Azole-resistant Aspergillus fumigatus: A global phenomenon originating in the environment?

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Résistance d'*Aspergillus fumigatus* aux antifongiques azolés : un phénomène mondial d'origine environnementale ?

Azole-resistant *Aspergillus fumigatus*: a global phenomenon originating in the environment?

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Résumé

Aspergillus fumigatus est le principal agent étiologique des aspergilloses invasives (AI), infections fongiques difficiles à prendre en charge et associées à un fort taux de mortalité. Si les antifongiques azolés et plus particulièrement le voriconazole ont permis d'améliorer considérablement le taux de survie des patients atteints d'AI, leur efficacité se voit cependant menacée par l'émergence de souches résistantes aux antifongiques azolés qui s'est accentuée ces 10 dernières années.

Le principal mécanisme de résistance, probablement d'origine environnementale, associe une ou des mutations ponctuelles dans la séquence codante du gène *cyp51A* et l'insertion d'une séquence répétée en tandem dans le promoteur de ce gène entraînant sa surexpression (TR_{34}/L98H et TR_{46}/Y121F/T289A). Cependant, l'émergence de souches résistantes cliniques et environnementales d'*A. fumigatus* dépourvues de mutations du gène *cyp51A* laisse supposer l'existence d'autres mécanismes (surexpression de pompes d'efflux, etc.).

Le développement de résistance peut être lié à l'utilisation au long court d'antifongiques azolés chez des patients atteints d'aspergilloses chroniques (voie médicale) ou à la pression de sélection exercée par les fongicides azolés dans l'environnement (voie environnementale). Cette voie d'acquisition de la résistance, médiée par l'utilisation des fongicides azolés, serait responsable de résistance chez les patients atteints d'AI et naïfs de traitements antifongiques.

Cette revue de la littérature a pour objectif de résumer les connaissances actuelles et les découvertes récentes concernant la résistance aux antifongiques azolés d'*A. fumigatus* et de comprendre l'importance du rôle de la voie environnementale dans l'acquisition de la résistance.

Abstract

Aspergillus fumigatus is the predominant etiological agent of invasive aspergillosis (IA), a difficult-to-manage fungal disease associated with a high case fatality rate. Azole antifungals, particularly voriconazole, have significantly improved the survival rate of patients with IA. However, the clinical advances made possible through the use of medical azoles could be threatened by the emergence of azole-resistant strains which has been reported in an ever-increasing number of countries over the last 10 years.

The major resistance mechanism, that combines point mutation(s) in the coding sequence of *cyp51A* gene and an insertion of a tandem repeat in the promoter region of this gene which leads to its overexpression (TR_{34}/L98H and TR_{46}/Y121F/T289A), is presumed to be of environmental origin. However, the emergence of clinical and environmental azole-resistant strains without the *cyp51A* gene mutation suggests that other mechanisms could also be responsible for azole resistance (for example, overexpression of efflux pumps).

The development of resistance may be linked to either long-term use of azole antifungals in patients with chronic aspergillosis (patient-acquired route) or selection pressure of the fungicides in the environment (environmental route). The fungicide-driven route could be responsible for resistance in azole-naive patients with IA.

This literature review aims to summarize recent findings, focusing on the current situation of azole-resistance in *A. fumigatus*, and provides better understanding of the importance of the environmental route in resistance acquisition.

Introduction

Invasive fungal infections (IFI) have been increasingly reported in many countries over the past 30 years [1]. This is mainly due to the development of antineoplastic and immunosuppressive therapies, resulting in an increasing number of patients at risk of IFI, including hematopoietic stem cell or solid organ transplant recipients, patients receiving immunosuppressive therapy, and cancer patients [2,3]. Among invasive filamentous fungal diseases, invasive aspergillosis (IA) which is mainly due to *A. fumigatus,* is the most frequently reported, especially in patients with hematological malignancies [1, 2]. IA − the most severe manifestation of *Aspergillus* disease − is difficult to manage, with a case fatality that can reach 65% [4,5]. The development of azole antifungal drugs, particularly voriconazole, in the early 2000s improved IA survival rate [6]. Two other more recently available azole molecules play an important role in the management of these diseases: isavuconazole which may be used as first-line therapy for treating IA just like voriconazole, and posaconazole as prophylaxis [7,8].

The recent clinical advances made possible by the use of azole antifungals could be threatened by the emergence of azole-resistance in *A. fumigatus* which has been reported in an ever-increasing number of countries over the last 10 years [6]. Since the first reports of clinical azole-resistant *A. fumigatus* strains in the United Kingdom and Sweden at the end of the 1990s [9,10], their number has been growing worldwide [3,6,11,12].

Two routes of resistance acquisition have been identified: long-term azole therapy (patient-acquired route) and the use of azole compounds in the environment (environmental route). Although these two routes have different characteristics, the prerequisites for azole resistance are the same: a favorable environment for the fungus development and the presence of azole compounds [6]. The development of resistance related to the long-term use of azole

antifungals, notably in patients with chronic aspergillosis, has been well known for many years. Understanding of the fungicide-driven route, still currently debated, has progressively improved over the last 10 years [6,13]. Indeed, the range of applications of azole fungicides and other demethylase inhibitors is broad. They include plant and crop protection and the preservation of a variety of materials, such as wood. *A. fumigatus* is very widespread in the environment. Regular use of azole fungicides, able to remain in soil for several months, can create an environment that contributes to the emergence of azole-resistant strains [14].

This review aimed to summarize current knowledge and recent findings on azole resistance in *A. fumigatus*, particularly the role of the environmental route in the emergence of azole-resistant strains.

Definition of azole resistance in *Aspergillus fumigatus*

Antifungal resistance is determined by the minimum inhibitory concentration (MIC), measured using broth microdilution methods. Two reference methods are used to measure MIC. The first was developed in the United States by the subcommittee on Antifungal Susceptibility Tests of the Clinical and Laboratory Standards Institute (CLSI) and the second was created in Europe by the European Union Committee on Antimicrobial Susceptibility Testing (EUCAST).

Determination of azole antifungal resistance is based on two threshold values: clinical breakpoints and epidemiological cut-off values (ECVs). These thresholds are defined for the four medical azoles used in *Aspergillus*-disease treatment (itraconazole, voriconazole, posaconazole, and isavuconazole) (Table 1) [15–18]. However, they do not all have the same meaning. Clinical breakpoints are established based on the pharmacokinetic and pharmacodynamic characteristics of antifungals, microbiological characteristics of the tested strains, and susceptibility data from

clinical trials associated with patient outcomes [16,19]. The susceptibility or resistance of *A. fumigatus* strains is determined by comparing the MICs of the four medical azoles with these clinical breakpoints. Currently, only EUCAST provides breakpoints for antifungals against *A. fumigatus.*

The ECV of a drug for a given species is based on the antifungal MIC distribution against a large panel of wild-type isolates generally encompassing 95% to 97.5% of the wild-type population. ECVs make it possible to classify strains into two groups: wild-type and non-wild-type. Strains classified as non-wild-type may have acquired resistance to the tested antifungal drugs [19].

Mechanisms of azole resistance described in the environment

Resistance mechanism linked to the *cyp51A* gene and its promoter

Resistance mechanisms of *A. fumigatus* are linked to mutations of the *cyp51A* gene and its promoter are the most commonly reported. These mechanisms may confer resistance to one or more azole antifungals ("multi-azole" resistance) [3,20].

Medical azole antifungals and azole fungicides share the same mechanism of action: they impact an important step of the ergosterol biosynthetic pathway. Ergosterol is a predominant sterol and essential membrane component that ensures the permeability and fluidity of the cell membrane [13]. Indeed, medical azole antifungals and azole fungicides inhibit lanosterol 14-alpha-demethylase. This important enzyme, encoded by the *cyp51A* gene, is a member of the P450 enzyme family and is responsible for lanosterol demethylation, which is necessary for the synthesis of ergosterol [21]. Inhibition of this enzyme interrupts ergosterol synthesis, resulting in

the accumulation of 14-alpha methyl sterols in the fungal cell. Thus, azole antifungals inhibit the ergosterol biosynthesis pathway and disrupt cell transport and fungal membrane structure [5,13].

Point mutations in the cyp51A gene

Positions G54, M220, and G448 of the *cyp51A* gene have been reported to be mutation hot spots, and mutations at these positions are frequently observed in patients with chronic aspergillosis treated with azole antifungals in the long term [3,19,20]. In addition, mutations at position G54 were reported for environmental strains isolated from various types of soil (agricultural fields, orchards, flowerbeds) in Romania, Tanzania, India, and Thailand [22,23]. Bader *et al.* [24] also reported an environmental strain carrying the M220I mutation.

Although less common, other point mutations associated with resistance to azole antifungals have also been reported: G138C, F219I, P216L, G432S, and G432A [3,25,26]. G138C and F219I have currently only been found in patients receiving long-term treatment with azole antifungals. However, the P216L mutation has also been reported for an environmental strain [27–29]. Strains carrying G432S and G432A mutations have been isolated from azole-naive patients [25,26]. A total of more than thirty *cyp51A* gene mutations have been identified, but the role of some of these mutations in azole antifungal resistance has yet to be proven.

Overexpression with or without point mutations in the cyp51A gene

Overexpression and point mutations in the *cyp51A* gene constitute an important second mechanism of azole resistance. The most commonly reported mutations, TR_{34}/L98H and TR_{46}/Y121F/T289A, combine one or several codon substitutions in the *cyp51A* gene with a tandem repeat of 34 (TR_{34}/L98H) or 46 base pairs (TR_{46}/Y121F/T289A) in the promoter of the gene, leading to its overexpression. This association is required to confer azole resistance [30,31]. This resistance mechanism is currently the most closely associated with the environmental route of resistance [6].

The number of strains carrying these mutations has been increasing over the last 20 years. Since the discovery of the first azole-resistant isolates carrying the TR_{34}/L98H mutation in the Netherlands and Italy in 1998, similar findings have been reported in many countries around the world and on every continent [6,11,32–34] (Figure 1). The more recent discovery of the TR_{46}/Y121F/T289A mutation confirms the rapid emergence of azole-resistant strains with complex mutations. Since 2009 clinical and environmental TR_{46}/Y121F/T289A strains have been reported in several European countries, such as the Netherlands, Belgium, Germany, France, Denmark, and Spain, as well as in India, the United States, and Colombia [6,11] (Figure 1).

The last three years have seen the emergence of TR_{34}/L98H strains from the environment in three Asian countries: China, Thailand, and Japan. In Japan, clinical and environmental TR_{34}/L98H strains were found almost simultaneously the same year [22,35,36] (Figure 1).

Several strains with one of the two tandem repeats associated with several point mutations have also emerged since 2014: TR_{34}/L98H/S297T/F495I in China and France, TR_{46}/Y121F/M172I/T289A in Germany, and $TR_{46}{}^{3}$/Y121F/M172I/T289A/G448S in the Netherlands [24,29,35,37].

Single tandem repeat with no mutation in the *cyp51A* gene, the TR_{53} mutation, is also considered to be responsible for multi-azole resistance. First reported in the Netherlands in 2009 in a young patient presenting with chronic granulomatous disease, this mutation was also found in environmental strains in the Netherlands and Colombia [38–40].

Resistance mechanisms not linked to the *cyp51A* gene

Other resistance mechanisms that do not involve the *cyp51A* gene and its promoter were described [3]. Such *cyp51A*-independent mechanisms are also thought to be responsible for

treatment failure [3,19]. They were first identified in clinical strains, but it is not unlikely that they exist in environmental strains as well. Fungal cells possess two families of efflux pumps: ATP Binding Cassette (ABC) transporters and Major Facilitator Superfamily (MFS) transporters. These membrane proteins mediate the efflux of toxic compounds, notably azole molecules, out of fungal cells. Their overexpression leads to resistance to azole antifungals due to a decrease in the intracellular concentration of the fungicide [13].

The Cdr1B transporter is an ABC transporter overexpressed by certain azole-resistant *A. fumigatus* strains. Deletion of the *Cdr1B* gene in resistant strains results in greater susceptibility to medical azoles [2,13].

Meneau *et al*. [41] highlighted the importance of two other ABC transporters, AtrF and AtrI, and one MFS transporter, MdrA, in *A. fumigatus* azole resistance. In this study, the expression of these three transporters was assessed in environmental strains with high itraconazole MICs (MIC ≥8 mg/L). These strains showed approximately three times AtrF expression than the reference susceptible strain [41]. The AtrF transporter could contribute to increased itraconazole MICs in environmental strains and may thus be responsible for azole resistance in certain strains that do not have mutations in the *cyp51A* gene.

Mutations in transcription factors may also play a role in the azole resistance of clinical strains and are therefore likely to be found in environmental strains: the P88L mutation in the CCAAT-binding transcript factor complex subunit HapE or mutations in the recently described AtR transcription factor [3,42].

Routes of azole resistance development: the role of azole fungicide use in the environment

Extensive use of azole fungicides

The environmental route probably plays an important role in the emergence of azole-resistant *A. fumigatus* strains. This path of resistance is linked to azole fungicide selection pressure; azole fungicides are largely used in farming to protect cereal and wine crops from phytopathogenic molds [5,43]. They are also used in the processing of vegetable crops and ornamental plants and to preserve a variety of materials, such as wood [13,43]. They are used on a global scale, particularly in Europe and Asia, where they represent one of the most commonly used pesticide classes. In Europe 40% of all pesticides sprayed on crops are fungicides and over one-third of all fungicides sold are azole fungicides [12]. The percentage of fungicide consumption in India and China may be as high as 23% [35,44].

Treatment with azole fungicides is less common in North America, where less than 5% of the total crop area is treated in the United States [5]. In South America, Alvares *et al.* [38] reported significant demethylase inhibitor consumption in Colombia, where azole fungicides are among the most predominant pesticides.

Five azole molecules largely used in farming (propiconazole, tebuconazole, difenoconazole, epoxiconazole, and bromuconazole) are particularly involved in the development of azole-resistant strains. Several studies reported cross resistance to medical azoles and these five azole molecules for TR_{34}/L98H strains [29,45,46]. These five demethylase inhibitors exhibited molecular characteristics that were very similar to those of medical azole antifungals, resulting in the most similar binding modes and the greatest level of cross-resistance [46]. Finally, the coincidence between authorization for use of these demethylase inhibitors and the first detection of the TR_{34}/L98H mutation supports the hypothesis of a fungicide-driven route for azole resistance [46].

IA due to azole-resistant strains in azole-naive patients

The hypothesis of an environmental route for resistance was first suggested in the Netherlands when IA cases caused by azole-resistant strains were reported in patients who had never been treated with azole antifungals [33]. No previous azole treatment was reported for 50% to 71% of aspergillosis cases caused by azole-resistant strains [12,20].

Aspergillosis is an infectious disease for which person-to-person transmission seems unlikely. Patients infected by a resistant strain and naive for medical azoles likely become infected by inhaling azole-resistant strains present in the environment [13,47]. Findings from two studies support this hypothesis. Lavergne *et al*. [48] reported an IA case caused by an *A. fumigatus* strain carrying the TR_{46}/Y121F/T289A mutation in a retired farmer. The patient was treated with infliximab for rheumatoid arthritis and was azole-naive. An environmental investigation, performed in and close to the patient's home (inside the house, vegetable garden, and crop fields), revealed strains with the TR_{46}/Y121F/T289A mutation and an indistinguishable genotype − using multiple-locus variable number tandem repeat (VNTT) analysis − from that of clinical strain isolated from the patient [48]. In Japan, a first case of IA caused by a strain carrying the TR_{34}/L98H mutation was reported in a patient with hepatocellular carcinoma. The patient's main hobby, who had never been treated with medical azoles before IA diagnosis, was gardening [49]. An environmental survey performed the same year, which consisted of investigating the air of 10 public parks, found a strain carrying the TR_{34}/L98H mutation with a genotype identical to that of the clinical strain isolated from the Japanese patient mentioned above [36]. Thus, patients' environment could be a potential source of azole-resistant *A. fumigatus* strains.

Mechanisms of resistance with overexpression of the *cyp51A* gene

Tandem repeats are found in many phytopathogenic molds that develop resistance through exposure to demethylase inhibitors, such as *Mycosphaerella graminicola* (now called

Zymoseptoria tritici), *Rhynchosporium secalis,* and *Botrytis cinerea* [12]. The insertion of a tandem repeat is a complex process that most likely takes place in the environment during sexual reproduction. Sexual reproduction, recently described in *A. fumigatus*, seems to be more common in the environment and does not appear to be involved in fungal diseases in humans [5,50].

Zhang *et al*. [37] recently reported a new complex mutation in *A. fumigatus* isolated from samples that came from azole-containing composts: TR_{46}^{3}/Y121F/M172I/T289A/G448S. This complex mutation is similar to the TR_{46}/Y121F/T289A mutation; it contains an additional repetition of the TR_{46} motif in the *cyp51A* gene promoter and two additional point mutations: M172I and G448S. In this study, the number of azole-resistant strains was higher in samples from azole-containing composts. Thus, these composts could be an important ecological niche for the development of azole-resistant strains [37]. The recent finding of the first environmental strains carrying TR_{34}/L98H in the south-eastern part of the United States supports this hypothesis. Indeed, these strains were isolated from compost made from harvest crop debris from peanut fields treated with propiconazole and tebuconazole [51]. Moreover, sexual reproduction of *A. fumigatus* may have been involved in the development of this complex mutation, as already suggested for the TR_{34}/L98H and TR_{46}/Y121F/T289 mutations [37]. The possible role of sexual reproduction in the emergence of the mutation was suspected due to the high genetic diversity of strain genotypes in compost containing azole residues. The TR_{46}^{3} mutation was also found after *in vitro* sexual reproduction; this mutation could come from a sexual cross between two TR_{46} strains, possibly through unequal crossing-over between the double tandem repeats during meiosis [37].

It was recently reported that *A. fumigatus* has three different reproduction modes [50]. Although asexual reproduction is the predominant mode, there may also be sexual and parasexual reproduction. This could increase genetic diversity via mutations and genotypic recombination, which could allow better adaptation of *A. fumigatus* to stress caused by azole fungicides, thus explaining the various resistance mechanisms developed by this fungus [50].

Relation between the use of azole fungicides and the development of resistance

Many arguments suggest the importance of the environmental route in the development of azole-resistant strains. However, the relation between the use of azole fungicides and the development of resistance has not yet been demonstrated by *in vitro* experiments. Indeed, *in vitro* exposure of wild-type strains or those with the TR_{34} or L98H mutation to several demethylase inhibitors (propiconazole, epoxiconazole, tebuconazole, difenoconazole, and bromuconazole) did not generate the TR_{34}/L98H mutation [46].

However, a recent Chinese study suggested a direct link between demethylase inhibitor use in farming and the development of azole-resistant strains. Initially susceptible environmental *A. fumigatus* strains became resistant to medical azoles after exposure to five demethylase inhibitors used in cereal crop processing (epoxiconazole, propiconazole, tebuconazole, hexaconazole, and metconazole). The TR_{46}/Y121F/T289A mutation was the most frequent resistance mechanism found in azole-resistant strains induced by *in vitro* exposure to these demethylase inhibitors [52]. This result requires confirmation by additional studies.

Clinical implications

Changes in treatment practices

A. fumigatus azole resistance is a worrisome and widespread phenomenon which can significantly compromise the management of patients, resulting in treatment failure and a high case fatality rate [4,28,40].

Voriconazole and more recently isavuconazole are the two azole molecules currently used as first-line therapy for treating IA. However, a group of experts recommended that these two azole molecules should no longer be used as monotherapy in areas where the prevalence of azole resistance is ≥10% [53]. Isavuconazole shows clinical efficacy and a spectrum of antifungal activity close to that of voriconazole for treating IA. It is also probably better tolerated. However, high MICs and MICs similar to those of voriconazole have been reported for azole-resistant *A. fumigatus* strains carrying the TR_{34}/L98H and TR_{46}/Y121F/T289A mutations [19,29]. Thus, isavuconazole may not be suitable for managing patients with IA caused by azole-resistant strains.

In regions with environmental resistance rates ≥10%, experts recommend amphotericin B for the first-line therapy. If susceptibility is unknown, because of negative cultures, or if there has been no susceptibility assessment, cautious de-escalation to voriconazole or posaconazole monotherapy after two weeks of amphotericin B may be considered, if the patient is responding on a clinical level [53].

Amphotericin B has many adverse effects, especially nephrotoxicity, despite the availability of the liposomal form with improved tolerability. Moreover, its sole administration by the intravenous route limits its long-term use [19]. An azole-echinocandin combination is suggested as a second treatment alternative when azole-resistance is ≥10%. Echinocandins are recommended as a salvage treatment for treating IA when first-line therapies fail. Some studies even reported that the azole-echinocandin combination has better efficacy than medical azoles in monotherapy, but additional studies are required to confirm the clinical benefit of the azole-echinocandin combination [7,53].

Determining the prevalence of azole-resistant *A. fumigatus* strains: an important challenge

Good knowledge of azole-resistant strain prevalence is necessary because it determines therapeutic management. However, the prevalence may vary greatly from one country to another and is difficult to measure. Garcia-Rubio *et al.* [3] recently reported a global prevalence between 0.55% and 30% based on the literature; this finding is comparable to that of Vermeulen *et al.* in 2013 [3,54].

The prevalence can also vary within a given country. For example, studies in Germany reported prevalence ranging from 1.1% to 30% [55,56]. This is also true for France, where resistance was found to be stable by a Parisian research team with a prevalence of less than 1% between 2006 and 2014 [25,57] whereas another French study, led by a Nantes research team, reported a prevalence of 8% [58]. Prevalence in the Netherlands ranges from 1.8% to 40% [33,59]. These national and international variations may be due to the studied diseases (chronic pulmonary aspergillosis in patients with chronic obstructive pulmonary disease or cystic fibrosis, or IA in patients presenting with hematological malignancies). This variability may also be due to the geographical location of the study. Azole fungicide selection pressure may indeed be different in urban and rural areas.

Such differences in prevalence could also be related to biases due to different laboratory practices. Prevalence varies depending on whether the study involves clinical strains of *A. fumigatus* collected in laboratories and not previously selected, clinical strains from patients with aspergillosis (chronic or invasive manifestation), or clinical strains isolated from a cohort of patients with an underlying and previously defined disease and who later developed IA. The source of the strains can lead to a 30-fold difference in the estimated prevalence [60].

The lack of standardized procedures and the low sensitivity of cultures for the detection of azole-resistant strains could also explain these variations. The low positivity rate of *A. fumigatus*

cultures from an infected site is well known for patients with chronic infections and for neutropenic patients [60], particularly those undergoing prophylactic antifungal treatment or for whom the detection of fungal biomarkers (*Aspergillus* antigen, PCR) led to the early initiation of antifungal therapy.

Fungal culture only provides a picture of viable microorganisms in the samples at a given time and is limited to microorganisms that can grow on culture media. A negative culture is not sufficient to completely rule out the presence of azole-resistant strains. Moreover, a recent study reported that patients can be co-infected by azole-resistant and susceptible strains. Thus, a positive *Aspergillus* culture may contain mixed phenotypes (wild-type and resistant), making it more difficult to identify patients with IA due to an azole-resistant strain [61].

Clinical and environmental azole-resistant strains can be detected by the use of culture media containing an azole antifungal such as itraconazole or voriconazole. Although some research teams chose to screen with media containing low azole antifungal concentrations (1 mg/L for voriconazole and 2 mg/L for itraconazole) to improve the sensitivity of azole-resistant strain detection, others use higher concentrations such as 4 mg/L [24,29,40,51]. Under these conditions, the detection of clinical and environmental strains with a particular resistance phenotype may be limited.

Standardizing laboratory practices and procedures and accessing data from national and international surveillance programs using standardized protocols are probably necessary to generate reliable surveillance data and to determine the prevalence of azole resistance for different countries and patient groups [57,60].

Molecular biology and the detection of azole resistance

Azole-resistant strains should be detected as soon as possible to better adapt antifungal therapy and to improve patient management. However, the detection of resistance is limited by the lack of sensitivity of *Aspergillus* cultures. Some laboratories have developed direct molecular biology detection techniques from samples, such as respiratory tissues or secretions. Molecular biology provides an interesting alternative to improve both the identification of azole-resistant strains and patient management. However, such molecular tools are not yet routinely used in all laboratories [6]. A PCR assay (AsperGenius™, Pathonostics, the Netherlands) has just become commercially available. This assay targets *Aspergillus* spp. DNA (*A. fumigatus* and *A. terreus*) in respiratory samples in combination with the detection of the most prevalent mutations TR_{34}/L98H and TR_{46}/Y121F/T289A [62-63].

Conclusion and perspectives

The importance of azole-resistant *A. fumigatus* strains and the high rates of azole resistance in some countries or regions support further development of national and international surveillance programs. A better understanding of the conditions that allow the selection of azole-resistant strains in the environment is necessary to implement adequate prevention measures. Studies focusing on professions that use azole fungicides may be useful for assessing the emergence and spread of new resistance mechanisms. Indeed, TR_{34}/L98H and TR_{46}/Y121F/T289A mutations are currently the most prevalent mutations associated with the environmental route, but azole-resistant *A. fumigatus* strains without *cyp51A* gene mutations may also emerge from the environment.

The evidence demonstrating the role of azole fungicides in the environmental emergence of azole-resistant strains is constantly increasing. However, although discontinuing the use of azole fungicides in the environment would be desirable − particularly in farming − it is currently unlikely

because of the significant economic losses and food shortages that would result from crop damage without their use [13]. It is thus essential to change processing practices to reduce the use of azole fungicides and their spread in the environment that results in cross resistance with medical azoles. The use of fungicide formulations without azole molecules, those combining molecules that induce less resistance or new adsorption processes to limit the diffusion of azole fungicides into the environment are new approaches that must be rapidly developed to preserve the efficacy of medical azoles [64]. Medical azoles are essential for the management of patients with *Aspergillus* diseases.

Disclosure of interests

The authors declare no conflict of interests.

Authors' contribution

AJ, SR, and LM established the plan of this general review. AJ carried out the literature review and summarized recent findings, focusing on the azole-resistance of *A. fumigatus*. AJ and SR wrote the review. AP, GR, and LM provided their expertise in medical mycology and *A. fumigatus* resistance in the final version. All authors read and approved the final version of the article.

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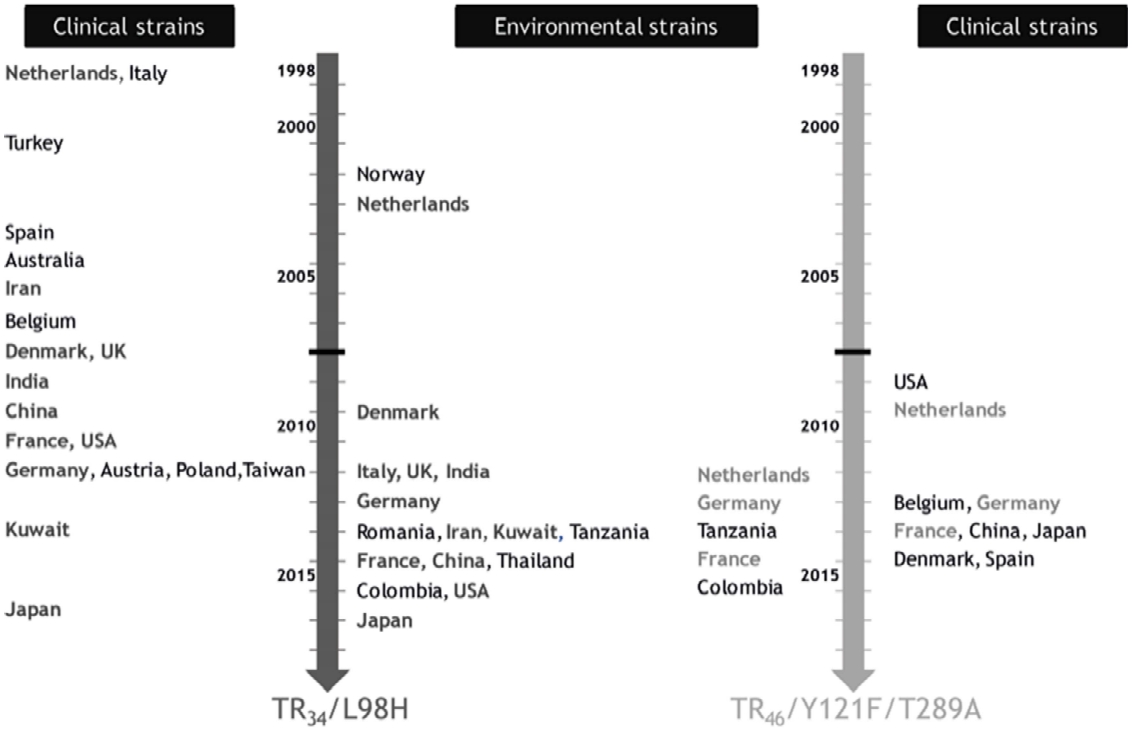
Figure 1. Chronologie de l'émergence des souches d'*Aspergillus fumigatus* possédant les mutations TR_{34}/L98H et TR_{46}/Y121F/T289A : pays et année de découverte initiale

Figure 1. Emergence of the TR_{34}/L98H and TR_{46}/Y121F/T289A *Aspergillus fumigatus* strains: country and year of first recovery



in bold: countries where clinical and environmental strains have been described

bold line: year 2007 indicating the acceleration of the emergence of TR_{34}/L98H and TR_{46}/Y121F/T289A strain

Tableau I. Valeurs seuils cliniques et épidémiologiques des quatre azolés médicaux pour *Aspergillus fumigatus*

Table I. Clinical breakpoints and epidemiological cut-off values of four medical azoles for *Aspergillus fumigatus*

Medical azoles	CLSI ECVs (mg/L)	EUCAST ECVs (mg/L)	EUCAST clinical breakpoints (mg/L)
itraconazole	1	1	S ≤ 1 ; R > 2
voriconazole	1	1	S ≤ 1 ; R > 2
posaconazole	0.5	0.25	S ≤ 0.125 ; R > 0.25
isavuconazole	1	2	S ≤ 1 ; R > 1

S=susceptible; R=resistant; EUCAST: European Union Committee on Antimicrobial Susceptibility Testing; CLSI: Clinical and **Laboratory Standards Institute; ECV:** epidemiological cut-off values.