this, it is important to identify all resistance mechanisms, including those not mediated through the *CYP51A*-gene.

Clinical data regarding the treatment and outcome of patients with resistant infection should be registered, to help in the re-evaluation of the current first-line management strategies in regions with a high resistance prevalence.

CONCLUSION

Resistance in *A. fumigatus* caused by environmental resistant strains is a growing public health concern

Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.

ED_013165_00000353-00006

with global dimensions. A new environmental resistance mechanism was recently described and illustrates how the unchanged selection pressure of azole fungicides in the environment leads to an evolving situation. More research is needed on the geographical spread and molecular mechanisms of resistance development in *Aspergillus* and the exact role of fungicides herein. A network with central registration of resistance in *Aspergillus* and clinical data on alternative management strategies are warranted.

Acknowledgements

E.V. receives a personal grant from the Research Foundation Flandres (Fonds Wetenschappelijk Onderzoek Vlaanderen).

Conflicts of interest

There are no conflicts of interest.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:
▪ of special interest
▪▪ of outstanding interest

1. Chryssanthou E. In vitro susceptibility of respiratory isolates of *Aspergillus* species to itraconazole and amphotericin B: acquired resistance to itraconazole. Scand J Infect Dis 1997; 29:509–512.
2. Denning DW, Venkateswarlu K, Oakley KL, *et al.* Itraconazole resistance in *Aspergillus fumigatus*. Antimicrob Agents Chemother 1997; 41:1364–1368.
3. Mellado E, Diaz-Guerra TM, Cuenca-Estrella M, Rodriguez-Tudela JL. Identification of two different 14-alpha sterol demethylase-related genes (cyp51A and cyp51B) in *Aspergillus fumigatus* and other *Aspergillus* species. J Clin Microbiol 2001; 39:2431–2438.
4. Verweij PE, Mellado E, Melchers WJ. Multiple-triazole-resistant aspergillosis. N Engl J Med 2007; 356:1481–1483.
5. Mellado E, Garcia-Effron G, Alcázar-Fuoli L, *et al.* A new *Aspergillus fumigatus* resistance mechanism conferring in vitro cross-resistance to azole antifungals involves a combination of CYP51A alterations. Antimicrob Agents Chemother 2007; 51:1897–1904.
6. Howard SJ, Cerar D, Anderson MJ, *et al.* Frequency and evolution of azole resistance in *Aspergillus fumigatus* associated with treatment failure. Emerg Infect Dis 2009; 15:1068–1076.
7. Verweij PE, Snelders E, Kema GH, *et al.* Azole resistance in *Aspergillus fumigatus*: a side-effect of environmental fungicide use? Lancet Infect Dis 2009; 9:789–795.
8. Bueid A, Howard SJ, Moore CB, *et al.* Azole antifungal resistance in Aspergillus fumigatus: 2008 and 2009. J Antimicrob Chemother 2010; 65:2116–2118.
9. Lagrou K, De Vleeschouwer J, Meersseman W, *et al.* Triazole resistance among clinical *Aspergillus fumigatus* isolates. 3rd Advances Against Aspergillosis Congress; 16–19 January 2008; Miami Beach, Florida; poster 33.
10. Mortensen KL, Jensen RH, Johansen HK, *et al.* *Aspergillus* species and other molds in respiratory samples from patients with cystic fibrosis: a laboratory-based study with focus on *Aspergillus fumigatus* azole resistance. J Clin Microbiol 2011; 49:2243–2251.
11. Burgel PR, Baixench MT, Amsellem M, *et al.* High prevalence of azole-resistant *Aspergillus fumigatus* in adults with cystic fibrosis exposed to itraconazole. Antimicrob Agents Chemother 2012; 56:869–874.
12. Snelders E, van der Lee HA, Kuijpers J, *et al.* Emergence of azole resistance in *Aspergillus fumigatus* and spread of a single resistance mechanism. PLoS Med 2008; 5:e219.
13. van der Linden JW, Snelders E, Kampinga GA, *et al.* Clinical implications of azole resistance in *Aspergillus fumigatus*, The Netherlands, 2007–2009. Emerg Infect Dis 2011; 17:1846–1854.
14. ▪ van der Linden JW, Camps SM, Kampinga GA, *et al.* Aspergillosis due to voriconazole highly resistant *Aspergillus fumigatus* and recovery of genetically related resistant isolates from domiciles. Clin Infect Dis 2013; 57 (4):513–520.

The emergence of a new environmental resistance mechanism is shown, which highlights that resistance is an evolving problem.

15. Alastruey-Izquierdo A, Mellado E, Peláez T, *et al.*, FILPOP Study Group. Population-based survey of filamentous fungi and antifungal resistance in Spain (FILPOP Study). Antimicrob Agents Chemother 2013; 57:3380–3387.
16. ▪ Escribano P, Peláez T, Muñoz P, *et al.* Is azole resistance in *Aspergillus fumigatus* a problem in Spain? Antimicrob Agents Chemother 2013; 57:2815–2820.

This article is one of the few epidemiological studies that give resistance data for patients with relevant aspergillus disease, instead of data for clinical isolates irrespective of relevance.

17. Alanio A, Sitterlé E, Liance M, *et al.* Low prevalence of resistance to azoles in *Aspergillus fumigatus* in a French cohort of patients treated for haematological malignancies. J Antimicrob Chemother 2011; 66:371–374.
18. ▪▪ Morio F, Aubin GG, Danner-Boucher I, *et al.* High prevalence of triazole resistance in *Aspergillus fumigatus*, especially mediated by TR/L98H, in a French cohort of patients with cystic fibrosis. J Antimicrob Chemother 2012; 67:1870–1873.

This article shows how environmental resistance also plays an important role in resistance in patients under chronic azole therapy.

19. ▪ Bader O, Weig M, Reichard U, *et al.* CYP51A-based mechanisms of *Aspergillus fumigatus* azole drug resistance present in clinical samples from Germany. Antimicrob Agents Chemother 2013; 57 (8):3513–3517.

Large German epidemiological surveillance study reporting azole resistance, reporting the presence of TR_{34}/L98H among other *CYP51A* mutations, but also of a high percentage of unexplained non-*CYP51A*-mediated resistance.

20. ▪ Tashiro M, Izumikawa K, Minematsu A, *et al.* Antifungal susceptibilities of *Aspergillus fumigatus* clinical isolates obtained in Nagasaki, Japan. Antimicrob Agents Chemother 2012; 56:584–587.

This study describes a high resistance rate among clinical isolates from patients from the Pneumology Department, Japan. This is the first report of resistance attributable to an I266N CYP51A mutation.

21. ▪ Chowdhary A, Kathuria S, Randhawa HS, *et al.* Isolation of multiple-triazole-resistant *Aspergillus fumigatus* strains carrying the TR/L98H mutations in the cyp51A gene in India. J Antimicrob Chemother 2012; 67:362–366.

The first report of TR_{34}/L98H in India, in clinical isolates from two azole-naive patients.

22. ▪ Seyedmousavi S, Hashemi SJ, Zibafar E, *et al.* Azole-resistant *Aspergillus fumigatus*, Iran. Emerg Infect Dis 2013; 19:832–834.

The emergence of TR_{34}/L98H strains in Iran is described, which are genotypically unrelated to European isolates. These data are important for the understanding of the selection and spread of this resistance mechanism.

23. Baddley JW, Marr KA, Andes DR, *et al.* Patterns of susceptibility of *Aspergillus* isolates recovered from patients enrolled in the Transplant-Associated Infection Surveillance Network. J Clin Microbiol 2009; 47:3271–3275.
24. ▪ Rath PM, Buchheidt D, Spiess B, *et al.* First reported case of azole-resistant *Aspergillus fumigatus* due to the TR/L98H mutation in Germany. Antimicrob Agents Chemother 2012; 56:6060–6061.

The first clinical case of TR_{34}/L98H resistance in Germany.

25. Hamprecht A, Buchheidt D, Vehreschild JJ, *et al.* Azole-resistant invasive aspergillosis in a patient with acute myeloid leukaemia in Germany. Euro Surveill 2012; 17:20262.
26. ▪ Mellado E, De La Camara R, Buendia B, *et al.* Breakthrough pulmonary *Aspergillus fumigatus* infection with multiple triazole resistance in a Spanish patient with chronic myeloid leukemia. Rev Iberoam Micol 2013; 30:64–68.

The first clinical case of TR_{34}/L98H resistance in Spain.

27. Tortorano AM, Venier A, Prigitano A, *et al.* Azole resistant *Aspergillus fumigatus* in the environment in Italy [abstract]. 18th Congress of the International Society for Human and Animal Mycology; 11–15 June 2012; Berlin, Germany; abstract P745.
28. Lockhart SR, Frade JP, Etienne KA, *et al.* Azole resistance in *Aspergillus fumigatus* isolates from the ARTEMIS global surveillance study is primarily due to the TR/L98H mutation in the cyp51A gene. Antimicrob Agents Chemother 2011; 55:4465–4468.
29. ▪▪ Chowdhary A, Kathuria S, Xu J, *et al.* Clonal expansion and emergence of environmental multiple-triazole-resistant *Aspergillus fumigatus* strains carrying the TR_{34}/L98H mutations in the cyp51A gene in India. PLoS One 2012; 7:e52871.

This article shows evidence for the occurrence of sexual reproduction in *Aspergillus* in nature, and a possible role of recombination in the development of Indian resistant TR_{34}/L98H strains, which are genotypically different from European isolates.

30. Badali H, Vaezi A, Haghani I, *et al.* Environmental study of azole-resistant *Aspergillus fumigatus* with TR(34) /L98H mutations in the cyp51A gene in Iran. Mycoses 2013. [Epub ahead of print]
31. ▪▪ Camps SM, Rijs AJ, Klaassen CH, *et al.* Molecular epidemiology of *Aspergillus fumigatus* isolates harboring the TR_{34}/L98H azole resistance mechanism. J Clin Microbiol 2012; 50:2674–2680.

This article documents the probability of a common ancestor of the European resistant *Aspergillus* isolates and subsequent geographical migration rather than an independent emergence of TR_{34}/L98H on different locations in Europe.

32. ▪ Vermeulen E, Maertens J, Schoemans H, Lagrou K. Azole-resistant *Aspergillus fumigatus* due to TR46/Y121F/T289A mutation emerging in Belgium, July 2012. Euro Surveill 2012; 17:48.

A fatal case due to new TR_{46}/Y121F/T289A mutation in Belgium, providing additional evidence for a rapid geographical spread of this environmental resistance mechanism.

Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.

ED_013165_00000353-00007

33. European Centre for Disease Prevention and Control. Risk assessment on the
■■ impact of environmental usage of triazoles on the development and spread of resistance to medical triazoles in *Aspergillus* species. Stockholm: ECDC 2013. http://ecdc.europa.eu/en/publications/Publications/risk-assessment-impact-environmental-usage-of-triazoles-on-Aspergillus-spp-resistance-to-medical-triazoles.pdf [Accessed 28 May 2013]

This ECDC report gives a comprehensive review of all evidence for a fungicide-driven environmental route of resistance selection.

34. Bowyer P, Denning DW. Environmental fungicides and triazole resistance in
■ *Aspergillus*. Pest Manag Sci 2013. [Epub ahead of print].

This article addresses the potential implications of *Aspergillus* azole resistance for both human health and food security.

35. Snelders E, Camps SM, Karawajczyk A, *et al.* Triazole fungicides can induce
■■ cross-resistance to medical triazoles in *Aspergillus fumigatus*. PLoS One 2012; 7:e31801.

This article provides proof for the fungicide-driven route of environmental resistance selection.

36. Camps SM, van der Linden JW, Li Y, *et al.* Rapid induction of multiple
■ resistance mechanisms in *Aspergillus fumigatus* during azole therapy: a case study and review of the literature. Antimicrob Agents Chemother 2012; 56:10–16.

This study provides insight into the kinetics of resistance development in *Aspergillus* cultured from patients under chronic azole treatment.

37. Stensvold CR, Jorgenson LN, Arendrup MC. Azole-resistant invasive aspergillosis: relationship to agriculture. Curr Fungal Infect Rep 2012; 6:178–191.

38. Gemayel R, Vinces MD, Legendre M, Verstrepen KJ. Variable tandem repeats accelerate evolution of coding and regulatory sequences. Annu Rev Genet 2010; 44:445–477.

39. Fraczek MG, Bromley M, Buied A, *et al.* The cdr1B efflux transporter is
■ associated with noncyp51a-mediated itraconazole resistance in *Aspergillus fumigatus*. J Antimicrob Chemother 2013; 68:1486–1496.

A new non-CYP51A-mediated resistance mechanism is proposed.

40. Camps SM, Dutilh BE, Arendrup MC, *et al.* Discovery of a HapE mutation that
■■ causes azole resistance in *Aspergillus fumigatus* through whole genome sequencing and sexual crossing. PLoS One 2012; 7:e50034.

A new method for identification of azole resistance mechanisms in *A. fumigatus* is described as well as a new non-CYP51A-mediated resistance mechanism.

41. Buied A, Moore CB, Denning DW, Bowyer P. High-level expression of
■ cyp51B in azole-resistant clinical *Aspergillus fumigatus* isolates. J Antimicrob Chemother 2013; 68:512–514.

Not only CYP51A but also (the rate of expression) CYP51B appears to play a role in azole resistance.

42. Paul S, Klutts JS, Moye-Rowley WS. Analysis of promoter function in
■■ *Aspergillus fumigatus*. Eukaryot Cell 2012; 11:1167–1177.

This work provides an important new tool for analysis of gene expression in *A. fumigatus*. With this technique, new insights into the regulation and function of the CYP51A promoter are obtained.

43. European Committee on Antimicrobial Susceptibility Testing (EUCAST). Antifungal Agents - breakpoint tables for interpretation of MICs. EUCAST 2012; version 4.1. http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/AFST/Antifungal_breakpoints_v_4.1.pdf [Accessed 4 July 2013]

44. Denning DW, Park S, Lass-Florl C, *et al.* High-frequency triazole resistance found in nonculturable *Aspergillus fumigatus* from lungs of patients with chronic fungal disease. Clin Infect Dis 2011; 52:1123–1129.

45. van der Linden JW, Snelders E, Arends JP, *et al.* Rapid diagnosis of azole-resistant aspergillosis by direct PCR using tissue specimens. J Clin Microbiol 2010; 48:1478–1480.

46. Georgiadou SP, Kontoyiannis DP. The impact of azole resistance on aspergillosis guidelines. Ann N Y Acad Sci 2012; 1272:15–22.

47. Denning DW, Bowyer P. Voriconazole resistance in *Aspergillus fumigatus*:
■■ should we be concerned? Clin Infect Dis 2013; 57 (4):521–523.

This editorial concisely summarizes the key questions regarding the management of aspergillus resistance and proposes a local resistance threshold that has to be reached before adapting first-line therapy.

48. Pappas PG, Kauffman CA, Andes D, *et al.*, Infectious Diseases Society of America.. Clinical practice guidelines for the management of candidiasis: 2009 update by the Infectious Diseases Society of America. Clin Infect Dis 2009; 48:503–535.

49. Arendrup MC, Perkhofer S, Howard SJ, *et al.* Establishing in vitro-in vivo correlations for *Aspergillus fumigatus*: the challenge of azoles versus echinocandins. Antimicrob Agents Chemother 2008; 52:3504–3511.

50. Seyedmousavi S, Melchers WJ, Mouton JW, Verweij PE. Pharmacodynamics
■ and dose-response relationships of liposomal amphotericin B against different azole-resistant *Aspergillus fumigatus* isolates in a murine model of disseminated aspergillosis. Antimicrob Agents Chemother 2013; 57:1866–1871.

A dose–response relationship for L-AMB was observed against azole-resistant invasive aspergillosis, independent of the azole resistance mechanism.

51. Seyedmousavi S, Brüggemann RJ, Melchers WJ, *et al.* Efficacy and pharma-
■ codynamics of voriconazole combined with anidulafungin in azole-resistant invasive aspergillosis. J Antimicrob Chemother 2013; 68:385–393.

The combination of voriconazole and anidulafungin was shown to be synergistic in voriconazole-susceptible invasive aspergillosis, in a nonneutropenic murine model, but only additive in voriconazole resistance.

52. Seyedmousavi S, Meletiadis J, Melchers WJ, *et al.* In vitro interaction of voriconazole and anidulafungin against triazole-resistant *Aspergillus fumigatus*. Antimicrob Agents Chemother 2013; 57:796–803.

53. Seyedmousavi S, Brüggemann RJ, Melchers WJ, *et al.* Pharmacodynamics of anidulafungin against clinical *Aspergillus fumigatus* isolates in a nonneutropenic murine model of disseminated aspergillosis. Antimicrob Agents Chemother 2013; 57:303–308.

54. Jeans AR, Howard SJ, Al-Nakeeb Z, *et al.* Combination of voriconazole and anidulafungin for treatment of triazole-resistant *Aspergillus fumigatus* in an in vitro model of invasive pulmonary aspergillosis. Antimicrob Agents Chemother 2012; 56:5180–5185.

Copyright © Lippincott Williams & Wilkins. Unauthorized reproduction of this article is prohibited.

ED_013165_00000353-00008