Emergence of Triazole Resistance in *Aspergillus* spp. in Latin America

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Abstract

Purpose of Review Azole resistance in *Aspergillus* spp. is becoming a public health problem worldwide. However, data about this subject is lacking in Latin American countries. This review focuses in the epidemiology and molecular mechanisms of azole resistance in *Aspergillus* spp. emphasizing in Latin America. Data on *Aspergillus fumigatus* stands out because it is the most prevalent *Aspergillus* spp. pathogen.

Recent Findings Azole resistance in *Aspergillus* spp. emergence was linked with intensive use of these antifungals both in the clinical setting and in the environment (as pesticides). Reports on azole-resistant *A. fumigatus* strains are being constantly published in different countries. Molecular mechanisms of resistance mainly involve substitution in the azole target (*CYP51A*) and/or overexpression of this gene. However, several other non-*CYP51A*-related mechanisms were described. Moreover, intrinsically resistant cryptic *Aspergillus* species are starting to be reported as human pathogens.

Summary After a comprehensive literature review, it is clear that azole resistance in *Aspergillus* spp. is emerging in Latin America and perhaps it is underestimated. All the main molecular mechanisms of azole resistance were described in patients and/or environmental samples. Moreover, one of the molecular mechanisms was described only in South America. Cryptic intrinsic azole-resistant species are also described.

Keywords Aspergillus · Triazole · Resistance · Latin America

Introduction: An Overview of Aspergillosis: Clinical Aspects, Importance, Prevalence, and Treatment Options

Aspergillosis is a group of opportunistic, heterogeneous, and non-contagious mycoses that have in common their causative agent, the species of the genus *Aspergillus*. These molds are saprophytic fungi that participate in the recycling of carbon and nitrogen. Their hydrophobic conidia can disperse and survive in different environments [1••, 2]. This genus includes more than 200 species, of which only some are human pathogens causing a diverse group of mycoses named aspergillosis

[3••]. The main agents of human aspergillosis are *A. fumigatus*, followed by *A. flavus*, *A. terreus*, *A. niger*, and *A. nidulans* [4, 5]. Recently, the spectrum of human pathogens within the genus was spanned including the so-called cryptic species of the sections Fumigati, Flavi, Terrei, and Nigri [3••, 6–8]. Despite this apparent diversity of causative agents, more than 70% of invasive aspergillosis is caused by *A. fumigatus*.

There are different clinical forms of aspergillosis and its presentation and importance depends, fundamentally, on factors related to the host. The most benign forms of infection are observed in immunocompetent patients, and the most severe forms with a worse prognosis, such as invasive aspergillosis and disseminated aspergillosis, occur in immunosuppressed patients. Other important groups of patients at risk of suffering aspergillosis are patients with chronic obstructive pulmonary diseases (especially cystic fibrosis patients and asthma) [4, 9–11]. A newer group of patients at risk of aspergillosis are severely ill Covid-19 and influenza patients (8–20% of the ICU admitted have co-infections with this viral pathogens) [12, 13•, 14].

The estimation of the burden of aspergillosis is difficult since these infections are not in the list of notifiable diseases

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in almost any country. However, a relatively good estimation can be made considering that the attack rate of *Aspergillus* spp. (invasive infections) in hematological population is at least 10%. Thus, considering only the worldwide cases of acute myeloid leukemia (around 120.000 new cases in 2017), a conservative estimation would be 12,000, but the real burden is higher since other type of patients are at risk [15–17].

The treatment options of these mycoses are limited. The 2016 ISDA practice guidelines for diagnosis and management of aspergillosis recommended triazoles as the preferred agent for treatment of invasive aspergillosis. Within these group of azoles, voriconazole (VRC) is recommended as the primary antifungal treatment. However, other triazoles as itraconazole (ITC), posaconazole (PSC), and isavuconazole (ISA) are also recommended [18••].

Known Molecular Mechanisms of Triazole Resistance in *Aspergillus* spp.

As with other microorganisms, resistance of filamentous fungi to antifungals is a broad concept that can be classified into clinical resistance and microbiological resistance. Microbiological resistance depends on the particular characteristics of the microorganism. The results obtained in the in vitro susceptibility tests to antifungals give an idea of this resistance. It that can be subdivided into (i) intrinsic resistance, which is that exhibited by all strains of the same species of a fungus and is not related to exposure to the antifungal, as in *Aspergillus* spp. and its resistance to fluconazole and (ii) secondary or acquired resistance, which is defined as the loss of activity of an antifungal agent that was once effective against a particular organism. These resistances develop after exposure to antifungal agents and are related to stable phenotypic and genotypic alterations. Azole resistance in *Aspergillus* spp. is an increasing worldwide problem and it is emerging in South America [19].

Azole antifungals target fungal sterol 14-demethylases, key enzymes in the biosynthetic pathway of ergosterol. These enzymes are encoded in *Aspergillus* spp. by two paralog genes named *CYP51A* and *CYP51B* [20••]. Molecular mechanisms of triazole resistance in *Aspergillus* spp. can be crowded into to two groups named *CYP51A*-related resistance and non-*CYP51A*-related resistance [21•]. Intrinsic fluconazole resistance is a common characteristic for all *Aspergillus* spp. and it was linked exclusively with *CYP51A*-related mechanism (More details below) [22•]. Other triazole intrinsic resistance is seen in some of the cryptic species of the section Fumigati, Flavi, and Terrei [18••, 23–25]. As an example, *A. lentulus* (cryptic Fumigati species) intrinsic triazole resistance was also linked with *CYP51A* sequence [26]. Turning to the secondary triazole resistance mechanisms, both *CYP51A* related and non-*CYP51A* related were described. However, the first group of mechanisms is the most prevalent worldwide [19, 21•]. In the following points of this review, we will be focusing on the particular mechanisms of azole resistance in *Aspergillus* spp. but as a partial conclusion, we can state that both intrinsic and secondary triazole resistance can be explained by similar molecular mechanism mostly linked with *CYP51A* substitutions.

Mechanisms of Intrinsic Resistance in *Aspergillus* spp.

All *Aspergillus* spp. are intrinsically resistant to fluconazole (FLC). The mechanism underlying this phenotype has been molecularly studied in *A. fumigatus*. This phenotype is related to a single nucleotide polymorphism in *CYP51A* sequence. A natural polymorphism at residue 301 isoleucine (I301) in *A. fumigatus* Cyp51Ap was confirmed to be implicated in it FLC-resistance since when its substitution by threonine (I301T) led to a 32-fold decrease in FLC MIC [22•]. This residue is a threonine in FLC susceptible wild type *Candida albicans* (T315 at Erg11p) and alanine (T315A) in FLC resistant strains (acquired resistance) [27, 28].

Non-*A. fumigatus Aspergillus* species may exhibit primary resistance to other antifungal drugs currently used for treatment and prophylaxis of aspergillosis, as they show high minimal inhibitory concentrations (MICs) when compared to *A. fumigatus* [18••, 23–25]. Sibling species of *A. fumigatus* in section *Fumigati* may be intrinsically resistant to one or more antifungals. High MIC values for amphotericin B (AMB) and azoles are being reported for *A. fumigatiaffinis*, *A. viridinutans*, and *A. pseudofischeri*, while *A. udagawae* shows reduced susceptibility to AMB and VRC. *A. lentulus* is referred as a multidrug-resistant species as all the strains of this species exhibit AMB, azole, and echinocandin reduced susceptibility [24, 25, 29–31]. The implicated mechanism of azole resistance in this species was strictly linked with its *CYP51A* gene sequence since when its *CYP51A* was transformed into an *A. fumigatus* strain, the recipient strain mimicked exactly the triazole resistance phenotype of *A. lentulus* [26].

Primary AMB resistance was described in species of the section *Terrei*, and it was supported by clinical and animal studies. *A. terreus* and cryptic species such as *A. citrinoterreus*, *A. hortai*, and *A. alabamensis* exhibit high AMB MICs, but are susceptible to azoles [32–34]. AMB resistance has been associated with stress response pathways, increased catalase production, and low membrane ergosterol content [35]. Sections *Flavi* and *Nidulantes* also show reduced susceptibility to AMB [36–39]. Cases of treatment failure have been reported with this polyene against *A. flavus* and its cryptic species, *A. alliaceus* [40].

A. niger sensu stricto and *A. tubingensis* (section *Nigri*) exhibit variable susceptibility patterns, with reduced susceptibilities to azoles [41–43]. Finally, multidrug-resistance is

exhibited by the species of the section *Usti*. *A. ustus* and *A. calidoustus* are intrinsically resistant to the triazoles; they show high MIC values for AMB and variable results when tested against caspofungin [43, 44]. Molecular mechanisms underlying these susceptibility profiles in particular remain unknown.

Mechanisms of Secondary Resistance in *A. fumigatus* and in Other *Aspergillus* spp.

A. fumigatus is normally susceptible to azoles drugs; however, since the first case of azole-resistance in 1997, secondary resistance has been increasingly reported around the world, becoming a worldwide problem [19]. The development of this type of resistance occurs after azole exposure in two possible ways named the patient and the environmental route. The patient route involves the selection of resistant strains after long-term azole treatment in patients with aspergillosis. The environmental route is related to azole-resistant *A. fumigatus* selected after exposure to azole antifungals used in agriculture [19]. As described earlier, the mechanisms of azole resistance in *A. fumigatus* can be divided into two main groups of mechanisms, those related with *CYP51A* alterations (CYP51A dependent) and those related with other genes (*CYP51A* independent).

The most prevalent azole resistance mechanisms in *A. fumigatus* involve mutations in the *CYP51A* gene, which encode the protein 14α sterol demethylase, the target of azole drugs [45]. Mutations in *CYP51A* gene include single-nucleotide polymorphisms (SNPs), tandem repeats in the promoter, or both. The SNPs at the Cyp51Ap that affect the affinity of the enzyme to azoles are mostly located close to the opening of one of the two ligand access channels of the protein [46]. The most frequent SNPs are those involving the residues G54, G138, G448, and M220, associated with cross-resistance to ITC and POS, resistance to ITC and VRC, VRC resistance, and different patterns of triazole resistance, respectively [21•]. Other less common SNPs, such as P216L, F219C, F219I, A284T, Y431C, G432S, and G434C, have also been occasionally reported related to azole resistance [47]. All of these point mutations are generally described in strains isolated from patients that have been undergoing azole treatment [48, 49]. These mutations were grouped based on their location in three regions named hot spot 1 (includes G54 and G138), hot spot 2 (P216, F219, and M220), and hot spot 3 (Y431, G432, and G434) [21•] (Fig. 1).

The other group of *CYP51A* mutations are the tandem repeats in the promoter, which lead to overexpression of the gene and the subsequent development of pan-azole resistance. Tandem repeats of 34 and 46 bp are the most frequently reported and consists in duplications of 34 and 46 bp in the *CYP51A* promoter, respectively [21•]. The TR34 is always in combination with the L98H substitution

conferring high level of resistance to ITC and elevated MIC values to the other triazoles [50], while TR46 has been found together with the Y121F/T289A substitutions in isolates highly resistant to VRC with simultaneous increase of azole MIC [51]. Both tandem repeated promoter sequences and both amino acid substitutions (L98H and Y121F/T289A) alone do not alter the MIC values. These facts were molecularly confirmed [50, 51] (Fig. 1). In addition, a 53-bp repeat has been found in isolates that show cross-resistance to VRC and ITC, without any other alterations in Cyp51Ap [52]. Recently, a novel combination of *CYP51A* mutations was reported in *A. fumigatus* isolated from an Argentinian cystic fibrosis patient. This newly described mutation combination consists of TR34-R65K-L98H and confers pan-azole resistance [53]. These resistance mechanisms involving tandem repeats at *CYP51A* gene 5′UTR or promoter are mainly found in environmental *A. fumigatus* strains [54–58] (Fig. 1).

On the other hand, a few *CYP51A*-independent mechanisms have been reported in *A. fumigatus* to contribute to clinical azole resistance, including overexpression of efflux pumps, *CYP51B* overexpression, cholesterol import, HapE mutations, and mutations in 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase-encoding gene, *HMG1*. Among them, the most important is the overexpression of efflux pumps, specifically ABC and MFS transporters, associated with azole resistance by reduction of intracellular concentration of azoles [59–61]. *CYP51B*-mediated azole resistance is rare, as for October 2020, only one study reported a clinical azole-resistant isolate showing an overexpression of *CYP51B* [60]. Another described mechanism is the overexpression of SrbA, a sterol-regulatory element binding protein that increases FLC and VRC resistance by *CYP51A* upregulation [62, 63]. Also, it was seen that after azole exposure, a P88L substitution in the HapE, a CCAAT-binding transcription factor, confers resistance to azoles. This has been described as another resistance mechanism and was molecularly proven [64]. One of the latest described molecular mechanisms of azole resistance in *A. fumigatus* is the presence of mutations at the *HMG1* gene that encodes an enzyme that participate in the earlier steps (pre-squalene) of ergosterol biosynthesis [65••].

Regarding non-*fumigatus* species, reports identifying mechanism of secondary azole resistance are limited. As in *A. fumigatus*, most of these reports described substitutions in Cyp51 proteins. Such is the case of *A. flavus* and the Y319H substitution in the Cyp51C protein [66] and several other substitutions in its Cyp51A and Cyp51B proteins [67]. In the same way, the linkage between *A. lentulus* azole resistance and its *CYP51A* was molecularly demonstrated [26] together with other single substitutions at *A. terreus*, *A. niger*, and *A. tubingensis* Cyp51A [21•].
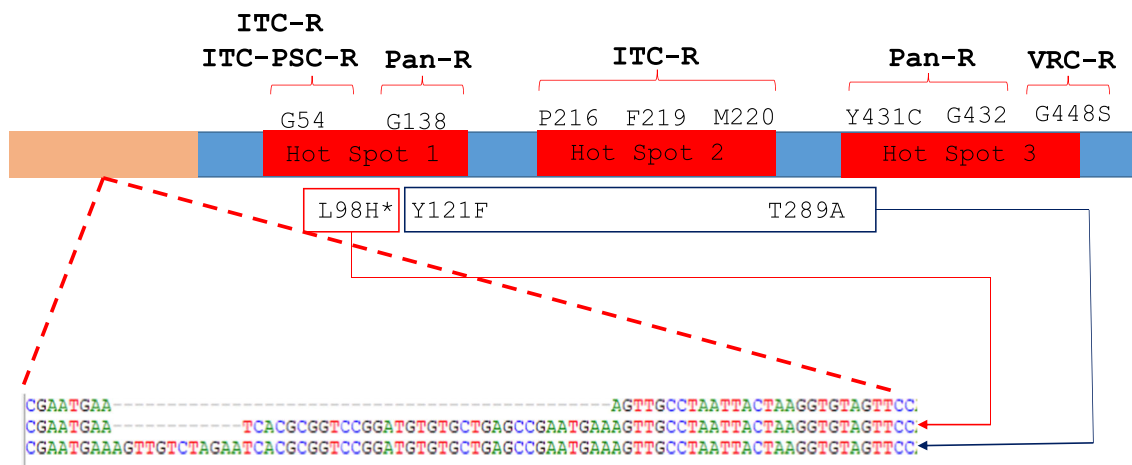
Fig. 1 CYP51A-related azole resistance mechanisms. The named hot spots are based on the names suggested by Dudakova et al. (ref). ITC, itraconazole; PSC, posaconazole; VRC, voriconazole; Pan-R, pan-azole resistance; ITC-R, resistance to itraconazole alone; ITC-PSC-R, cross itraconazole-posaconazole resistance; VRC-R, voriconazole resistance. *L98H was described to be either alone or together with other substitutions

Worldwide Resistance to Triazole: Epidemiology, Prevalence, and Most Affected Regions

Azole-resistant *Aspergillus* spp. isolates went from being mere anecdotes in the late twentieth century to being a public health problem in 2020 [48, 50, 68]. Europe is currently the focal point of the problem since resistant isolates were reported in most countries and it is the continent that shows the highest prevalence. However, azole resistance is being described worldwide [19]. The first isolates showed mutations that were thought to be geographically restricted as TR34/L98H and TR46/Y121F/T289A (the Netherlands) [50, 51, 68] and different non-TR Cyp51Ap substitutions (UK) [48]. It was rapidly clear that almost all mechanisms were worldwide distributed. Point mutations at G54, G138, and M220 residues were reported in different countries but TR-mutants (TR34/L98H and TR46/Y121F/T289A) are the most commonly described *CYP51A*-related mechanisms of resistance in clinical settings [19]. Non-CYP51A-related mechanisms are being increasingly reported. The proportion of these isolated compared with *CYP51A* mutants varies depending on the analyzed study. As examples, we can describe the report of 6.5% of resistant *A. fumigatus* isolates with no *CYP51A* mutations in the UK [48], 25% in a US study [69], and 44% reported in Germany [70].

The prevalence of triazole resistance in *Aspergillus* spp. in the clinical setting is difficult to establish since it is not mandatory to report these infections in almost any country. Few international surveillance studies were performed. Van der Linden et al. reported a 3.2% prevalence of resistance (3788 strains from 22 hospitals of 19 countries) [71]. Data on resistance prevalence usually came from individual center describing one population (e.g., resistance prevalence in cystic fibrosis patients) [72, 73]. In addition, differences on the diagnostics have to be taken into account. Some health professionals rely on non-culture-based tools (mainly galactomannan detection) for invasive aspergillosis diagnostics. Thus, no culture is obtained, and as a consequence, no resistance prevalence can be determined [74]. The conclusions obtained from published reports on this topic depend on where the study was carried out and ranged from considering that resistance is a serious problem to that it is a minimal or emerging problem. Most report from clinical setting in Europe stated the first idea. In two hospitals from Germany, resistant *A. fumigatus* strains were isolated in 29.6% of the hematopoietic stem cell transplantation patients [75], while in the UK, prevalence was similar (28%) [76]. Similarly, >20% resistance was described in the Netherlands in high-risk patients [77], whereas in Spanish [78] and Dutch [79] general population, this prevalence seems to be as low as 1.8% and 4%, respectively. On the other hand, in other continents, azole resistance prevalence in high-risk patients was lower: 1.7% in India [80], 0.6–11.8% in the USA [69, 81], 2.6% in Australia [82], and 1.8% in Brazil [83]. These differences in azole resistance frequencies can be skewed due to studied populations, center complexity, and even partly due to the used denominator. Moreover, azole treatment seems to have little influence in the resistance development since most resistance is believed to be acquired in the environment (-TR-mediated resistance) and azole-naïve patients may be infected by a resistant isolate. Taking this panorama into account, Dutch experts recommended to established the local resistance rates (hospital and environmental) and use the standard empirical treatment (VRC) if the resistance rate is lower than 10%. If this prevalence is higher, VRC treatment should be revised [84].

Azole resistance prevalence seems to be high in cystic fibrosis patients due to the extensive use of azole antifungals. In

a French cohort, 20% of the cystic fibrosis exposed to ITC showed azole-resistant *A. fumigatus* in their sputum samples [85], while in Argentina, this percentage was 8% [72], and in Germany, resistant strains were isolated in 2.71% of the patients (TR34/L98H allele was the most frequent followed by M220L and TR46/Y121F/T289A) [73].

Epidemiology of Triazole Resistance in *A. fumigatus* in Latin American Countries

As in other continents, reporting of fungal diseases in Latin America is not mandatory. As for October 2020, there are no reports of triazole resistance published in www.pubmed.gov from Bolivia, Chile, Ecuador, Guyana, Paraguay, and Uruguay. Thus the following paragraphs will include data limited to Argentinian, Brazilian, Colombian, and Peruvian reports (Fig. 2).
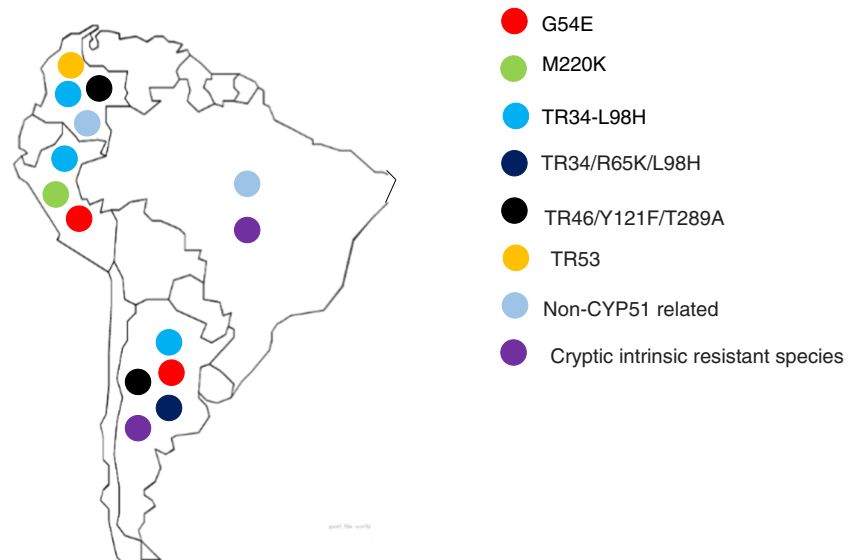
In Argentina, three retrospective surveys on fungal infections were conducted in 2004, 2008, and 2010, and one prospective surveillance between 2010 and 2012 [86, 87]. Moreover, in 2018, invasive aspergillosis and chronic pulmonary and allergic bronchopulmonary aspergillosis rates were calculated [88]. All studies agreed that *A. fumigatus* is the most predominant species causing invasive mold disease. Susceptibility testing of the isolates showed low azole MIC values [86, 87]. In a 2011 study, 23 isolates of the section *Fumigati* were characterized and three were ITC resistant (MIC >16 μg/ml), but no further characterization was done [89]. In a report published in 2019, an 8.1% ITC resistance rate was described in a single Argentinian institution [90•]. However, no nationwide epidemiology studies on antifungal resistance in clinical settings were carried out. Yet, some specific case reports were published. In 2017, the first *A. fumigatus* strain harboring a G54E substitution (ITC MIC >16 μg/ml) in

Latin America was reported in corneal lesion produced by an accidental inoculation of a vegetal detritus of the azole naive patient. This patient was treated with natamycin drops resolving the infection [91]. In 2018, the first South American isolate harboring a TR46/Y121F/T289A allele was described. This strain in particular was isolated in 2009 but reported in 2018. It was isolated from a brain biopsy in an acute lymphoblastic leukemia patient. Microbiological cure was achieved after the recuperation of the patient's immune status and antifungal treatment with AMB combined with caspofungin and ITC [92]. Finally, it was recently reported a case where three different *A. fumigatus* strains were isolated from a cystic fibrosis patient receiving long-term triazole therapy. Strains harbored the resistance allele TR34-L98H-S297T, which confers pan-azole resistance. In addition, one of the strains also carried a R65K substitution in Cyp51Ap. This new mutation was confirmed to increase MIC values for all triazoles [53]. These strains were reported again as part of a study designed to report the incidence of triazole resistance in cystic fibrosis patients in Argentina [72]. In a recent report, triazole resistance in *A. flavus* was reported in clinical and environmental Argentinian strains linked with *CYP51C* mutations [93].

Reports on triazole resistance in *Aspergillus* spp. from Brazil are scarce. Negri et al. affirmed in 2017 that azole resistance was not emerging in Brazil, but these authors reported under that statement that they found 1.8% of the *A. fumigatus* isolated from invasive aspergillosis have VRC MICs above the epidemiological cut off values [83].

In a prospective descriptive study performed in 2019 which includes 143 *A. fumigatus sensu stricto* strains isolated in two tertiary hospital from Peru, triazole resistance prevalence was 2.09% (*n*=3). The isolates harbored different mutations including M220K, G54E, and TR34-L98H. The resistant strains were isolated from patients diagnosed

Fig. 2 Distribution of the described azole resistant strains in South America

- G54E
- M220K
- TR34-L98H
- TR34/R65K/L98H
- TR46/Y121F/T289A
- TR53
- Non-CYP51 related
- Cryptic intrinsic resistant species

Table 1 Molecular mechanisms of azole resistant in *A. fumigatus* described in South America

Cyp51A aa. Substitutions/ Cryptic specie	Country of isolation	Patient/origin	Sample	n	MIC (μg/ml)				Ref
					ITC	VRC	PSC	ISA	
G54E	Argentina	Azole naïve	Cornea	1	>8.00	0.25	0.12	ND	[91]
G54E	Peru	Itraconazole exposure/chronic pulmonary aspergillosis	Undefined respiratory sample	1	>16	0.50	0.06	ND	[101]
M220K	Peru	Itraconazole exposure/chronic pulmonary aspergillosis	Undefined respiratory sample	1	>16	1.00	1.00	ND	[101]
TR34-L98H	Argentina	Itraconazole/exposure Cystic fibrosis	Sputum	2	>16.00	8.00	4.00	4.00	[53]
TR34/L98H	Colombia	Flower fields	Soil	1	Grew [a]	Did not grew [a]	ND	ND	[56]
TR34/L98H	Colombia	Vegetable fields	Soil	1	>8.00	8.00	ND	ND	[95]
TR34/L98H	Peru	Itraconazole exposure/chronic pulmonary aspergillosis	Undefined respiratory sample	1	>16	2.00	0.50	ND	[101]
TR34/L98H	Brazil	Strain collection	Sputum	2	>8.00	2.00	1.00	ND	[100]
TR34/R65K/L98H	Argentina	Itraconazole exposure/cystic fibrosis	Sputum	1	>16.00	2.00	1.00	1.00	[53]
TR46/Y121F/T289A	Argentina	Voriconazole exposure/acute lymphoblastic leukemia	Brain biopsy	1	8.00	>16.00	0.50	ND	[92]
TR46/Y121F/T289A	Colombia	Flower fields	Soil	17	Grew [a]	Grew [a]	ND	ND	[56]
TR46/Y121F/T289A	Colombia	Vegetable fields	Soil	8	1.00	>16	ND	ND	[95]
TR53	Colombia	Flower fields	Soil	2	Grew [a]	Did not grew [a]	ND	ND	[56]
TR53	Colombia	Vegetable fields	Soil	1	>8.00	4.00	ND	ND	[95]
Wild type	Brazil	Strain collection	Undefined	4	ND	2.00	ND	ND	[83]
Wild type	Colombia	Flower fields	Soil	1	Grew [a]	Did not grew [a]	ND	ND	[56]
A. calidoustus	Brazil	Clinical sample	Undefined	1	>32	4.00	4.00	ND	[44]
A. ochraceous	Brazil	Clinical sample	Undefined	1	4.00	1.00	0.50	ND	[44]
A. thermomutatus	Brazil	Clinical sample	Undefined	1	2.00	16.00	0.50	ND	[44]
A. lentulus	Brazil	Kidney transplantation	Respiratory	1	2.00	4.00	ND	ND	[99]
A. lentulus	Argentina	Kidney transplantation	Respiratory	1	ND	2.00	ND	ND	[97]

MIC minimal inhibitory concentration; *ITC* itraconazole; *VRC* voriconazole; *PSC* posaconazole; *ISA* isavuconazole; *MCZ* miconazole; *ND* no data available

[a] No antifungal susceptibility testing assays were performed. These strains were isolated in ITC- and VRC-containing agar plates and were classified as able or not able to growth in those plates

with chronic pulmonary aspergillosis, and those harboring M220K and G54K substitutions in its Cyp51Ap were exposed to long-term ITC treatment (>300 days) [101].

In Colombia, several *A. fumigatus sensu stricto* strains were isolated from soil of flower fields. Authors demonstrated that all the isolated harbor promoter alterations (-TR) including 17 strains with TR46/Y121F/T289A allele, 2 strains with the TR53 promoter alteration, and 1 strain with the TR34/L98H [56]. The same data was published by the same authors 1 year later [94]. In 2019, the same group described the isolation of 18 resistant mutants in vegetable fields. Eight of the strains harbor TR46/Y121F/t289A, 1 TR34/L98H, and 1 TR53. The other 8 strains showed no *CYP51A* mutations but no further studies were done [95].

Intrinsic-Resistant Species Distribution in Latin America (Clinical Reports and Environmental)

Some pathogen *Aspergillus* cryptic species show high triazole MIC values suggesting that they are intrinsic resistant to some of these drugs [96]. The molecular confirmation of this statement was reported by Mellado et al. for *A. lentulus* (section Fumigati) [26]. Thus, it would be useful to know the prevalence of these species in Latin America both in the environment and in the clinical setting.

Data about this important issue in Latin America is scarce, and as for *Aspergillus* spp. triazole resistance, few reports were published. Using "cryptic *Aspergillus*" plus the name

of the country to search in different reference data bases (Scopus, PubMed, Google Scholar), we only found papers published by Argentinian and Brazilian groups regarding cryptic species with high azole MIC values.

In Argentina, different species of the Fumigati section were isolated in clinical and environmental samples, including *A. lentulus*, *A. udagawae*, and *A. fumigatiffinis*. In 2009, Montenegro et al. reported the first Argentinian isolation of *A. lentulus* from a patient with probable invasive aspergillosis and the first Argentinian case of simultaneous infection by *A. fumigatus* and *A. lentulus* [97]. More recently, Giusiano et al. evaluated 17 soil samples from two Argentinian semi-desert areas having different geological characteristics. *A. fumigatus sensu stricto* and *A. fumigatiaffinis* were the most frequent species, followed by isolates closely related to *A. felis* and *A. udagawae* [98].

In Brazil, an analysis of the distribution of cryptic *Aspergillus* spp. was done analyzing samples from 133 patients of 12 different medical centers. Only three of the several cryptic species described in this report showed high azole MIC values (one *A. thermomutatus* of the section Fumigati, one *A. ochraceous* of the section Circumdati and one *A. calidoustus* of the section Usti) [44] (Table 1). In 2014 in Sao Paulo, a kidney transplant patient was diagnosed with a pulmonary invasive aspergillosis due to a *A. lentulus* strain showing high azole MICs [99](Table 1).

Conclusions and Research Gaps

After analyzing the data presented in this review, it is clear that azole resistance is emerging in Latin America and that it is an issue that needs to be extensively studied. Comprehensive molecular identification of clinical strains has to be performed in order to uncover the prevalence of potentially intrinsically resistant Aspergillus cryptic species in the clinical setting and in the environmental. Moreover, *A. fumigatus* harboring –TR resistance mechanisms linked with environmental route of resistance acquisition were described in different countries of South America. However, few data is available considering that the agriculture pesticides are increasingly used in the whole continent and prolonged azole therapies are being prescribed in South American patients.

We consider that epidemiological studies including antifungal resistance surveillance are needed in our region in order to improve patient management.

Declarations

Conflict of Interest   Macedo Daiana, Leonardelli Florencia, Gamarra Soledad, and Garcia-Effron Guillermo declare that they have no conflict of interest.

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

Refereences

Papers of particular interest, published recently, have been highlighted as:
• Of importance
•• Of major importance

1.•• Latgé, J.P., Chamilos, G. *Aspergillus fumigatus* and aspergillosis in 2019. *Clin. Microbiol. Rev.* 2020; *33*: doi:https://doi.org/10.1128/CMR.00140-18. **A good review written by one of the most renowned researcher on *Aspergillus fumigatus*. It includes a comprehensive and actual description of the biological cycle; spectrum of disease, immune response, virulence, diagnostic tools and antifungal treatments. In adition new concepts are described.**

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