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Author for correspondence:
Jacques F. Meis
e-mail: jacques.meis@gmail.com

Clinical implications of globally emerging azole resistance in *Aspergillus fumigatus*

Jacques F. Meis[1,2,3], Anuradha Chowdhary[4], Johanna L. Rhodes[5], Matthew C. Fisher[5] and Paul E. Verweij[2,3]

[1]Department of Medical Microbiology and Infectious Diseases, Canisius Wihelmina Hospital (CWZ), Nijmegen, The Netherlands
[2]Department of Medical Microbiology, Radboud University Medical Center, Nijmegen, The Netherlands
[3]Radboudumc/CWZ Centre of Excellence in Mycology, Nijmegen, The Netherlands
[4]Department of Medical Microbiology, Division of Mycology, Vallabhbhai Patel Chest Institute, University of Delhi, Delhi, India
[5]Department of Infectious Disease Epidemiology, Imperial College School of Public Health, St Mary's Campus, London, UK

JFM, 0000-0003-3253-6080; AC, 0000-0002-2028-7462; JLR, 0000-0002-1338-7860; MCF, 0000-0002-1862-6402

Aspergillus fungi are the cause of an array of diseases affecting humans, animals and plants. The triazole antifungal agents itraconazole, voriconazole, isavuconazole and posaconazole are treatment options against diseases caused by *Aspergillus*. However, resistance to azoles has recently emerged as a new therapeutic challenge in six continents. Although de novo azole resistance occurs occasionally in patients during azole therapy, the main burden is the aquisition of resistance through the environment. In this setting, the evolution of resistance is attributed to the widespread use of azole-based fungicides. Although ubiquitously distributed, *A. fumigatus* is not a phytopathogen. However, agricultural fungicides deployed against plant pathogenic moulds such as *Fusarium*, *Mycospaerella* and *A. flavus* also show activity against *A. fumigatus* in the environment and exposure of non-target fungi is inevitable. Further, similarity in molecule structure between azole fungicides and antifungal drugs results in cross-resistance of *A. fumigatus* to medical azoles. Clinical studies have shown that two-thirds of patients with azole-resistant infections had no previous history of azole therapy and high mortality rates between 50% and 100% are reported in azole-resistant invasive aspergillosis. The resistance phenotype is associated with key mutations in the *cyp51A* gene, including TR_{34}/L98H, TR_{53} and TR_{46}/Y121F/T289A resistance mechanisms. Early detection of resistance is of paramount importance and if demonstrated, either with susceptibility testing or through molecular analysis, azole monotherapy should be avoided. Liposomal amphotericin B or a combination of voriconazole and an echinocandin are recomended for azole-resistant aspergillosis.

This article is part of the themed issue 'Tackling emerging fungal threats to animal health, food security and ecosystem resilience'.

1. Introduction

Aspergillus fungi cause a spectrum of clinical diseases in humans and animals. The disease spectrum ranges from colonization, allergic conditions such as allergic bronchopulmonary aspergillosis (ABPA) to invasive disease. ABPA is primarily seen in patients who manifest with chronic *Aspergillus* colonization, such as patients with cystic fibrosis and chronic granulomatous disease. *Aspergillus* can colonize a pre-existing cavity in the lung, such as occurs post-tuberculosis, which is then referred to as an aspergilloma. An aspergilloma can be asymptomatic; however, aspergilloma generally gives rise to persistent symptoms and lung haemorrhage. Patients with lung diseases such as chronic obstructive pulmonary disease (COPD) can develop chronic pulmonary aspergillosis (CPA),

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when the fungus becomes locally invasive with tissue damage [1]. This results in chronic inflammation, which can result in the occurrence of more cavities, or ultimately pulmonary fibrosis. At the other end of the spectrum is acute invasive aspergillosis (IA) primarily observed in patients with haematological malignancies such as acute myeloid leukemia (AML), and in patients who have undergone solid organ transplantation [2]. The disease is also increasingly diagnosed in critically ill patients with COPD and/or severe influenza [3,4].

2. Treatment of disease caused by *Aspergillus* infections

In the past 15 years, azoles have gained an important role in the prevention and treatment of *Aspergillus* diseases [5]. The azole antifungals target ergosterol that is present in the fungal cell membrane. This essential component is synthesized from lanosterol by removing the methyl group at C14, and is catalysed by the enzyme alpha-demethylase [6]. This enzyme, a member of the cytochrome P450 family, is encoded by the *cyp51A* gene, a coding region of 2 048 base pairs found on chromosome 4. Azoles inhibit the biosynthesis pathway for ergosterol, thereby interfering with the integrity of the fungal cell membrane [6]. Azole resistance can be an intrinsic phenotype, as it is known to occur in cryptic *Aspergillus* species related to *A. fumigatus*, specifically *A. lentulus* and *A. pseudofischeri* [7] whereas wild-type *A. fumigatus* and *A. flavus* are sensitive to these drugs. However, azole resistance is an acquired trait that occurs after azole exposure during medical treatment, or after fungicide exposure in the field. There are multiple mechanisms of acquired resistance to azoles, with the most common mutations that are involved being located in the *cyp51A* gene [8–10].

Primarily, itraconazole is used in the treatment of CPA [1], while voriconazole is used as first-line therapy of IA [5]. Recently, another azole, isavuconazole, was approved for the primary treatment of IA [11]. Posaconazole is indicated as prophylaxis in high-risk patients such as AML and stem cell transplant patients with graft-versus-host-disease [12]. Itraconazole, voriconazole, posaconazole and isavuconazole are available as oral and intravenous formulations, and azoles are the only class of antifungals available as oral options for treatment of *Aspergillus* diseases. Another class of compounds, the echinocandins, are also active against *Aspergillus* and have been used as salvage therapy [13,14]. This class has three registered drugs (caspofungin, anidulafungin and micafungin), which inhibit the synthesis of the fungal cell wall, a structure which is absent in human cells. The echinocandins have minimal side effects, but the effectiveness against *Aspergillus* is limited due to the fungistatic nature of the drug (fungistasis may be the net result of some cidal and some resistant growth). Clinical studies have shown that echinocandins are effective in patients with CPA [1], but they seem to be less effective as primary treatment of neutropenic patients with IA [15]. However, the echinocandins are suitable in combination with an azole for primary treatment against infections caused by *Aspergillus* [16].

3. Resistance to azoles

In retrospect, the first azole-resistant *A. fumigatus* isolates date back to the late 1980s on the West Coast of the USA in California, and were cultured from two patients treated with itraconazole [17]. Later, a Dutch study reported three itraconazole-resistant *A. fumigatus* recovered in 1997 from a lung transplant recipient after long-term itraconazole treatment [18]. Following these reports, a French study found four itraconazole-resistant isolates in 1999 [19]. Subsequently in 2007, a comprehensive series of nine cases of azole-resistant IA showed that four out of nine patients had previously never received azole therapy [20]. Further, the resistant *A. fumigatus* isolates exhibited the same resistance mechanism as that found in environmental and airborne isolates [21]. Remarkably, the genetic relatedness of European clinical and environmental azole-resistant *A. fumigatus* isolates measured using a panel of highly polymorphic genetic markers showed a close relationship, and it was hypothesized that these patients had acquired azole-resistant *A. fumigatus* strains from the environment [22]. This indicated that a second route for the induction of resistance had developed through a fungicide-driven route, due to exposure and selection of the fungus to azoles in the environment [8]. The characteristics of these two routes was clearly established in the last decade [23,24] (figure 1).

While a spectrum of resistance mechanisms to azoles has been characterized in *A. fumigatus* [25,26], azole resistance is frequently the result of mutations in the *cyp51A* gene. Many azole-resistant isolates have non-synonymous point mutations at codons in this gene, for example at positions G54, M220 and G138 [9], which are primarily found in patients who have been treated for long periods with azoles [24]. These point mutations induce changes in channels giving the azole access to a heme molecule to which the azole binds [27,28]. Protein models of lanosterol 14α-demethylase indicate that these mutations change the shape of the channel so that the azole molecule can no longer bind [27,28]. Many of these mutations result in resistance to multiple, if not all, anti-*Aspergillus* triazoles [24,29]. Patients harbouring azole-resistant *A. fumigatus* isolates with point mutations have primarily been observed in lung cavities, such as aspergilloma. Chronic cavitary lung diseases have been hypothesized as a risk factor for resistance development [30] because asexual sporulation, which takes place in a fungal lung cavity, could potentially facilitate the formation of mutations [31,32]. Within such patients, genotypically different colonies of *A. fumigatus* may grow that manifest varied resistance mutations and *in vitro* susceptibility tests suggest that multiple colonies should be investigated when exploring *in vivo* resistance [33,34]. It needs to be emphasized that, in comparision to the in-host development of azole resistance, the *A. fumigatus* strains from the environment have, in addition to the mutations in the *cyp51A* gene, a tandom repeat (TR) duplication in the promoter region. The presence of the TR repeat has been shown to drive overexpression of *cyp51A* by increasing the binding activity of the sterol regulatory element binding protein resulting in increased tolerance of azoles [35]. To date, three resistance mechanisms involving promotor duplications (TR_{34}/L98H, TR_{53} and TR46/Y121F/T289A) and single mutations (G54 and M220) have been found in *A. fumigatus* isolates from soil and air samples and in clinical specimens of patients (table 1) [24,60–62].

Surveillance by University Medical Centers (UMCs) in The Netherlands increasingly show that the environmental route of infection is an important source for azole-resistant

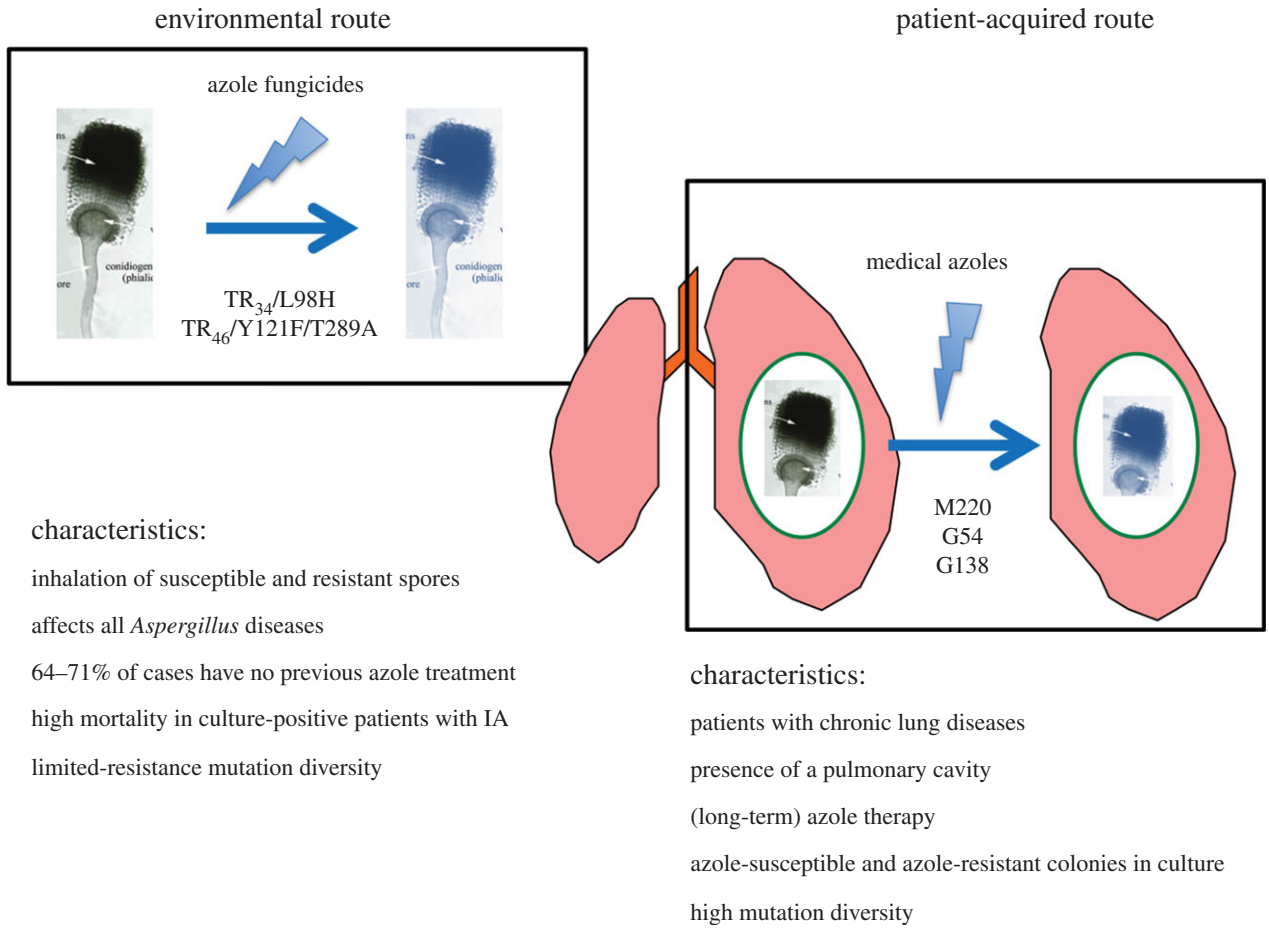

Figure 1. Characteristics of azole-resistant *Aspergillus* infections by the environmental and patient-acquired route.

aspergillosis owing to the fact that between 80 and 90% of clinical drug-resistant strains contain one of the above mechanisms [63]. In addition to the *cyp51A*-mediated resistance mechanisms, about 10% of the resistant strains found in nature have no mutations in the *cyp51A* gene. It is highly likely that induced azole efflux mechanism is also involved in non-*cyp51A*-mediated resistance [26]. There are potentially (many) other, undiscovered, mechanisms that lead to azole resistance such as seen in the human fungal pathogen *C. albicans* where induced aneuploidy and isochromosome formations can occur [64]. One mechanism has been recently described in a patient with aspergillosis on long-term azole treatment, namely a P88 L substitution in *HapE*, but this mechanism currently appears to be rare and has not yet been found in the environment [65]. Although resistance to antifungals is expected to be accompanied by a 'fitness cost', it appears that for the *cyp51A*-mediated resistance, fitness is not affected and the virulence of resistant isolates is similar to that of wild-type strains [66,67].

4. Population structure of azole-resistant *Aspergillus fumigatus*

Aspergillus fumigatus is one of the most frequently occurring eukaryotes on the planet owing to its high prevalence as a saphrophyte on decaying plant matter; its airborne conidia are among the most numerous eukaryotes in air and soil samples [68]. The highly aerosolized and dispersive character of *A. fumigatus* has led to the hypothesis that it is a truly 'globalized' organism. Identification of the sexual cycle further suggested that this species may be a panmictic (globally unstructured and randomly interbreeding) organism [69]. The implication of being a cosmopolitan sexual organism is that there are little or no barriers to gene flow; the corollary being that a unique allele (such as TR_{34}/L98H), that has a selective advantage over wild-type alleles, can theoretically penetrate all genetic backgrounds. If true, then this means that azole-resistance alleles that carry a selective advantage in nature have an essentially unlimited potential to disperse in space. A previous study by Rydholm *et al.* [70], analysing the genetic structure of *A. fumigatus*, suggested that *A. fumigatus* is cosmopolitan and randomly breeding across a global scale. A subsequent study, while confirming these aspects of the population genetic structure of *A. fumigatus*, also found evidence of a genetic barrier to gene flow, suggesting *A. fumigatus* contained two (or perhaps more) cryptic and morphologically identical species [68]. More recent studies have confirmed the occurrence of a separate sexual species, *A. lentulus* [71] and have hinted at the existence of further genetic diversity within *A. fumigatus sensu stricto*.

The advent of next-generation whole-genome sequencing (WGS) allows the sequencing of entire genomes for a cost that is similar to conventional multi-locus genotyping. WGS therefore heralds a new dawn in our ability, not only to genotype clinical and environmental genomes of *A. fumigatus*, but to also analyse patterns of natural selection and gene flow in nature. To this end, we recently sequenced and analysed 24 genomes of *A. fumigatus* from across the world. These isolates were rationally chosen to include an informative selection of clinical and environmental isolates that were wild-type, or known to carry the TR_{34}/L98H allele

Table 1. Common resistance mechanisms reported in the *cyp51A* gene of clinical and environmental *Aspergillus fumigatus*.

promotor tandem repeat (TR) insertion and point mutation	country	medical azoles affected	reference
TR34/L98H	The Netherlands, UK, Germany, Belgium, France, Spain, Italy, Austria, Denmark, Poland, Ireland China, USA, India, Japan, Colombia, Taiwan, Turkey, Kuwait, Iran, Pakistan, Australia, Tanzania, Romania	itraconazole, voriconazole, posaconazole, isavuconazole	[24,36–54]
TR46/Y121F/T289A	The Netherlands, UK, Germany, Belgium, France, Spain, Denmark, Ireland, China, USA, India, Japan, Colombia, Tanzania	itraconazole (variable), voriconazole, posaconazole (variable), isavuconazole	[24,36,37,41, 54–58]
TR53	The Netherlands, Colombia	itraconazole, voriconazole, posaconazole, isavuconazole	[24,58]
G54	The Netherlands, UK, Germany, France, Spain, India, Romania, China, USA, Tanzania	itraconazole, posaconazole	[24]
M220	The Netherlands, UK, Germany, Belgium, France, Spain, China, USA	itraconazole, posaconazole	[24]
Y121F	France	voriconazole	[59]

[72]. This population genomic analysis showed that *A. fumigatus* was broadly panmictic, with as much genetic diversity found within a country as is found between continents. However, a striking exception to this pattern was shown in India, where isolates containing TR_{34}/L98H were found to be highly related despite being isolated from both clinical and environmental sources across more than 1000 km. This high degree of relatedness across India suggested a recent selective sweep of a highly fit genotype that is associated with the TR_{34}/L98H allele [73,74]. We found that the isolates that we sequenced all show the hallmarks of genetic recombination, showing that azole-resistant alleles are segregating into diverse genetic backgrounds. However, despite linkage equilibrium being high across the global sample (except for the Indian subset), TR_{34}/L98H alleles did cluster suggesting that they are not in linkage equilibrium with the global set of allelic diversity.

We then combined the 24 *A. fumigatus* genomes with 17 genomes recently published from clinical isolates in Japan [75]. Phylogenetic analysis (figure 2) revealed that the majority of Japanese isolates clustered together, with the exception of one isolate that clustered with the Indian isolates. These Japanese isolates were separated from the Indian isolates by over 50 000 SNPs. Further analysis revealed that none of the Japanese isolates contained known resistance mutations for azole drugs, and that the phylogeny showed evidence for two clades, only one of which was enriched for the TR_{34}/L98H allele. Investigation into MAT-locus typing, which was carried out computationally, revealed that 58% of Japanese isolates were MAT1-2; indeed, 70% of the Japanese 'cluster' were typed as MAT1-2. This cluster also contained three UK isolates, one isolate from The Netherlands, and only 1/20 isolates carrying the TR_{34}/L98H allele compared with 15/20 for the non-Japanese clade (figure 2). This preliminary population genomics analysis suggests that Pringle *et al.* [68] are correct and that cryptic and morphologically identical populations of *A. fumigatus sensu stricto* do occur, and that this genetic structure leads to substantial linkage disequilibrium for at least two important genetic markers, the mating types and azole-resistance alleles.

Although the sample of genomes that we analysed was small, our analysis shows that a broader analysis of globally occurring *A. fumigatus* genomes that are azole sensitive, azole resistant, and of known clinical or environmental provenance is urgently needed in order to determine worldwide patterns of linkage disequilibrium and the occurrence of cryptic species within *A. fumigatus*. At the time of writing, the evidence suggests that TR_{34}/L98H at least is a relatively recent and novel evolutionary innovation, and that it is perturbing the natural population genetic structure of *A. fumigatus* as selective sweeps, which are imposed by this allele, occur. Dating the origin(s) of this and other azole-resistance alleles as they are discovered, is possible given a large enough sample of global *A. fumigatus* genomes. These data will also allow a description of the fundamental population genetic parameters that are needed to enable the use of genome-wide association studies to identify the contribution of SNP diversity to the generation and spread of azole resistance in this medically significant fungus, as well as describing where, when and how any genetic barriers to gene flow (cryptic species) occur.

5. The environmental route of dissemination

Evidence for an environmental route of azole-resistant *A. fumigatus* acquisition was first found in The Netherlands. Azoles are widely used outside medicine for crop protection and as biocides, with the aim of deterring mould infestation of crops and materials. *Aspergillus fumigatus* is a saprophyte and not a plant pathogen, and is therefore an 'innocent bystander' when exposure of crops to fungicides occurs. Although azole fungicides are not employed to target *A. fumigatus*, it transpires that many of these fungicides have activity against *A. fumigatus*, a condition that led to the development of resistance [76,77]. More than 30 azole

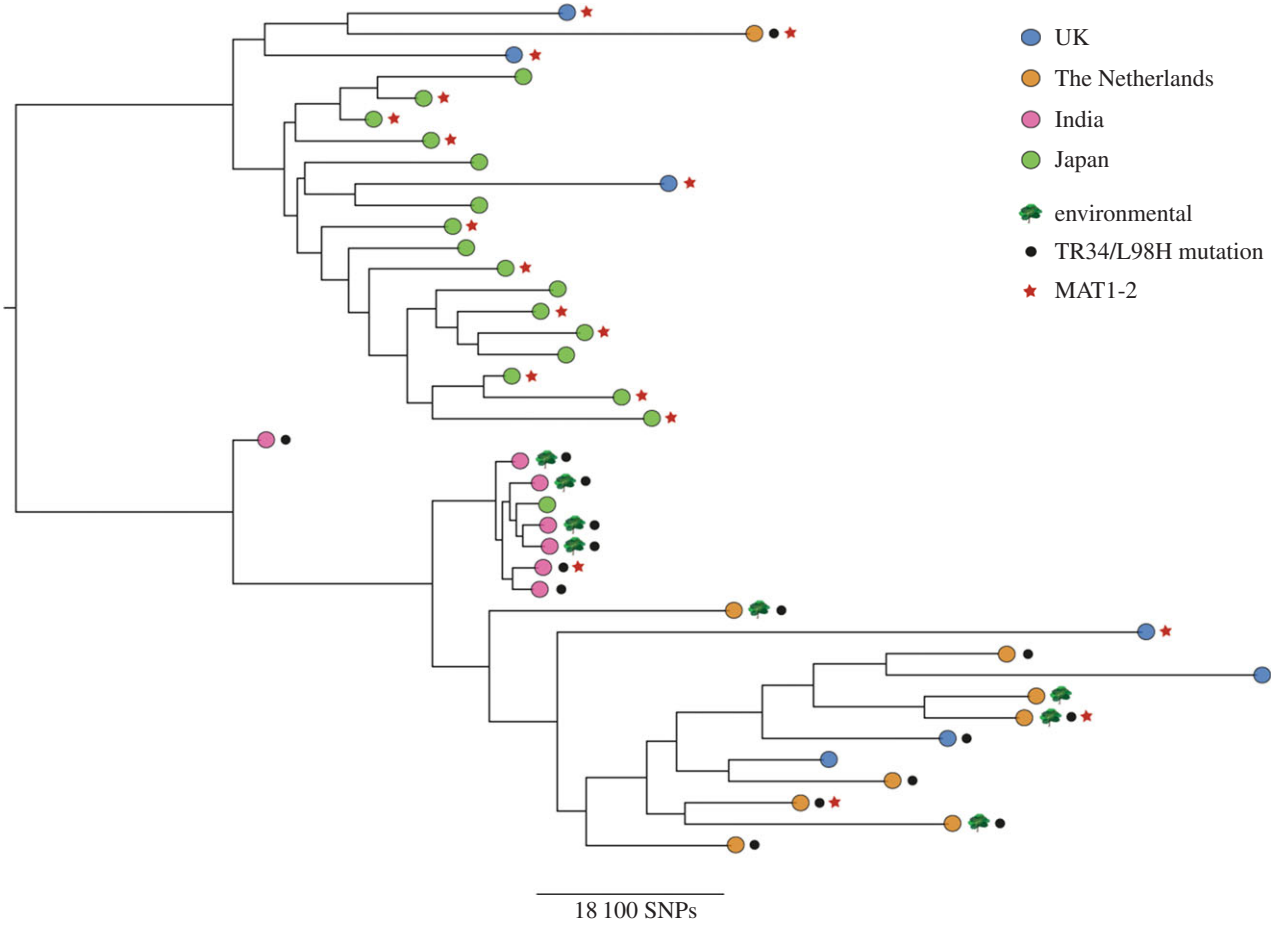

Figure 2. Phylogenetic analysis of azole-susceptible and -resistant *A. fumigatus* isolates from UK, The Netherlands, India and Japan [68,71]. Bootstrap analysis using 100 replicated was performed on WGS SNP data to generate a maximum-likelihood phylogeny, with all branches supported to 68% or higher. Branch lengths represent the number of SNPs between taxa.

fungicides have been tested for their *in vitro* activity against *A. fumigatus* [76,77]. Many of these fungicides were able to inhibit wild-type strains and some azole fungicides were active against wild-type strains but ineffective against isolates with the TR_{34}/L98H mutations. Structural modelling showed that there is a spatial molecular similarity between medical triazoles and the agricultural triazole fungicides such as propiconazole, bromuconazole, epoxiconazole, difenconazole and tebuconazole [76]. These fungicides were introduced in The Netherlands between 1990 and 1996, just before the first clinical TR_{34}/L98H strain was found in 1997. Azoles are also frequently used as biocides in paint and coatings, wall-paper paste, clothing, and wood preservation. Currently, the hypothesis is that *A. fumigatus* becomes resistant through the inevitable exposure to these agents in the environment [77,78]. Vulnerable patients can become exposed to resistant spores in the air and, if these hosts develop *Aspergillus* disease, the medical azoles are no longer effective due to the structural similarity of the molecules. It is still unclear how and where resistance develops in the environment and which applications of fungicides are inducing the development of resistance most successfully. It has not been routine practice in microbiological laboratories to carry out *in vitro* susceptibility testing of filamentous fungi. However, this has changed in the last few years and presently many centres now test *A. fumigatus* for resistance to azoles. As a result, it has become evident that azole resistance has a potentially global distribution, and is a worldwide problem [24,36]. The mutations associated with the environmental route are found in many geographically diverse countries and continents (figure 3). In Europe, many countries including Belgium, France, Spain, Denmark, Italy, The Netherlands, Norway, Germany, Ireland, United Kingdom, Poland, Romania and Austria have reported azole resistance [37–45,55,56]. Resistance is also reported outside Europe in Turkey, Iran, Kuwait, Japan, China, Taiwan, Pakistan, India, Tanzania and Australia [46–53,57], so far mainly associated with TR_{34}/L98H but increasingly also TR_{46}/Y121F/T289A. In the United States and Colombia, azole resistance owing to environmental mutations was reported recently [54,58]. Extensive asexual sporulation, the ability to survive in very different environmental conditions, and the hydrophobicity of spores, faciliating airborne dispersibility, probably enable the global spread of resistance from its centres of origin [31,79]. At present, the development of azole resistance is mainly associated with *A. fumigatus* and less so with human pathogens *A. flavus* [80] and *A. terreus* [81]; however, further surveillance is warranted.

6. Clinical implications of resistance

Although no randomized studies have been conducted, it is increasingly clear that patients with azole-resistant *Aspergillus* infections have a high risk for therapy failure. In a Dutch surveillance study, eight patients were identified with azole-resistant IA, seven of whom died within two months after diagnosis [60]. Five patients had a

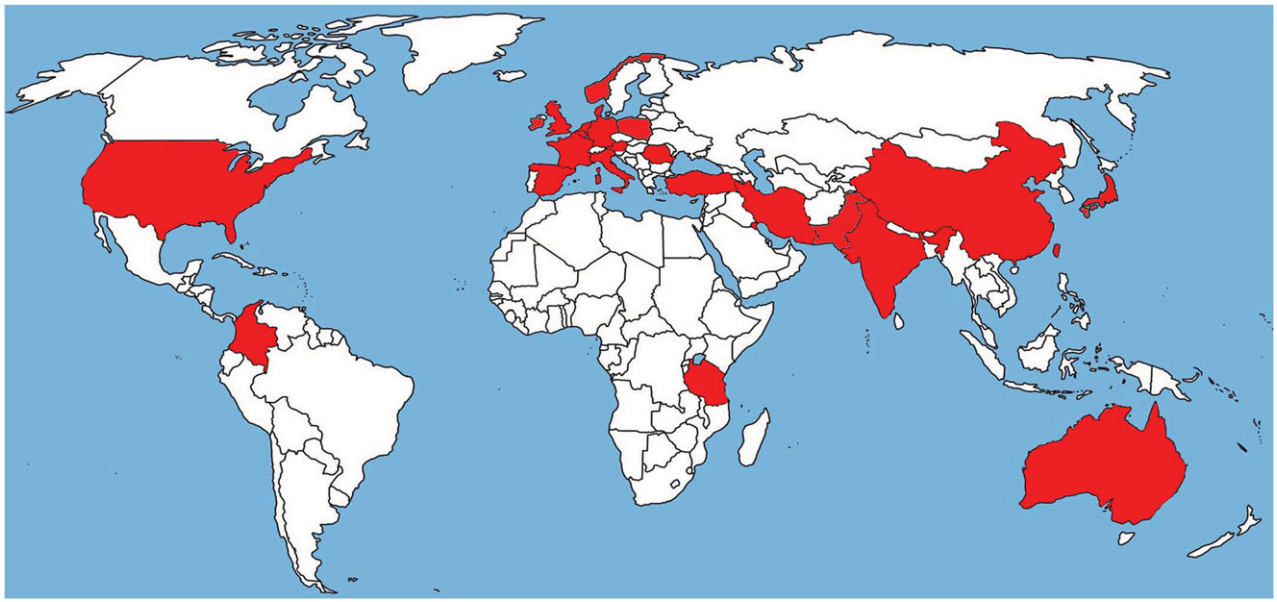

Figure 3. Worldwide map of azole resistance in *Aspergillus fumigatus*. The red highlighted countries have reported azole resistance.

culture-positive pulmonary aspergillosis and failed treatment with voriconazole. Other case series confirmed this observation, such as a recent study from Germany where seven of eight patients with azole-resistant IA failed on therapy and died, [44] and it was also shown that patients with CPA due to azole resistance failed azole therapy [45]. However, there are many other factors that can cause failure of therapy for aspergillosis such as patients who fail to come into remission of the underlying leukemia, have insufficient voriconazole exposure, or have an already far advanced infection, among others [82]. As a result, at present, the contribution of a high MIC in therapy failure is unclear, warranting case-control investigations to ascertain the exact nature of the relationship. Animal models, however, support the observation that an increased MIC leads to loss of effectiveness of azoles [83].

7. Diagnosis of azole resistance

Azole resistance in Europe and particularly in The Netherlands is widespread. The National Institute for Public Health and the Environment in The Netherlands is supporting surveillance of resistance in UMCs and the data are published annually in Nethmap [63]. On average, in 7% of the patients who are culture-positive, azole resistance has been detected. This percentage differs between the UMCs ranging from 4.3% to 13.3% in 2014, and from 6.7% to 16.3% in 2015 [63]. The reason for this variation between the centres is unclear at present. In a first surveillance study, in large teaching hospitals, similar high resistance rates were observed [84].

It is recommended also to perform *in vitro* susceptibility testing on multiple colonies when patients have a positive *A. fumigatus* culture and the clinical intention is to treat. The problem with this approach is that MIC determinations of *Aspergillus* are not performed routinely in many microbiological laboratories, at least not in The Netherlands [85]. Therefore, a screening method based on an agar-based test has the advantage of selecting resistant strains with unknown mechanisms of resistance. Multiple colonies are sub-cultured on a four-well plate with a growth control and itraconazole, voriconazole and posaconazole added to the agar (VIP Check™, Beneden-Leeuwen, The Netherlands). This approach detects with high sensitivity and specificity potential resistance in the isolates in a simplified way, i.e. isolates growing only on the growth-control well excludes resistance. However, growth on drug-containing wells very probably represent a resistant isolate and a MIC-determination should be done. This approach is, in addition to surveillance studies, also useful for clinical management of patients with aspergillosis. The agar-based test can help to select the strains for *in vitro* susceptibility testing. Furthermore, growth of *A. fumigatus* on the agar with added azoles makes the likelihood of azole resistance so high that modification of therapy should be considered, even without confirmation with MIC testing. Alternatively, isolates can also be screened with the Etest method. This screening can best be done with itraconazole, although some resistance mechanisms can be missed. Both the EUCAST and CLSI have a published reference method for *in vitro* susceptibility testing of conidia-producing fungi [86,87]. The CLSI used an epidemiological cutoff value for detection of non-wild-types while the EUCAST, based on a number of parameters such as standard dose, pharmacokinetics/dynamics and clinical outcome of treatment, defined clinical breakpoints.

8. Detection of azole resistance in patients with negative cultures

Conventional cultures of bronchoalveolar lavage (BAL) fluid and other respiratory samples are negative in the majority (up to 90%) of aspergillosis patients [88]. Thus, determination of resistance in culture-negative patients is difficult. Although tests such as galactomannan antigen and beta-D-glucan are indicative of aspergillosis, they give no information about azole sensitivity of the infecting species. The detection of resistance mechanisms by means of PCR directly from tissue was first described in 2010 in a patient with culture-negative cerebral aspergillosis [89]. Other researchers have confirmed that in culture-negative patients, direct detection

of mutations in clinical samples is possible [90]. Recently, a multiplex real-time PCR for detection of some *Aspergillus* species became available (AsperGenius®, PathoNostics, Maastricht, The Netherlands) which also detects two mechanisms of resistance associated with the environment, TR_{34}/L98H and TR_{46}/Y121F/T289A. This test showed a good sensitivity and specificity for the detection of *Aspergillus* in BAL fluid [91]. In patients with haematologic malignancies, the sensitivity and specificity was 88.9% and 89.3%, respectively, and in ICU patients 80% and 93.3%. Of 77 BAL fluid samples, 22 patients with IA (2 proven, 9 probable and 11 non-classifiable) were included. In another study, AsperGenius-PCR in serum of patients with IA [92] reported 100% sensitivity and 78.6% specificity, but no cases of azole-resistant IA were diagnosed. Because the *cyp51A* gene exists in a single copy per cell, the detection of mutations is difficult in serum due to the limited sensitivity of the test. In addition, the AsperGenius-PCR only allows two mechanisms of resistance to be identified. Therefore, a negative test result does not rule out the presence of an azole-resistant infection. Yet the results of molecular tests are encouraging and may be of added value in clinical settings, particularly in the rapid analysis of BAL fluid samples. Excepting a recent consensus document from an expert panel [23], there are currently no guidelines available in which the treatment of azole-resistant aspergillosis is described. Guidelines on the treatment of *Aspergillus* diseases with ESCMID and ECMM are in preparation, including treatment of azole-resistant disease [93].

9. Future trends for treating azole-resistant *Aspergillus* disease

The use of azoles in different application areas, namely crop protection, material conservation and treatment of fungal diseases in humans and animals, has resulted in a complicated policy situation. Azole-resistance mechanisms found in the environment are also involved in incurable human infections. Arguably, without a change of policy the successful use of azoles as a first line of defence against diseases caused by *Aspergillus* is threatened, constituting a broad impact on public health. Clearly, it is very important to investigate the development of azole resistance by *A. fumigatus* in the environment and to focus on the questions 'where', 'when', 'how' and 'how often' it occurs. This could, for instance, identify high-risk situations (hot spots) in which wild-type isolates are able to develop resistance in each of the application areas of azoles. This may lead to insights into how to decrease the rate at which resistance develops. Given the huge worldwide applications of azole fungicides in farming practices, measures should also be considered on a worldwide scale to achieve any effect and to curb the selection for resistance. However, this policy intervention is complicated, as food security against fungal epidemics is predicated on the widespread deployment of azoles on crop monocultures, and trade-offs occur depending on policy recommendations.

Regarding the diagnosis and treatment of patients with aspergillosis, if possible, the presence of azole resistance should be vigorously investigated as there are no obvious patient risk factors known which predict infection with *Aspergillus* that may harbour an environmentally acquired mutation. Therefore, intensive monitoring of patients treated with azole monotherapy is necessary and when there is suspicion of clinical failure, new diagnostic interventions should (rapidly) be considered. When azole resistance is demonstrated therapy should be adjusted to include liposomal amphotericin B, or alternatively an echinocandin should be added to voriconazole. If no proof of azole resistance has been obtained and there is a high prevalence of resistance in the hospital or the specific department, the proper approach to (empirical) therapy is currently unclear and is a public health concern that should be debated. During a recently held consensus meeting, most experts felt that first-choice (empirical) therapy should not consist of voriconazole monotherapy when a prevalence of 10% resistance due to environmental azole resistance was present [23] and such reflections may constitute the public health response that will be needed to combat this infection into the future.

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