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Epidemiology and Prevalence of Azole-Resistant *Aspergillus fumigatus*: What Is Our Understanding of the Situation?

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Abstract

Purpose of the Review Azole-resistant *Aspergillus fumigatus* is an emerging clinical problem. Resistance can develop both with clinical exposure and with environmental exposure to azole and azole-like fungicides used against phytopathogens. Here, our current understanding of the epidemiology and prevalence of this emerging problem is reviewed.

Recent Findings Azole-resistant *A. fumigatus* is now a worldwide problem, as it has been documented in clinical and environmental samples on all but one continent. Although Europe has been the center of this issue, hotspots are now emerging in Asia. However, there are several limitations to our current understanding of the extent of this problem, including issues with surveillance strategies and our reliance on phenotypic methods for detecting resistance.

Summary Azole-resistant *A. fumigatus* is an emerging issue both clinically and in the environment that requires greater attention in order to preserve this very useful class of antifungal agents for the treatment of aspergillosis.

Keywords *Aspergillus fumigatus* · Azoles · Antifungal resistance · *CYP51A* · TR_{34}/L98H · TR_{46}/Y121F/T289A

Introduction

Aspergillus species are fungi that are ubiquitous in nature and feed on dead or decaying organic material. In certain patient populations, these fungi are able to cause a myriad of conditions, including allergic bronchopulmonary aspergillosis, sinusitis, chronic pulmonary aspergillosis, aspergillomas, and acute invasive aspergillosis, with the respiratory route being the most common means of entry. Although over two hundred individual species within this genus have been described, the most common species cultured from humans include *A. fumigatus*, *A. flavus*, *A. terreus*, and *A. niger*, with *A. fumigatus* being the most prevalent [1–3]. In individuals who require treatment of infections caused by *Aspergillus* species, the azole antifungals itraconazole, voriconazole, posaconazole, and isavuconazole are commonly used due to their in vitro activity against *Aspergillus* species, efficacy in clinical studies, and ability to be administered orally, which is important given the lengthy durations of therapy that are often required in patients with aspergillosis. However, as with most microbes, antifungal resistance in these fungi can develop with exposure to these agents. Prior to the late 1990s, azole-resistant *A. fumigatus* was not seen as a clinical problem. However, over the last 15 years, this situation has changed, and numerous studies have documented azole resistance in countries throughout the world, both in clinical and environmental specimens. Clinically, this is important as azole-resistant invasive aspergillosis has been associated with increased mortality in highly immunocompromised individuals, especially in ICU settings [4–6]. Here, the epidemiology of azole-resistant *A. fumigatus* is reviewed focusing on new information regarding the prevalence of this emerging issue in clinical and environmental isolates from countries in different geographic areas of the world.

Early History of Azole Resistance in *Aspergillus fumigatus*

The first clinical cases of azole-resistant *A. fumigatus* began to appear in the literature in the late 1990s, with reports from the USA (California) and Sweden [7, 8]. However, the cases from California were retrospective evaluations of isolates that had been cultured from patients with chronic pulmonary

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aspergillosis who had received long-term azole therapy in the 1980s [8]. Thus, resistance had developed earlier than when first reported. Following these early reports, additional descriptions of clinical failures associated with microbiological resistance continued to emerge. For example, in the UK, the rate of azole-resistant *A. fumigatus* cultured primarily in patients who received long-term azole therapy for chronic aspergillosis and submitted to the Mycology Reference Centre Manchester increased from 5 to 7% for the period of 2005 to 2007 up to 20% in 2009 [9, 10]. In the Netherlands, over a 14-year period, from 1994 to 2007, itraconazole-resistant *A. fumigatus* was found in 32 of 1219 patients, and the annual prevalence ranged from 1.7% to 6% [11]. Interestingly, in these early studies from the UK and the Netherlands, resistance was not detected until after 1999 [10, 11]. Since then, numerous studies from institutions in countries around the world have reported azole resistance in clinical isolates of *A. fumigatus* [12].

Many of these early clinical reports were in patients who had received long-term azole therapy due to chronic aspergillosis. Azole resistance with long-term patient exposure is known to occur in individuals with allergic bronchopulmonary and chronic pulmonary aspergillosis, those receiving long-term antifungal prophylaxis, individuals with pre-existing lung cavities (e.g., due to tuberculosis or sarcoidosis), and cystic fibrosis patients [12–14]. Numerous spontaneous mutations can occur during asexual sporulation; thus, these individuals may harbor several strains of *A. fumigatus* that are genetically distinct, including those that are phenotypically susceptible and resistant to different antifungals [9, 10, 13, 15]. These nonsynonymous point mutations that can develop with long-term azole exposure occur within the *CYP51A* gene, which encodes the Cyp51a enzyme (also known as lanosterol 14α-demethylase, the target of the azoles) [16–18, 19•]. The development of resistance in patients with long-term exposure to azoles is consistent with what is known about azole resistance development in different fungi, including *Candida* species. However, in 2007, reports began to emerge from the Netherlands of multiple-azole–resistant *A. fumigatus* invasive aspergillosis in azole-naïve patients [20]. This resistance was caused by a novel mechanism that consisted of a nonsynonymous mutation within the *CYP51A* gene along with a tandem repeat in the promoter region of this gene (i.e., TR_{34}/L98H). Because this occurred in patients without previous exposure to azoles, it was postulated that resistance may have developed with environmental exposure to azoles used in agriculture [20, 21].

Mechanisms of Azole Resistance in *Aspergillus fumigatus*

CYP51A mediated mechanisms of resistance

CYP51A-mediated mechanisms of resistance have been broadly categorized as patient-exposure or environmental exposure. As briefly described above, the patient exposure mechanisms involve nonsynonymous point mutations within the *CYP51A* gene that can occur during asexual sporulation [13, 19•]. These mutations can lead to amino acid changes within the Cyp51A enzyme, which in turn leads to modifications to the channels by which the azoles are able to access the active site and inhibit the enzyme [17, 22]. Amino acid positions commonly associated with patient exposure include G54 (posaconazole and itraconazole resistance), G138 (voriconazole, isavuconazole, and itraconazole resistance), M220 (itraconazole resistance with elevations in MIC values for the other mold-active azoles), and G448 (voriconazole, isavuconazole, and itraconazole) [19•, 22]. Several other less common mutations within Cyp51a have also been reported to cause azole resistance. Common features of patients from which isolates with these types of mutations are cultured include those with long-term azole exposure and the presence of pulmonary cavities [15].

Mutations associated with azole resistance following environmental exposure to azole and azole-like compounds (i.e., demethylase inhibitors) include nonsynonymous mutations within *CYP51A* that cause amino acid substitutions within the enzyme coupled with tandem base pair repeats within the promoter region of the gene. Azoles and azole-like fungicides are used in agriculture and horticulture against phytopathogens, and in various products to prevent rot in wood and other materials [23]. Although *A. fumigatus* is not a phytopathogen, it is ubiquitous in the environment. In addition, sexual or parasexual reproduction that can occur in the environment where extensive genetic reshuffling can occur through recombination may also play a role in resistance development [13, 15]. The most common mechanisms associated with environmental exposure that have been described include TR_{34}/L98H (pan-azole resistance) and TR_{46}/Y121F/T289A (voriconazole and isavuconazole resistance and attenuation of posaconazole and itraconazole susceptibility) [19•]. These tandem repeats are bound by transcription factors that increase the transcription of the gene so that more enzymes are produced [19•, 22]. For example, increased binding of the transcriptional regulator SrbA to the 34 base pair repeat leads to increased expression of the gene and also blocks binding of the transcriptional repressor CCAAT-DNA binding complex [19•, 24, 25]. Other reported mutations associated with environmental exposure include those with variations in the number repeat duplications (i.e., triplication of TR_{34} and TR_{46}) [15]. Interestingly, an isolate with an increased length of the repeat in the promoter region (i.e., TR_{120}) along with other mutations within Cyp51A was cultured from a patient with long-term azole exposure due to chronic pulmonary aspergillosis, raising the possibility of in vivo

acquisition of this mechanism rather than through environmental exposure [26]. It should also be noted that mutations leading to a G54E codon change within Cyp51A have also been documented in environmental isolates from different countries [27••, 28]. Thus, as noted by Buil et al., the boundaries between patient and environmental routes of triazole resistance selection are fading [15]. Interestingly, one study from the Netherlands that included 15 adult cystic fibrosis patients known to be colonized with *A. fumigatus* and collected sputum samples and cough plates following spirometry reported that *A. fumigatus* was able to be cultured from both the sputum and cough samples in some patients [29•]. This raises the possibility of aerosolized transmission of *A. fumigatus* from patient-to-patient and from patient-to-environment. Similarly, a report from France that used electrostatic dustfall collectors to monitor indoor airborne fungi in an ICU setting identified a patient's airways as the source of *A. fumigatus* within two separate rooms [30].

Non-*CYP51A* mediated mechanisms of resistance

However, not all phenotypically resistant isolates have been found to harbor *CYP51A* mutations, and other mechanisms of azole resistance are now known to occur in *A. fumigatus*, although their prevalence and contributions to clinical failures are not well understood. These include upregulation of *CYP51B*, the gene that encodes a second enzyme, Cyp51B, involved in ergosterol biosynthesis; overexpression of efflux pump genes, including members of the major facilitator (MFS) and ATP-binding cassette (ABC) superfamilies; gain-of-function mutations in transcription factors, such as the P88L substitution in the CCAAT-binding transcription factor HapE, which results in de-repression of *CYP51A* transcription; and mutations in the *HMG1* gene that affect a rate-limiting enzyme in the ergosterol biosynthetic pathway termed HMG-CoA reductase [19•, 31].

In most studies, TR_{34}/L98H has been the predominant mechanism of resistant detected in both clinical and environmental cultures of *A. fumigatus* [14, 32, 33••]. However, the true prevalence of this mechanism and other mechanisms of resistance of azole resistance in patients with aspergillosis is most likely underestimated. Cultures for filamentous fungi suffer from poor sensitivity [34••, 35]; thus, most patients with invasive aspergillosis are culture negative [15]. Therefore, the detection of antifungal resistance by phenotypic means and the subsequent determination of the mechanism of resistance are not possible. Although there are commercially available assays in some countries for the detection of common *Aspergillus* species and also the detection of TR_{34}/L98H and/or TR_{46}/Y121F/T289A mutations in direct specimens (AsperGenius, PathoNostics, the Netherlands, MycoGENIE, Ademtech, France, Fungiplex, *Aspergillus* Azole-R, Bruker Daltonics GmbH, Germany), they have not received regulatory clearance for clinical use in other countries, including the USA. Furthermore, as previously noted, between 15 and 50% of phenotypically resistant *A. fumigatus*, isolates have been reported to be *CYP51A* wild-type [9, 10, 15, 36, 37]. Thus, even if these commercial assays were more widely available, infections caused by strains harboring *CYP51A*-independent mechanisms of resistance would still be missed.

Prevalence of Azole Resistant *Aspergillus fumigatus*

Azole-resistant *A. fumigatus* in clinical and environmental settings is now a worldwide issue (Fig. 1). Unfortunately, the true clinical prevalence of azole-resistant *A. fumigatus* is unknown. In an early prospective, multicenter surveillance study conducted over 2 years between 2009 and 2011 that included 3788 isolates collected from 1450 patients at 22 centers in 19 countries, including Australia, North America, South America, and several European countries, the overall prevalence of azole-resistant *A. fumigatus* was 3.2% [32]. However, this rate differed markedly between centers, ranging from 0 to 26.1%, and resistance was widespread in Europe. The predominant mechanism of resistance identified was TR_{34}/L98H (48.8%). Of the 1450 patients from whom *A. fumigatus* was cultured; 353 were known to have aspergillosis, including 195 with invasive disease; and 158 with known noninvasive disease, including chronic pulmonary aspergillosis/bronchitis, allergic bronchopulmonary aspergillosis, and aspergillomas. Similarly, an international surveillance study (ARTEMIS Global Surveillance Study) that included 497 *A. fumigatus* isolates from 62 medical centers between 2008 and 2009 found 29 isolates (5.8%) with azole MICs above the established epidemiological cut-off values (i.e., non-wild-type azole MICs) [37]. Twenty-four of these isolates with elevated azole MICs came from different medical centers in one province in China, two from the Czech Republic, and one each from the USA, Brazil, and Portugal. Eight of these isolates harbored the TR_{34}/L98H mutation, with each of these coming from China, representing the first time this mechanism of resistance had been reported in this country and outside of Europe. Recently, another large international surveillance study (SENTRY Antimicrobial Surveillance Program) that included 1263 *A. fumigatus* clinical isolates between 2010 and 2017, with the majority coming from North America and Europe, reported overall low rates of non-wild-type *A. fumigatus*, ranging from 0.6 to 1.3% for itraconazole, 0.9 to 6.3% for posaconazole, and 0.4 to 1.1% for voriconazole [38]. The frequency of *A. fumigatus* isolates with non-wildtype azole MICs steadily increased in Europe over this timeframe but remained relatively constant in the Asia–Pacific, North American, and Latin American regions. Unfortunately, most studies have been from single

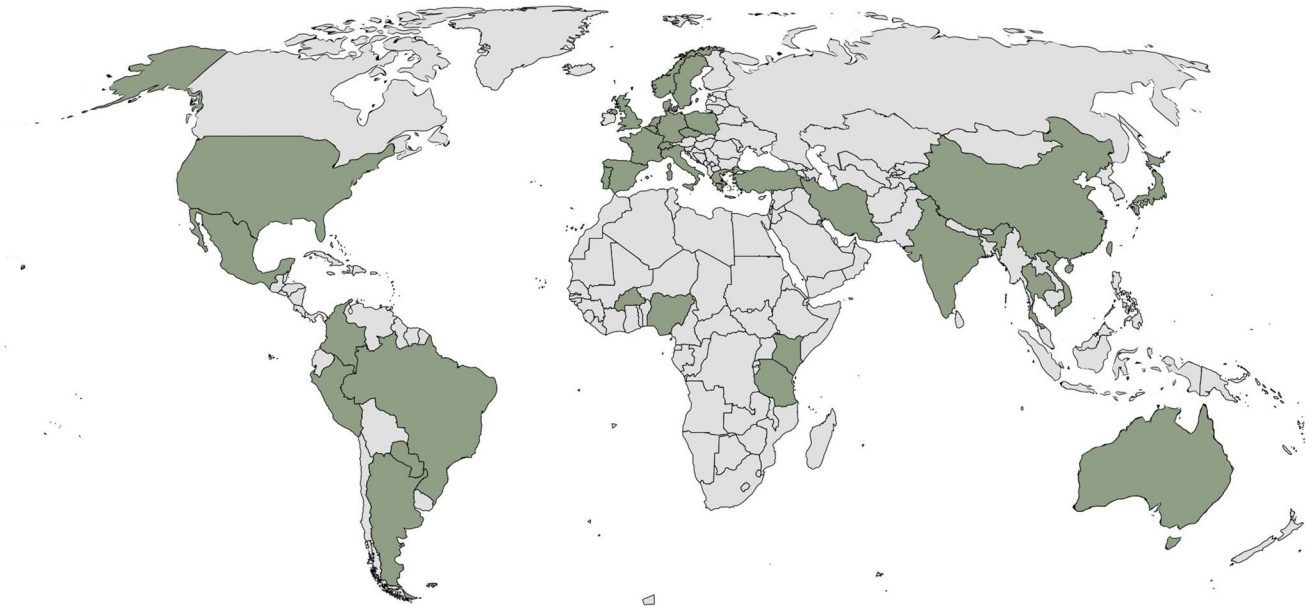

Fig. 1 Map showing many of the countries (in green) where multiple azole-resistant *A. fumigatus* isolates have been isolated in clinical and/or environmental settings in surveillance or point prevalence studies, including those with TR_{34}/L98H and TR_{46}/Y121F/T289A mutations

centers that often describe a single patient population or have included a limited number of institutions within a single country. Despite the lack of recent multicenter prospective studies, data has emerged regarding the reach of azole-resistant *A. fumigatus* in different parts of the world, both in clinical and environmental samples.

Europe

The issue of azole-resistant *A. fumigatus* in both clinical and environmental strains has been well documented in Europe. As noted previously, institutions in the UK and the Netherlands quickly became hot spots for azole-resistant clinical isolates, and the first reports of such resistance in azole-naïve patients and the isolates from the environment that harbored the TR_{34}/L98H mutation were described in the Netherlands [20]. This was soon followed by descriptions of clinical and environmental isolates that contained the TR_{46}/Y121F/T289A mutation [39, 40]. Overall, the prevalence differs from country to country within Europe, with rates of 0.6 up to 28% depending upon the study and the country [3, 9, 10, 32, 39, 41–48]. There can also be marked fluctuations over time. Over a 23-year period from 1994 to 2016 at one medical center in the Netherlands, the overall frequency of resistance was 4.2% [49]. However, this varied markedly between different periods, with a frequency of azole-resistant *A. fumigatus* as low as 0.79% between 1996 and 2001 and up to a high of 7.17% between 2007 and 2011. The clinical prevalence has also been reported to differ between institutions within the same countries and different patient populations. For example, several studies from Europe have reported elevated rates of azole-resistant *A. fumigatus* cultured from cystic fibrosis patients, which have ranged from 3.5 to 8.2%, depending upon the country [50–52]. However, rates as high as 9.1% were reported in one center in Germany 9.1%, and up to 20% in cystic fibrosis patients who had previous azole exposure at one center in France [51, 53].

Work from the UK has also demonstrated that the prevalence of azole-resistant isolates in the environment can differ markedly between different types of locations. In a study that sampled soils across southern England, the presence of azole-resistant *A. fumigatus* was detected in 6.7% of the total samples, but was markedly higher in those collected from urban centers (13.8%), including flower beds around hospitals and gardens, compared to just 1.1% in rural locations [54]. Similar findings have also been reported in Mexico, Vietnam, and India [55, 56••, 57]. One study from the Netherlands recently identified three environmental hotspots, including flower bulb waste, green waste material (organic waste material originating from landscaping), and wood chippings, all of which contained high counts of total and resistant *A. fumigatus* spores and trace amounts of azole fungicides [58].

North America

In North America, including Canada, Mexico, and the USA, the rate of azole-resistant *A. fumigatus* appears to be lower than that reported in parts of Europe, such as the UK and

the Netherlands. An early study that included 274 *Aspergillus* isolates collected from transplant recipients in the USA with proven or probably invasive aspergillosis reported azole resistance in 10 isolates (3.6%) [1]. However, resistance was found in only 1 of 181 *A. fumigatus* isolates, with the remaining being non-*fumigatus Aspergillus* species, including 5 *A. calidoustus* isolates, a species known to have reduced susceptibility to the azoles [59]. The US Centers for Disease Control and Prevention (CDC) conducted a passive surveillance study between September 2011 and September 2013, which included 1026 clinical *A. fumigatus* isolates [60]. Fifty-one isolates (4.9%) had an elevated itraconazole MIC value, of which 18 had a mutation within *CYP51A*. Interestingly, no TR_{34}/L98H or TR_{46}/Y121F/T289A mutations were found. However, a retrospective study of clinical *A. fumigatus* isolates received by a reference laboratory from institutions across the USA did identify two strains with TR_{34}/L98H and two with TR_{46}/Y121F/T289A mutations, with the earliest of these cultured from a patient in 2008 [61]. A subsequent passive surveillance study conducted by the CDC from 2015 to 2017 reported that 1.4% of *A. fumigatus* isolates had elevated azole MICs, with 5 isolates being identified as having the TR_{34}/L98H mutation [62]. Recently, results from a large surveillance study of over 2000 *A. fumigatus* clinical isolates collected from US institutions over a 52-month period (October 2015 to January 2020) was published [36]. Overall, the percentage of isolates classified as either resistant or non-wildtype to the azoles fluctuated between 3.33 and 6.58%, but remained relatively steady over this timeframe. Seven additional isolates harboring the TR_{34}/L98H and 7 with the TR_{46}/Y121F/T289A mutation were identified. Isolates containing these mutations have also now been identified in environmental samples in the USA. In an environmental sampling study conducted in 2015 in four separate peanut fields in Georgia that had been treated with azole fungicides, 38 of 200 *A. fumigatus* isolates (19%) were found to be itraconazole resistant, with 20 of these confirmed to have the TR_{34}/L98H mutation [63].

Limited data on the prevalence of azole-resistant *Aspergillus* from Canada and Mexico are also available. One study conducted between March 2018 and December 2018 that evaluated the susceptibility profiles of 113 *Aspergillus* isolates collected from 5 hospitals across the province of Quebec, Canada, of which 86 were *A. fumigatus*, found no azole resistance [64]. Another study screened 999 *Aspergillus* isolates for azole resistance that had been collected over a 14-year period (2000 to 2013) at a hospital in Montreal [65]. Of these, 985 were *A. fumigatus sensu stricto* and 14 cryptic species within *Aspergillus* section *Fumigati*. Ten isolates were identified as azole-resistant, but this was driven by isolates of cryptic species, 7 of which were azole-resistant. Only a single *A. fumigatus* isolate was considered to be non-susceptible to the azoles but with only mildly elevated azole MICs and no *CYP51A* mutations. The very low prevalence of azole resistance described in these two studies from the province of Quebec are in agreement with a study conducted in the neighboring province of Ontario, which included 194 *A. fumigatus* isolates, including 124 environmental isolates collected from agricultural and urban settings around the city of Hamilton and 71 clinical isolates, none of which were resistant to itraconazole or voriconazole [66]. Although these results suggest that the prevalence of azole resistance in Canada is rare, the data are limited.

Unfortunately, data available from Mexico are also limited. One study conducted at a tertiary care center in Mexico City included 43 isolates collected from 39 patients between 2014 and 2017, of which 24 were identified as *A. fumigatus* [67]. Only two *A. fumigatus* isolates were found to be azole resistant, with both containing the TR_{34}/L98H mutation that had been cultured from azole-naive patients who had presented with invasive aspergillosis. A recent environmental study included 198 soil samples collected from agricultural and urban areas in Mexico City and Guanajuato [55]. Azole resistance was found in 7 of 102 *A. fumigatus* isolates, all of which were collected from urban settings. Five of these contained TR_{34}/L98H mutations and one the TR_{46}/Y121F/T289A mutation.

South America

Data on azole resistance in South America is limited but is beginning to emerge. In a single-center study in Buenos Aires, Argentina, conducted between 2012 and 2016, of the 142 *Aspergillus* section *Fumigati* isolates, of which the majority were *A. fumigatus*, 13 isolates were found to be non-wildtype to itraconazole and 4 were non-wildtype to voriconazole [68]. In Peru, a prospective study performed in 2019 that included 143 *A. fumigatus* isolates from two tertiary care centers reported an azole resistance prevalence of 2.09% [69]. In both Argentina and Peru, the TR_{34}/L98H and TR_{46}/Y121F/T289A mutations have been identified in clinical isolates, including those from patients with prior azole exposure and those that were azole-naïve [33••]. Similarly, in a retrospective study that included 221 *A. fumigatus* isolates from patients with aspergillosis from six medical centers in Brazil between 1998 and 2017, only 4 isolates (1.8%) were identified as having elevated voriconazole MICs, none of which contained a *CYP51A* mutation [70]. Azole-resistant isolates have also been found within environmental samples from South America. In Colombia, numerous *A. fumigatus* isolates harboring alterations in the promoter regions of the *CYP51A* gene, most of which were TR_{46}/Y121F/T289A, have been found in the soil of flower and vegetable fields in and around Bogota [71, 72]. Similarly, azole-resistant *A. fumigatus* isolates have now been reported in environmental samples collected from both urban and rural areas within

Peru, all of which had the TR_{34}/L98H mutation, as well as in Paraguay [55].

Asia–Pacific

Reports have also begun to emerge of azole resistance in clinical isolates cultured from patients in the Asia–Pacific region. As previously noted, the ARTEMIS study documented the presence of azole-resistant isolates, including those with the TR_{34}/L98H mutation, in different medical centers in Hangzhou, China, as early as 2009 [37]. Similarly, azole resistance, including that caused by this same mutation, was found in 3.17% of clinical isolates of *A. fumigatus* from a single center in Nanjing, China, between 2012 and 2015 [73]. Similar rates of azole resistance in clinical isolates have now been reported in Japan and Taiwan, including isolates harboring TR_{34}/L98H and TR_{46}/Y121F/T289A mutations [74–80]. In Australia, the prevalence of azole-resistant *A. fumigatus* in clinical isolates collected at two hospitals from 2015 to 2017 was low (2%) [81].

What is more concerning, however, is that parts of Asia may become hotspots for azole resistance in the environment [82], as high rates have been reported in recent studies conducted in Vietnam and China. In an environmental sampling study that collected 450 samples from 150 locations across the Ca Mau province in the Mekong delta region of Vietnam, 324 *Aspergillus* species within section *Fumigati* were recovered, 62 of which were confirmed to be *A. fumigatus sensu strico* [56••]. Resistance to at least one azole was found in 95.2% of the *A. fumigatus* isolates. Fifty-six underwent *CYP51A* sequencing, the majority of which contained a TR_{34}/L98H mutation (60.7%). Interestingly, 18 of the azole-resistant *A. fumigatus* isolates (32.1%) were wildtype for *CYP51A*. The odds of itraconazole resistant *A. fumigatus* were highest in isolates collected from urban residential areas (odds ratio 9.31; 95% CI 2.52–41.71), followed by fruit farms (7.16; 1.53–43.36), rice farms (5.70; 1.80–19.71), and shrimp farms (3.15; 1.01–10.40). Previously, this same group reported that over 85% of *A. flavus* isolates tested from this same environmental sampling study were resistant to at least one azole [83]. High rates of azole-resistant *A. fumigatus* have also been reported in environmental isolates collected in China. Within 900 soil samples collected from greenhouses in Kunming in the Yunnan province of southwest China, itraconazole resistance was observed in 78.54% and voriconazole resistance in 33.91% of the 233 *A. fumigatus* isolates that were cultured [84••]. TR_{34}/L98H mutations were found in 28 isolates compared to three with TR_{46}/Y121F/T289A mutations and two that solely had a 53-base pair repeat in the promoter region (TR_{53}). Interestingly, in a cross-sectional study of 63 soil cores collected from agricultural fields in China, azole resistance was detected in 21 of 206 *A. fumigatus* isolates (10.2%) [85]. However, 18 of these were in samples collected from strawberry fields. Azole resistance has also been documented in environmental isolates collected in Taiwan and in agricultural products imported into Japan from the Netherlands [78, 86–88].

India and the Middle East

Initial reports from India suggested a low rate of azole-resistant *A. fumigatus* in clinical isolates. The first study to identify a TR_{34}/L98H mutation in this country reported it in only 2 of 103 isolates cultured from patients at a center in Delhi that caters to patients with chronic respiratory disease [89]. A subsequent surveillance study conducted at this same referral center, also in Delhi, between 2011 and 2014, reported a prevalence of 1.73% (12 of 695 isolates), of which the majority also contained the TR_{34}/L98H mutation [90]. These results were supported by another study in which 0.8% of immunocompromised patients with invasive aspergillosis were found to have infections caused by azole-resistant *A. fumigatus*, although the rate of resistance in cultured isolates was higher (4.9%) [91]. However, a more recent study from the referral center in Delhi, which used the commercially available AsperGenius TR_{34}/L98H, TR_{46}/Y121F/T289A assay, and a research that used only assay from this same company that detects G54 and M220 mutations, to detect azole resistance mechanisms in bronchoalveolar lavage samples from 160 patients with chronic respiratory disease, reported an azole resistance rate in 34% of culture negative samples and in 25% of all samples, of which only 23% were culture-positive [34••]. This finding highlights the potential for underestimating azole resistance due to the poor sensitivity of cultures for *Aspergillus* species. The majority of patients in which resistance mutations were found were those with chronic pulmonary aspergillosis or allergic bronchopulmonary aspergillosis. Resistance has also been documented in clinical isolates collected in Iran (4 of 124 isolates collected over a 6-year period from Tehran, 3 of which harbored TR_{34}/L98H) and in Turkey (3.3% of 392 *A. fumigatus* isolates collected from 12 centers) [92, 93].

Environmental samples from these countries have also noted the detection of azole-resistant strains. In an early environmental sampling study conducted in several regions of India between 2011 and 2012, 9% of all soil samples contained azole-resistant *A. fumigatus* strains [57]. The highest rate was found in urban samples, including those from tea gardens (33%) and from flower pots in hospital gardens (20%), all of which contained the TR_{34}/L98H mutation. A subsequent study from two regions of India reported that 7.6% of environmental *A. fumigatus* isolates were azole-resistant, including those with the TR_{46}/Y121F/T289A mutation. Environmental samples from Iran, Turkey, and Kuwait have also been found to

harbor the azole-resistant isolates, including those with TR_{34}/L98H and TR_{46}/Y121F/T289A mutations [93–96].

Africa

Recent data has also emerged regarding the prevalence of azole-resistant *A. fumigatus* on the African continent [27••]. Between 2000 and 2021, 11 studies from 7 African countries reported azole resistance in *A. fumigatus*. The majority of these were in environmental isolates (380) where the resistance rate ranged from 0 to 52% varying widely by country [27••, 55, 97–102]. The highest resistance prevalence was noted in the eastern countries of Tanzania and Kenya (27% to 52%), while the western countries of Nigeria and Burkina Faso had much lower rates (0 to 2.2%) [55, 97–99, 101, 102]. This difference has been postulated to be due to the higher use of azole fungicides in flower farming in Kenya and Tanzania [27••]. Many of these environmental isolates harbored the TR_{46}/L98H mutation, although several that contained a G54E mutation. Unfortunately, the clinical prevalence of azole resistance on this continent is poorly understood, as these studies included only 7 clinical *A. fumigatus* isolates, 5 of which were azole-resistant [99, 103].

Conclusion

Although much has been learned regarding the extent of azole-resistant *A. fumigatus* in both clinical and environmental specimens, our true knowledge of this emerging issue is fragmented. Most evaluations have been small point prevalence studies of limited duration that have included a limited number of institutions or sampling locations. Thus, they have not been able to adequately address trends in azole resistance over an adequate length of time. However, based on studies from the Netherlands, it is known that rates of azole-resistant *A. fumigatus* can fluctuate between different periods even though the overall has been an increase. In addition, the relationship between azole resistance and clinical outcomes must be better understood in different patient populations. Our understanding of the extent of this issue is also hampered by the fact that in most institutions and countries, susceptibility testing of molds, including *A. fumigatus*, is not routinely performed. Even if this were to improve, the rates of azole resistance may still be underestimated given the poor sensitivity of cultures for filamentous fungi, which are needed to perform phenotypic susceptibility testing. Thus, more emphasis is needed on the use of molecular assays, including the development of methods to detect *CYP51A*-independent mechanisms of resistance. Thus, more work is most definitely needed in order to grasp the extent and relevance of this emerging issue.

Declarations

Human and Animal Rights This article does not contain any studies with human or animal subjects performed by the author.

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- • Of importance
- •• Of major importance

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