Azole-resistant *Aspergillus fumigatus* as an emerging worldwide pathogen

Sofia Marisel Rivelli Zea[1], Takahito Toyotome[1,2,3]*

[1] Department of Veterinary Medicine, Obihiro University of Agriculture and Veterinary Medicine

[2] Diagnostic Center for Animal Health and Food Safety, Obihiro University of Agriculture and Veterinary Medicine

[3] Medical Mycology Research Center, Chiba University

* Corresponding author: Takahito Toyotome; tome@obihiro.ac.jp, ORCID ID 0000-0003-2822-2767

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Abstract

Aspergillus fumigatus, a ubiquitous pathogen, causes aspergillosis in humans, especially in immunodeficient patients. Azoles are frontline antifungal drugs for treating aspergillosis. The recent global emergence of azole resistance in *A. fumigatus* has become a serious problem worldwide. It has arisen through two routes: long-term azole medical therapy, called the patient route, and the use of azole fungicides in its habitats especially for agricultural activities, called the environmental route. Resistant strains developed through the latter route show cross-resistance to medical azoles because of the identical molecular target Cyp51A between azole compounds used for medical treatment and agricultural disease control. In azole-resistant strains arising through the environmental route, *A. fumigatus* is observed frequently possessing mutations in the *cyp51A* gene linked to tandem repeats in the promoter region such as TR_{34}/L98H and TR_{46}/Y121F/T289A. Results of microsatellite genotyping analyses of resistant *A. fumigatus* strains have suggested a transboundary spread of this microorganism in many countries. Diverse actors are involved in the global highway of transmission. Therefore, the matter must be addressed as a "One Health" issue. This review presents a background of azole resistance in *A. fumigatus* and introduces newly discovered difficulties generated as this pathogen spreads worldwide.

Key words: *Aspergillus fumigatus*, azole, drug resistance, One Health

Introduction

Fungi, which are important biological degraders and consumers in the soil, proliferate and survive in numerous and diverse ecosystems throughout the Earth. Furthermore, some are opportunistic pathogens, especially affecting animals and immunocompromised humans.

The invasive *Aspergillus* infections caused mainly by the ubiquitous fungus *Aspergillus fumigatus* are associated with at least 50% mortality of patients **(1)**. Azole antifungals are the front-line drug used to treat aspergillosis. The azole drug family includes imidazoles and triazoles whose structural difference is in the number of nitrogen atoms in the azole ring, two or three, respectively. The members of this group of drugs possess the same mechanism of action, which is based on disruption of the fungal cell membrane **(2)**. Beyond the beneficial oral and parenteral routes of azole administration, triazole compounds such as itraconazole, voriconazole, posaconazole, and isavuconazole have demonstrated broad spectrum and effective results in the treatment against *Aspergillus* spp. **(3, 4)**.

The azole drug family are available for human medicine and veterinary treatment. Moreover, most of them, designated as 14α-demethylase inhibitors (DMIs), are used extensively as fungicides to protect plants and agricultural products **(5)**. Recently, strains of *A. fumigatus* resistant to antifungal azole drugs are emerging and putting at risk the limited number of antifungal therapies available.

During the last 20 years, azole-resistant *A. fumigatus* (AR*Af*) strains have been detected in patients receiving prolonged azole therapies, and particularly in azole-naïve patients without prior azole treatment **(6–8)**. This finding has taken on new relevance in human health. In the period 2007-2011, two surveillance studies that focused on the epidemiology of resistance selection in *A. fumigatus* were carried out in the Netherlands **(9, 10).** They reported the AR*Af* isolation rates of

50% (four of eight patients) and 75% (six of eight patients) from patients who had not previously received azole treatment **(9, 10)**. Currently, there are two potential routes for the development of azole resistance in *A. fumigatus*: the patient route, related to long-term azole medical therapies, and the environmental route, related to use of azole fungicides in agricultural activities and habitats. As a result of AR*Af* emergence, the international commercialization of agricultural products that could be contaminated with such resistant strains increases their dissemination and threatens the effectiveness of azole drugs worldwide. This review presents the background of azole resistance in *A. fumigatus* as a current health issue and the transboundary spread of the AR*Af* strains as a global concern related to One Health.

Aspergillus and Aspergillosis

The genus *Aspergillus* includes more than 300 species, of which *A. fumigatus* is a common one in the environment, and possesses biological properties that allow it to adapt to surrounding changes. *A. fumigatus* is thermotolerant, capable of surviving and growing at temperatures of 12°C–65°C and being resistant to low pH (3.7–7.6). Furthermore, it has evolved to resist antifungal agents, and therefore it poses a higher risk to infect immunocompromised hosts **(11)**. The principal sterol compound of its cell membrane is ergosterol, which imparts to the membrane the vital functions of fluidity, regulation, and cell cycle control. The catalytic reaction of lanosterol 14α-demethylase (Cyp51), a cytochrome P450 protein, is the key step for ergosterol biosynthesis in *A. fumigatus*. This pathway constitutes the common target of azole antifungal drugs, with the goal of disrupting the cell membrane and inhibiting fungal growth **(12–14)**.

Fungi have both asexual and sexual life cycles. The sexual life cycle, exchanging alleles and creating genetic diversity among strains, is important for recombination. Nevertheless, the sexual life cycle of many fungal species has not been fully examined. In fact, details of *A. fumigatus* remained unknown until 2009, when the sexual cycle was discovered and characteristic structures of sexual reproduction such as cleistothecia, asci, and ascospores were shown **(15)**. However, the major reproduction mode of *A. fumigatus* is the asexual cycle, which generates and disseminates huge amounts of small, hydrophobic, asexual spores (or conidia, 2–3 μm diameter) into the air. These later grow into a vegetative mycelium to colonize a substrate. The spores, which can survive in hostile conditions, germinate when the environment becomes appropriate **(16)**. In addition, the parasexual cycle is a third type of reproduction mode. It is triggered when the environment contains stress factors such as nitrogen starvation, antifungal drugs, and host immune factors as *A. fumigatus* develops in patients with severe lung affections, in whom the distinctive diploidy of parasexual reproduction has been frequently observed **(17)**. The parasexual cycle is produced by the fusion of two compatible hyphae to form a vegetative heterokaryon containing two or more different nuclei for recombination. Firstly, the haploid nuclei undergo karyogamy to generate a heterozygous diploid nucleus. Later, random mitotic recombination of diploid nuclei occurs and produces diverse genotypes. Moreover, during diploid reproduction, chromosomes are lost continuously through mitotic nondisjunction, resulting in haploid and aneuploid cells with diverse progenies **(17, 18)**.

The airborne conidia involved in the asexual reproduction cycle are inhaled and are normally eliminated by the innate immune system of the host. However, immunocompromised patients who inhale the conidia contract invasive aspergillosis (IA) including pulmonary aspergillosis and systemic aspergillosis. Even other chronic forms can manifest, such as allergic bronchopulmonary

aspergillosis, aspergilloma, and chronic pulmonary aspergillosis with mortality rates that remains high (30%–90%) **(14, 19, 20)**. Nowadays, aspergillosis is regarded as the most common mold infection worldwide, and a point of concern is coinfection in cases of severe influenza or COVID-19 **(19, 21)**.

Azole resistance of *Aspergillus fumigatus* via the patient route

Currently, the Food and Drug Administration (FDA) has approved few classes of antifungal drugs for aspergillosis treatment: echinocandin, polyene, and azoles **(22)**. This last class includes major useful agents because of their availability as oral and intravenous formulations. The azoles are the only antifungal oral options available for treating *Aspergillus* diseases. For that reason, the spread of azole cross-resistance threatens the effectiveness of oral treatment **(23)**.

Drug resistance in *A. fumigatus* which developed in patients with long-term azole medical therapy is called the *de novo* or patient route **(24)** (Figure 1). The first isolation of azole-resistant *Aspergillus* spp. strains was reported in long-term itraconazole therapy patients from California (USA) in the late 1980s **(6)**. Almost a decade later, similar cases were identified around the world **(24)**. AR*Af* strains have exhibited high minimum inhibitory concentration (MIC) values for azoles **(25)**; this characteristic was associated with point mutations and substitutions in the sequences of the fungus **(26)**. It is noteworthy that the most frequent azole resistance mechanisms in *A. fumigatus* are linked to non-synonymous mutations in the *cyp51A* gene, resulting in structural modifications of the channels of azole access and consequently decreasing its affinity **(19, 27)**. In particular, the representative AR*Af* strains harbor single mutations at codons 54, 138, 220, 431, 434, and 448 **(23, 24)** (Table 1).

The position and type of amino acid substitution in the sequence of 14α-demethylase enzyme determine chemical changes in the interaction with azole drugs generating diverse patterns of azole cross-resistance. According to earlier findings for the gene *cyp51A*, a clinical isolate with a G138C mutation was confirmed to be a multi-azole (itraconazole, voriconazole, and posaconazole) AR*Af* strain **(28)**. Additionally, high MICs for itraconazole and voriconazole were reported in a laboratory-selected strain with a G138R mutation. Isolates harboring mutations at positions Y431C and G434C have demonstrated resistance to voriconazole and posaconazole **(23)**. Moreover, a mutation at position G448S has been closely related to high MIC values for voriconazole and itraconazole **(19, 29, 30)**. Single mutations in the gene *cyp51A* at position G54 with amino acid substitutions G54E, G54V, and G54R have been widely reported as related to itraconazole and posaconazole resistance **(19, 31)**. Lastly, mutations at the 220th position M220, M220K, or M220T have shown high MIC values for itraconazole, voriconazole, and posaconazole **(19, 23)**.

AR*Af* strains resistant to multiple azole drugs without mutations in the *cyp51A* gene have been reported **(32),** suggesting alterations in different genes, as well as other resistance mechanisms **(32)**. The *A. fumigatus* genome includes genes encoding efflux transporters of multidrug resistance (MDR) comprising the major facilitator superfamily (MFS) and the ATP-binding cassette (ABC) superfamily **(33)**. Their upregulations as a response to itraconazole treatment have been reported in *A. fumigatus* wild-type strains from clinical samples **(32)**. Moreover, an earlier study explored MDR efflux transporters and resulted in the amplification of the genes *mdr1* and *mdr2* encoding the ABC transporters Mdr1 and Mdr2 in *A. fumigatus* by employing degenerate primers for polymerase chain reaction **(34)**. Later, the expressions of *mdr3* and *mdr4* with homology to members of the MFS were investigated, and the results indicated that the expressions were of a constitutively high level in laboratory-induced strains **(33)** (Table 1). Among clinical isolates,

153 *cdr1B* encoding the ABC transporter was associated with azole resistance. The basal expression
154 was found to be increased significantly **(32)**. A substitution (P88L) in the *hapE* gene was also
155 associated with azole resistance through the increase of the *cyp51* expression **(35)**. Furthermore,
156 mutations in the genes *hmg1* and *erg6* in AR*Af* have been explored; both are involved in the
157 ergosterol biosynthesis pathway, which is linked strongly with azole resistance **(36)** (Table 1).

158 Development of the patient route has been associated mainly with AR*Af* harboring single
159 mutations but not with the tandem repeat resistance mechanism. However, an AR*Af* strain
160 harboring tandem repeats of the 120-bp (TR_{120}) in the promoter region of the *cyp51A* gene was
161 isolated from a case of fatal aspergillosis with long-term azole treatment **(37)**. Although the
162 development of tandem repeat mechanisms in a patient route, as with the TR_{120} strain, remains
163 unknown, one hypothesis suggests that the tandem repeats in AR*Af* strains would derive from
164 single events of sexual reproduction of isogenic strains **(38)**. In addition, a recent study described
165 that parasexual recombination of *A. fumigatus* permitted its adaptation in cystic fibrosis (CF)
166 patients **(17)**. Long-term hyphal colonization can be established through parasexual mitotic
167 recombination, giving rise to the formation of diploid spores **(16, 17, 39)**. Parasexual reproduction
168 allows the fungus to persist regardless of stressful changes in the environment. Subsequent
169 evolutionary modifications that occur in the transition from heterokaryon to diploid mitotic
170 recombination could generate azole-resistant strains which have increased competence to infect
171 immunocompromised hosts with CF **(17)**. This is a hypothetical mechanism of tandem repeats
172 development, and more in-depth research is necessary to advance our understanding of azole-
173 resistance mechanisms and novel AR*Af* strains.

174

175 **Azole resistance of *Aspergillus fumigatus* in the environment**

Fungicides are vitally important for the protection of agricultural products, whether on farms or in gardens, from phytopathogenic fungi. For that reason, azole fungicides have become commercialized globally. All azole fungicides share the same primary mechanism of action against the fungal ergosterol biosynthetic pathway described above **(40)**. The five triazole DMIs of propiconazole, tebuconazole, epoxiconazole, difenoconazole, and bromuconazole show similar molecular characteristics to those of medical azoles such as itraconazole and voriconazole; consequently, identical binding modes and the highest level of cross-resistance are seen **(5)**. Although *A. fumigatus* is neither an important phytopathogen nor the target of azole application in agriculture, it undergoes stress by the pressure exerted from fungicide use in agricultural fields, thus allowing it to acquire azole resistance **(19, 41)** and producing AR*Af* strains that are present in the environment **(42)**. Because medical front-line drugs are not effective against AR*Af* strains, the likelihood of failure of conventional aspergillosis treatment increases in immunocompromised patients, even in azole-naïve patients **(19)** (Figure 1). Therefore, it is important to ascertain whether the patient is infected with environmental AR*Af* or not, followed by prompt selection of an appropriate drug therapy.

Environmental AR*Af* strains possess tandem repeats (TR) in the promoter region of the *cyp51A* gene, which lead to gene overexpression **(24)**. The common TR strains harbor two or three repetitive units composed of 34 base pairs (bp), 46 bp, or 53 bp **(43)**. They also possess amino acid substitutions TR_{34}/L98H **(44)** and TR_{46}/Y121F/T289A **(45)**. Strains with the TR_{53} mutation have not evinced substitution in the coding region. Further mutations have been detected in AR*Af* isolates worldwide, thus increasing the genetic variability: TR_{34}/L98H/S297T/F495I **(46)** and TR_{46}/Y121F/M172I/T289A/G448S **(38)**. In particular, a phylogenetic analysis demonstrated the predominance of the TR34 / L98H allele among clinical and environmental samples in India **(44)**.

Although the samples were collected from unconnected settings, all of them were genotypically identical regardless of their origins. The researchers postulated an Indian genotypic cluster generated by a selective sweep process **(44)**. The TR alterations constitute non-synonymous mutations with irreversible consequences such as increased gene expression and decreased affinity for the azole ligand. Additionally, single mutations in the codon G54 with amino acid substitutions E, A, and R have been detected from environmental samples **(47–50)** (Table 1).

Mutations generally entail a fitness cost, but experimental data on *ARAf* is still scant in this regard. The first fitness study was performed using constructed isogenic *A. fumigatus* strains harboring G54W or M220K mutation and parental strains **(41)**. No adverse fitness cost was demonstrated comparing both growth rates together in vitro and in vivo under azole-free conditions **(41)**. Indeed, the worldwide spread might indicate a low fitness cost for AR*Af* with mutations related to the environmental route **(51)**. In contrast, azole-resistant isolates from the patient route were found to be unable to compete with wild-type in absence of triazoles. The growth rate and morphology of AR*Af* strains were affected, and they also lacked sporulation **(52)**. However, their ability to prevail through alternative reproductive cycles and mutations has been reported **(53)**.

In addition, azole-resistant and azole-susceptible *A. fumigatus* strains harboring non-synonymous mutations F46Y, M172V, N248T, D255E, and E427K in the *cyp51A* gene have been isolated repeatedly from environmental and clinical samples throughout the world **(54–57)**. These amino acid substitutions have been shown to be located in the peripheral regions of the CYP51A protein, without interfering in the access channels for the binding of the azole compounds **(58)**. Therefore, the five mutations are regarded as polymorphisms of the *cyp51A* gene. Their detection is not correlated intrinsically with resistance to azoles, despite a tendency to present high MIC values compared to wild types **(59)**.

Hot spots and transboundary spread of azole-resistant *A. fumigatus*

At this time, abundant AR*Af* strains are detected in environmental samples recognized as "hotspots" because of the favorable conditions for resistance selection in *A. fumigatus*. Specifically, flower bulbs, stockpiles of green waste, wood chips, and sawmills have been scanned as potential hotspots **(39, 60)**. Fungicide treatments are commonly applied to seeds and wood, bulbs may be dipped in fungicides as preplanting treatment, and fruits often receive post-harvest antifungal protection.

To illustrate common features of environments designated as hotspots, a risk assessment model was postulated for the selection of resistance to DMI in *A. fumigatus*. A hotspot has been described as an environment having the following features: (1) its physical, biotic, and abiotic conditions facilitate fungal growth and its propagation; (2) growth can take place for prolonged periods, during which the fungus can complete all stages of its growth cycle; and (3) azoles are present in concentrations sufficient to select populations and combinations **(61)**. A composting technique under controlled aerobic conditions was shown to reduce the level of AR*Af*. Aerobic and high-temperature conditions during composting are efficient at eliminating pathogenic microorganisms including *A. fumigatus*, which does not survive at the high temperatures reached, usually >70°C. Asexual spores are unable to survive at temperatures higher than 60°C **(62)**, but if the compost has non-uniform conditions, it could become a hotspot for strains resistant to medical triazoles. In fact, the detection of AR*Af* strains cross-resistant to fungicides and genetically related to clinical isolates have been reported in compost samples from the Netherlands **(27)**. Aside from these findings, a recent study has postulated the aerosol patient-to-environment transmission of AR*Af* in chronically infected patients, who can be regarded as possible hotspots that facilitate its spread through an environmental route **(63)**.

After detection of the environmental AR*Af* strains first reported in the Netherlands and Italy **(64, 65)**, several other European countries and other continents have uncovered AR*Af* strains to triazoles. As a result, their existence has been recognized as an emergent microorganism threatening public health worldwide. That is true principally in the Netherlands, where it has a mortality rate of 88% in triazole-resistant IA cases. According to a report from 2015–2017, two-thirds of patients with triazole-resistant IA have no history of prior treatment with medical triazoles **(66)**; this finding underscores the high risk of environmental exposure to this pathogen. Moreover, 80% to 90% of those clinical AR*Af* strains harboring resistance mutations were found to be associated with the environmental route, and the number of cases of therapy failure subsequently tends to increase **(66)**. The Netherlands has an intensive ornamental plant production, providing approximately 60% of the world's supply of flower bulbs. Its trading companies account for 85% of the international trade in these agricultural products **(67)**. In this context, many investigations have been conducted in different countries to explain the connection between bulb production and the spread of *ARAf* through international trade, especially in the Netherlands and their major markets, which include China, the EU, Japan, the US, and the Scandinavian countries **(68)**. Global scattering of soil-associated fungi including *A. fumigatus* has been postulated. AR*Af* strains with TR_{34}/L98H and TR_{46}/ Y121F/T289A were detected from samples of plant bulbs imported from the Netherlands to Ireland **(69)**. Another investigation examined environmental samples in rural and urban zones from the UK and revealed that a higher prevalence of ARA*f* was obtained in urban city centers, especially flower beds and gardens, than in rural areas **(70)**. In France, a study was conducted in environments surrounding a hospital because of the notable increase of clinical cases of CF related to AR*Af* strains and the existence of ornamentation with outdoor flower beds **(71)**. Consequently, they isolated AR*Af* from tulip pots imported from the Netherlands and other environmental hotspots **(71)**. The results imply the risk involved when contaminated plants with

AR*Af* are in gardens of hospitals and at dwellings of immunocompromised patients; conditions become potential hotspot sources of infection. On the other side of the globe, AR*Af* strains (TR_{34}/L98H and TR_{46}/ Y121F/T289A) were isolated from plant bulbs imported from the Netherlands to Japan, suggesting intercountry transmission from Europe to Asia **(42)** (Figure 2). It is particularly interesting that a low frequency of AR*Af* isolation was reported in agricultural land in the UK; only two isolates were detected in samples collected from rural areas there **(70)**. Additionally, as described in an earlier report of a study from Japan, AR*Af* was not isolated from agricultural lands, where protectant azole fungicides had been used to prevent plant diseases **(29)**. All the above findings indicate that AR*Af* expands broadly through the cultivation of horticultural crops, principally vegetables and ornamentals such as flowers.

Microsatellite genotyping has been used widely to explore the population structure and the genetic relationship between AR*Af* strains through identification of identical Short Tandem Repeat (STR) patterns and close multilocus genotypes (MLGs) **(72–74)**. A genetic study examining a global collection of 4,049 *A. fumigatus* strains revealed the strains as divisible into two divergent clades and unevenly distributed the allele richness of AR*Af* harboring TR_{34}/L98H and TR_{46}/ Y121F/T289A across the two clades **(74)**. Furthermore, it showed that the genetic diversity of AR*Af* was lower than that of wild-type strains. Another study demonstrated that strains with mutations TR_{34}/L98H extend across many STR genotypes other than wild-type STR, and this might support the hypothesis of multiple origins of the mutation **(75)**. The multiple progenies might develop independently by azole selection in the environment or by sexual or parasexual recombination between an imported resistant strain and a domestic susceptible strain **(75)**. AR*Af* strains have been detected in soil samples from Denmark, India, Iran, Romania, and Tanzania, some of which were genetically related to Dutch isolates by microsatellite genotyping analysis **(47, 75–78)**. However, although researchers from China and the US have reported AR*Af* strains in soil

292 samples, they did not find genetic relatedness to isolates of the Netherlands **(66, 79–81)**.
293 Investigations have also been carried out in Latin American countries to determine the presence of
294 AR*Af* strains. Colombia is the second most prolific exporter of cut flowers in the world and an
295 important consumer of triazole fungicides. AR*Af* strains were isolated from soil samples of
296 Colombian flower fields, and all of them presented wide genotypic diversity **(43)**. Despite some
297 strains such as *A. fumigatus* NAN077 showing an almost identical genotype to a clinical isolate
298 from the Netherlands, no genetic relatedness was found between them **(43)**. Moreover,
299 environmental samplings conducted in Mexico and Peru revealed ARA*f* isolates harboring the
300 prevalent alleles TR_{34}/L98H and TR_{46}/Y121F/T289A that were genetically related to one another
301 **(54)** but not to the Dutch AR*Af* strains. Similar results have also been reported for other countries
302 from Asia, Africa, and Oceania, such as Australia, Burkina Faso, Kenya, Kuwait, New Zealand,
303 and Thailand, which are not genetically related or had an unknown genetic link to those AR*Af*
304 isolated in Netherlands **(49, 55, 82–85)** (Figure 2). Beyond the data which have been accumulated,
305 additional information related to the environmental dispersion of AR*Af*, including genetic
306 relationships, is necessary to produce a comprehensive global overview. Although STR has
307 superior discriminatory power to that of other developed typing techniques, the interpretation and
308 the validity of the data require certain assumptions (**74, 86)**. Whole-genome sequencing analysis
309 is expected to reveal single-nucleotide polymorphisms in each gene and to provide a map of
310 crossing-over because of parasexual recombination.

311 The extensive scale of international trade related to agricultural products and live animals
312 constitutes a route for global dissemination of emerging infectious diseases (EIDs). Most EIDs
313 derive from changes in the microbe-host-environment epidemiological triangle, with potentially
314 deleterious physical and social effects **(87)**. Therefore, elucidating the evolutionary history of

315 emerging pathogens is crucially important to ascertain shifts in organisms with potential virulence
316 (**88)**. Similar to the transboundary spread of ARA*f*, the etiological agent of chytridiomycosis
317 infectious diseases was found to be responsible for a decrease of amphibian species worldwide
318 through international trade in amphibians for food, pets, ornamental garden animals, scientific
319 research, and zoo animals, as well as when they are introduced or released into the environment
320 **(89)**. All of those conditions have played important roles in its dissemination. The link between
321 the emergent fungus *Batrachochytrium dendrobatidis* (Bd) and steadily declining amphibian
322 populations was reported in 1998 **(90)**. However, studies based on evolutionary history confirmed
323 the ancient existence of Bd and postulated that it originally came from southern Africa **(91).**
324 Furthermore, a wide phylogenetic diversity of Bd preceding the global declination of amphibians
325 was elucidated by genomic sequencing analysis of a global panel of isolates **(88)**. Therefore, Bd is
326 regarded as an endemic pathogen with a complex background of dynamic genetic evolution, while
327 the inter-regional spread of Bd is associated with the generation of a global panzootic lineage and
328 putatively endemic Bd lineages that comprise novel genetic variations **(88)**. Although the
329 transmission is established primarily by motile waterborne zoospores or through direct contact
330 with infected amphibians, Bd can also grow on sterile bird feathers, arthropod exoskeletons,
331 keratinous paw scales of waterfowl, and the gastrointestinal tracts of crayfish; all of these serve to
332 amplify the potential environmental reservoirs **(92)**. Detection of Bd has been reported in countries
333 from Africa, Asia, Europe, Middle East, North and South America. Moreover, all of them were
334 confirmed to be active nodes in the international trade of amphibians through multi-destination
335 marketing **(89)**. Aiming to evaluate the spread of chytridiomycosis infectious diseases and their
336 effects on wild and farmed amphibian populations, as well on the trade in live amphibians and its
337 products, the World Organization for Animal Health (OIE) formed a group on amphibian diseases
338 to report and advise the OIE Aquatic Animal Health Standards Commission about

emergent diseases **(89)**. Based on that group's findings, this pathogen was approved as an addition to the list of the Aquatic Animal Health Code (Aquatic Code). Reporting the detection of Bd to the competent veterinary authority and thence to the OIE is mandatory. Furthermore, member countries must report the presence or absence of each disease and guarantee disease surveillance programs **(89, 93)**.

Implementing effective measures to control AR*Af* is an urgent matter requiring sanitary safety of international trade processes by restoration of the contaminated environments, diagnosis and prevention of AR*Af* infection in azole-naïve patients, and development of new antifungals for AR*Af* infection. In this regard, some countries with evidence of international AR*Af* spreading have also evaluated possible strategies to control the *ad hoc* risk implied by plant bulbs contaminated with AR*Af* **(42, 94)**. French researchers investigated the change of AR*Af* distribution in plant pots placed in outdoor areas surrounding the hospital by changing the ornamental plants from bulbs treated with azole fungicides to organic agriculture **(94)**. Subsequent results showed a marked decrease in the percentage of azole-resistant isolates, from 71% in 2019 to less than 3% in 2020 **(94)**. The hazard posed to the hospital environment, where numerous patients at risk are hospitalized in the hematology intensive care unit (ICU), was reduced considerably **(94)**. Hagiwara proved the effectiveness of benomyl and prochloraz at eliminating AR*Af* **(42)**. These measures are expected to mitigate the spread of AR*Af* into naïve environments and to reduce the risk in occupied environments. In the last decade, molecular tools to detect AR*Af* have been developed extensively, some of which have been commercialized **(95, 96)**. Their further development and evaluation are expected to contribute to the control of AR*Af* infections. Although several antifungals have been available for aspergillosis, oral antifungal drugs are limited; therefore, new antifungal drugs other than azoles are anticipated. Surveillance data studies and networks of multidisciplinary research

362 are expected to be crucial to identify hotspots, improve diagnosis targets, and clarify epidemiologic
363 descriptions related to the extensive factors and numerous interested parties involved in the global
364 spread of AR*Af*.
365
366

Figure legends

Figure 1. Resistance selection routes of *A. fumigatus.*

Figure 2. AR*Af* reported in soil and plant bulb samples **(42, 43, 47–50, 54, 55, 57, 69, 70, 75–79, 81–85)**. The map was prepared using a web tool (https://n.freemap.jp/).

List of acronyms

ABC: ATP-binding cassette

AR*Af:* azole resistant *Aspergillus fumigatus*

Bd: *Batrachochytrium dendrobatidis*

bp: base pairs

COVID: Coronavirus disease

CF: Cystic fibrosis

DMI: demethylation inhibitor

EID: emerging infectious diseases

FDA: Food and Drug Administration

IA: invasive aspergillosis

ICU: intensive care unit

MDR: multidrug resistance

MFS: major facilitator superfamily

MIC: minimum inhibitory concentration

MLGs: multilocus genotypes

OIE: World Organization for Animal Health

SNPs: single-nucleotide polymorphisms

STR: Short Tandem Repeat

TR: tandem repeats

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