Emerging threat of triazole-resistant *Aspergillus fumigatus*

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Invasive aspergillosis is a leading cause of morbidity and mortality among immunocompromised populations and is predicted to cause more than 200 000 life-threatening infections each year. *Aspergillus fumigatus* is the most prevalent pathogen isolated from patients with invasive aspergillosis, accounting for more than 60% of all cases. Currently, the only antifungal agents available with consistent activity against *A. fumigatus* are the mould-active triazoles and amphotericin B, of which the triazoles commonly represent both front-line and salvage therapeutic options. Unfortunately, the treatment of infections caused by *A. fumigatus* has recently been further complicated by the global emergence of triazole resistance among both clinical and environmental isolates. Mutations in the *A. fumigatus* sterol-demethylase gene *cyp51A*, overexpression of *cyp51A* and overexpression of efflux pump genes are all known to contribute to resistance, yet much of the triazole resistance among *A. fumigatus* still remains unexplained. Also lacking is clinical experience with therapeutic options for the treatment of triazole-resistant *A. fumigatus* infections and mortality associated with these infections remains unacceptably high. Thus, further research is greatly needed to both better understand the emerging threat of triazole-resistant *A. fumigatus* and to develop novel therapeutic strategies to combat these resistant infections.

Introduction

Invasive aspergillosis is a leading cause of morbidity and mortality among immunocompromised patient populations, such as patients receiving solid organ or stem cell transplants, patients with haematological malignancies and those taking immunosuppressive agents.¹ *Aspergillus*, the causative agent of invasive aspergillosis, is a genus of septate, saprophytic mould containing hundreds of identified species. Of these, only *Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus terreus* and *Aspergillus niger* commonly cause disease in humans, with *A. fumigatus* representing more than 60% of all clinical isolates.² Worldwide, invasive aspergillosis is conservatively estimated to cause more than 200 000 life-threatening infections each year.^{1,2} Mortality rates range considerably, from 30% to 90%, varying by patient population as well as severity and duration of immunosuppression.^{3–5} Unfortunately, the treatment of infections caused by *A. fumigatus* has recently been further complicated by the global emergence of triazole resistance among both clinical and environmental isolates.^{6–9}

Mould-active triazoles

Since the widespread introduction of itraconazole to clinical practice in 1992, the mould-active triazole antifungals have been utilized as both front-line and salvage therapeutic agents for the treatment of invasive aspergillosis. Voriconazole is now considered the therapy of choice and is available in both intravenous

and oral formulations.¹⁰ Multiple studies comparing the efficacy of voriconazole with that of the previous standard of care, amphotericin B, have found voriconazole to be more efficacious and generally safer than either conventional (deoxycholate) or lipid formulations of amphotericin B.^{11,12} Isavuconazole was recently approved for the treatment of invasive aspergillosis, after demonstrating non-inferiority to the standard of care, voriconazole.^{4,10} Isavuconazole, as the prodrug isavuconazonium sulphate, is available in both intravenous and oral formulations.¹³ Posaconazole, often used as prophylaxis against invasive fungal infections in high-risk patients such as those with AML, is also employed as second-line or salvage therapy for invasive aspergillosis and is now available in both intravenous and delayed-release tablet formulations. Itraconazole is still used commonly worldwide, but its use in invasive aspergillosis has decreased owing to both lower anti-*aspergillus* potency and the withdrawal of intravenous formulations from some markets.¹⁰ Although all these agents possess a triazole active moiety (from which this class of agents gained its name), they can be grouped into two distinct pharmacophores (Figure 1). The compact triazoles such as voriconazole and isavuconazole consist of a triazole ring linked to aromatic groups such as difluorobenzene via a four-carbon backbone, while the extended side-chain triazoles, including itraconazole and posaconazole, possess both a halogenated phenyl ring as well as a long lipophilic side chain consisting of five- and six-membered aromatic rings terminating in a triazolone group. The structural distinctions between these agents are thought to contribute to differences in the stabilizing interactions with sterol demethylase enzymes.

Figure 1. Structures of the clinically relevant triazoles.

Mechanism of action of the triazoles

In fungi, ergosterol is a key membrane lipid similar to mammalian cholesterol. Under normal growth conditions, ergosterol is plentiful in the fungal cell membrane and contributes to membrane integrity and fluidity, as well as the appropriate positioning of fungal proteins within the membrane. The triazoles exert antifungal activity by blocking a key enzymatic step in the biosynthesis of ergosterol. Through direct interaction between *N*4 of the triazole ring and an iron atom present in the catalytic haem group, the triazoles competitively inhibit sterol-demethylase enzymes (Figure 2). Inhibition of sterol-demethylases, such as Cyp51A and Cyp51B in the case of *A. fumigatus*, leads to the diversion of ergosterol precursors from the normal biosynthetic pathway and accumulation of alternative sterols including toxic 14 α -methylated sterols. It is believed that this depletion of ergosterol and buildup of 14 α -methylated sterols ultimately leads to inhibition of fungal growth.¹⁴

A. fumigatus possesses two genes, *cyp51A* and *cyp51B*, that encode homologous but distinct sterol-demethylase enzymes. These genes share ~60% sequence identity; however, the triazole antifungals have been observed to have significantly lower binding affinity for the Cyp51A enzyme.^{15,16} This reduced binding affinity is due in large part to the threonine amino acid present at position 301 (T301) of Cyp51A, which replaces the more common isoleucine residue present at this position in other fungal sterol demethylase enzymes (including *A. fumigatus* Cyp51B).¹⁷ The intrinsic fluconazole resistance of *A. fumigatus* appears to be a result of this single residue substitution. Surveillance studies of the potency of the mould-active triazoles against *A. fumigatus* clinical isolates have found isavuconazole, itraconazole, posaconazole and voriconazole to all possess clinically relevant activity against *A. fumigatus*.¹⁸ In general, the MICs of isavuconazole and itraconazole are higher, with MIC₅₀ and MIC₉₀ values of 1 and 2 mg/L and 1 and 1 mg/L, respectively (range: 0.128–8 mg/L, isavuconazole; and 0.25–4 mg/L, itraconazole), whereas the MIC₅₀ and MIC₉₀ values of posaconazole and voriconazole are typically lower, at 0.25 and 0.5 mg/L for both agents (range: 0.06–1 mg/L, posaconazole; and 0.125–2 mg/L, voriconazole).¹⁸

The antifungal activity of the triazoles is concentration dependent. This activity has been shown to be best predicted by the

*f*AUC:MIC ratio.^{19,20} In neutropenic murine models of pulmonary aspergillosis, the *f*AUC:MIC needed to produce a 90% reduction in fungal burden (1-log reduction) was found to be roughly twice that of stasis for both posaconazole and isavuconazole. This was true for triazole-susceptible and triazole-resistant isolates.^{19,20} Additionally, the triazoles possess fungicidal activity at physiologically relevant concentrations against most triazole-susceptible *A. fumigatus*.²¹ However, this fungicidal activity is also concentration dependent and varies from isolate to isolate.²² Unlike amphotericin B, the mould-active triazoles do not possess a clinically relevant post-antifungal effect.²³

Mechanisms of triazole resistance

Considerable research has led to the identification of multiple mechanisms of triazole resistance among pathogenic fungi other than *A. fumigatus*.²⁴ The most predominant mechanisms responsible for clinically encountered triazole resistance can be grouped into three distinct categories: (i) mutations in the sterol-demethylase gene; (ii) overexpression of the sterol-demethylase gene; and (iii) overexpression of either ATP-binding cassette (ABC) or major facilitator superfamily (MFS) type drug efflux pumps.²⁴ Although relatively less is understood of the mechanisms of clinical triazole resistance among *A. fumigatus*, much of what is known focuses on these three paths to triazole resistance (Figure 2).^{9,25,26}

Mutations in the *A. fumigatus* sterol-demethylase gene *cyp51A*

The most commonly identified mechanism of triazole resistance among clinical isolates of *A. fumigatus* involves mutations in the sterol-demethylase gene, *cyp51A*.^{8,27} A great number of these mutations have been identified among both clinical and environmental isolates originating from across the globe, many of which are uniquely found in triazole-resistant isolates (Table 1).^{8,28,29} A smaller subset of these mutations has been shown to directly contribute to triazole resistance through genetic manipulations in the laboratory setting (Table 2).^{28,30–36} Conversely, no mutations that are associated with clinical triazole resistance have been found in the *cyp51B* sterol-demethylase gene to date.

Figure 2. Canonical mechanisms of triazole resistance in *A. fumigatus*. The most commonly identified mechanisms of triazole resistance in clinical isolates of *A. fumigatus*: mutations in the sterol-demethylase gene *cyp51A*; overexpression of *cyp51A*; and overexpression of drug efflux pumps.

Mutations in *cyp51A* associated with triazole resistance are believed to result in amino acid substitutions, which either diminish the binding affinity of triazoles with the sterol-demethylase enzyme or alter the enzyme structure in a manner that favours native substrate conversion and thus ergosterol biosynthesis.^{28,30–36} The majority of the known resistance-associated mutations can be grouped into distinct regions of the sterol-demethylase enzyme. The most common and best understood of these encode amino acid substitutions occurring along the ligand access channel (residues G54, P216, F219 and M220) or in residues that are located near the catalytic site (Y121 and G138).¹⁵ The ligand access channel-associated residue substitutions have been shown to greatly decrease *A. fumigatus* susceptibility to both itraconazole and posaconazole, while having relatively less impact on voriconazole and isavuconazole susceptibilities.^{30,31,36} It is believed that this agent-specific triazole resistance is a consequence of these amino acid substitutions interfering with stabilizing interactions between the long hydrophobic side chains present in itraconazole and posaconazole, but absent in the other two agents. Conversely, a mutation conferring a tyrosine to phenylalanine substitution at position 121 (Y121F) has been shown to greatly decrease voriconazole susceptibility while having minimal impact on itraconazole or posaconazole.^{32,34} In X-ray crystallography experiments voriconazole has been observed to directly interact with tyrosine 121, though it is unknown if any of the other triazoles also interact with this residue.¹⁵

In addition to mutations in the ORF of the *cyp51A* gene, sequence duplications forming a tandem repeat in the promoter region of *cyp51A* have also been observed.³⁷ These tandem repeats do not alter the Cyp51A enzyme itself, but significantly increase the level of *cyp51A* gene expression and in turn the amount of Cyp51A enzyme that must be inhibited by the triazoles to block

ergosterol biosynthesis.²⁶ The impact of these tandem repeat-associated mutations on triazole resistance will be further detailed in the subsequent section.

Overexpression of *cyp51A*

The expression of *cyp51A* and *cyp51B* is regulated by a complex system of interacting transcription factors controlled by environmental conditions such as nutrient availability and stress. Although differences are known to exist in the regulation of expression of the two sterol-demethylase genes, the majority of research has focused on *cyp51A*, which when overexpressed is known to contribute to clinical triazole resistance in *A. fumigatus*.^{9,26,34,35} It remains unknown if overexpression of *cyp51B* may also contribute to clinical resistance, but triazole-resistant clinical isolates of *A. fumigatus* have been observed to demonstrate increased expression of *cyp51B*.^{38,39}

The positive regulation of *cyp51A* expression is controlled by the sterol regulatory element binding protein SrbA, which binds to the promoter region of *cyp51A* at two specific binding sites as a homodimer (Figure 3a). One of these two binding sites is negatively regulated by both the multimeric CCAAT-binding complex (CBC) transcription factor (consisting of HapB, HapC and HapE) and the iron-responsive basic region leucine zipper transcription factor, HapX. It is believed that under environmental conditions that favour repression of ergosterol biosynthesis and thus repression of sterol-demethylase expression, the CBC and HapX cooperatively bind directly downstream of the 3' SrbA binding site and diminish the ability of SrbA to promote *cyp51A* expression. In the absence of either SrbA or the SrbA binding sites in the promoter region of *cyp51A*, *cyp51A* expression is essentially absent and *A. fumigatus* becomes hypersusceptible to the triazoles.²⁶

Table 1. *cyp51A* mutations associated with triazole resistance

Tandem repeat-associated <i>cyp51A</i> mutations	
TR ₃₄ /L98H	
TR ₄₆ /Y121F/T289A	
TR ₅₃	
TR ₅₃ /L98H	
Individual <i>cyp51A</i> mutations	
WT residue	amino acid substitution
N22	D
G54	E, K, R, V, W
G138	C, R, S
P216	L
F219	C, I, L, S
M220	K, I, R, T, V, W
A284	T
<u>I367</u>	F
Y431	C, S
G432	A, C, S
G434	C
T440	A
G448	S
<u>F495</u>	L
Y491	H

Residues shown in bold are located along the ligand access channel and residues that are underlined directly interact with voriconazole.

Two distinct mechanisms leading to overexpression of *cyp51A* have been identified in triazole-resistant clinical isolates of *A. fumigatus*. By far the most prevalent is the formation of a tandem repeat in the promoter region of *cyp51A*.^{8,27} Three versions of this tandem repeat sequence have been observed (TR₃₄, TR₄₆ and TR₅₃).^{33,37,40} Each version differs in the length of the repeated sequence (34–53 bases), but all result in the formation of two additional *SrbA* binding sites that are not effectively regulated by the CBC or HapX (Figure 3b). Thus, *cyp51A* expression is increased by 2-fold or more and triazole MIC subsequently increases by ~4-fold.²⁶ Additionally, the TR₃₄ and TR₄₆ variations are, with only one known isolate being the exception, universally found in conjunction with specific mutations in the *cyp51A* gene itself, resulting in the amino acid substitutions L98H and Y121F/T289A, respectively.³² Isolates possessing these mutations are often referred to as either having the TR₃₄/L98H or TR₄₆/Y121F/T289A genotype and are most commonly observed to be multi- or pan-triazole resistant. Worryingly, the prevalence of these tandem-repeat mutations in both environmental and clinical isolates appears to be increasing around the world.^{6,8,27,37,40}

In addition to alterations of the *cyp51A* promoter region, a single mutation in the *hapE* gene, encoding a subunit of the CBC multimer, has been shown to directly result in increased *cyp51A* expression. This mutation was originally identified in an isogenic pair of clinical isolates in which resistance developed on triazole therapy and resulted in a P88L amino acid substitution in HapE.⁹ A CBC containing the mutant HapE^{P88L} subunit binds less effectively

to the promoter region of *cyp51A*. Thus, *cyp51A* expression, driven by the unimpeded *SrbA*, is increased (Figure 3c). Similarly to what is observed with the tandem repeat *cyp51A* genotypes, this mutation in *hapE* leads to a 2-fold or greater increase in *cyp51A* expression and increases in MICs of all triazoles that have been tested.²⁶ While screening for this *hapE* mutation is not commonly performed, to date this mechanism of triazole resistance has only been identified in the original clinical isolates of *A. fumigatus* from which it was discovered.

Overexpression of drug efflux pumps

In other pathogenic fungi, efflux pump overexpression has been shown to contribute to clinical triazole resistance. The *A. fumigatus* genome is predicted to contain more than 300 efflux pumps, many of which have been observed to have their expression induced by exposure to the triazoles.²⁵ Furthermore, a number of these pumps have been observed to be constitutively overexpressed among triazole-resistant clinical isolates.^{25,41} However, at present relatively little is known about which of these efflux pumps contribute to clinical triazole resistance. In triazole-susceptible laboratory strains of *A. fumigatus*, the individual disruption of six drug efflux pump genes (*abcA*, *abcC*, *atrF*, *atrI*, *mdrA* and *mdr1*) has been observed to increase triazole susceptibility.^{25,42–45} However, only the efflux pump gene, *abcA*, has been demonstrated to contribute to triazole resistance when constitutively overexpressed in a triazole-susceptible laboratory strain of *A. fumigatus*.⁴⁵ Additionally, *abcC* is the only efflux pump gene that has been demonstrated to contribute to clinical triazole resistance. This ABC efflux pump of the G subfamily is a close homologue of well-characterized drug efflux pumps from other species of pathogenic fungi and has been observed to be overexpressed in a number of triazole-resistant clinical isolates of *A. fumigatus*. Disruption of *abcC* in a triazole-resistant clinical isolate of *A. fumigatus* decreased the itraconazole MIC by greater than 4-fold, whereas voriconazole and posaconazole susceptibility remained unchanged. This clinical isolate was observed to exhibit increased constitutive expression of *abcC* (>5-fold) relative to the susceptible reference strain.²⁵ Drug efflux pumps have therefore been proven to be a mechanism by which triazole resistance is mediated in clinical isolates of *A. fumigatus*. However, the genetic determinants of drug efflux pump overexpression in *A. fumigatus* remain elusive.

Recently, the zinc-cluster transcription factor AtrR was found to promote the expression of both *cyp51A* and *abcC*. AtrR is a close homologue of the *Saccharomyces cerevisiae* pleotropic drug response zinc-cluster transcription factor Pdr1 and *A. fumigatus* strains with disrupted *atrR* exhibit a dramatic reduction in expression of both *abcC* and *cyp51A*.⁴⁶ However, the presence of activating mutations in *atrR* among triazole-resistant clinical isolates has yet to be identified and its direct role in clinical triazole resistance remains unclear.

Emerging mechanisms of triazole resistance

In addition to the aforementioned canonical mechanisms of triazole resistance, additional potential pathways to resistance in *A. fumigatus* are beginning to be identified, the most notable of which are directly linked to the ergosterol biosynthetic pathway. Two different studies investigating mutations acquired in

Table 2. *cyp51A* mutations by residues and relative impact on triazole MIC^{29,31–37}

Triazole ^a	Ligand access channel residues				Tandem repeat-associated residues	
	G54	P216	F219	M220	TR ₃₄ /L98H	TR ₄₆ /Y121F/T289A
Voriconazole	–	–	–	+	+	++
Posaconazole	++	+	+	+	+	+
Itraconazole	++	++	++	++	++	+

–, minimal to no impact on MIC; +, increase in MIC; ++, large increase in MIC.
^aData for isavuconazole not currently available.

Figure 3. Regulation of *cyp51A* expression. (a) Regulation of *cyp51A* expression with SrBA homodimers under normal repression of the CBC and HapX. (b) Regulation of *cyp51A* expression in the presence of a tandem-repeat mutation creating two additional SrBA binding sites not effectively repressed by the CBC and HapX. (c) Regulation of *cyp51A* expression in the presence of a CBC containing the mutant HapE^{P88L}, which is unable to effectively repress the SrBA-mediated expression of *cyp51A*.

laboratory strains of triazole-susceptible *A. fumigatus* that develop triazole resistance upon triazole exposure have been successful in identifying potentially novel resistance mechanisms. In these experiments, mutations in the *hmg1* gene were found to confer resistance to voriconazole, itraconazole and posaconazole (isavuconazole was not tested). The *hmg1* gene encodes an *A. fumigatus* homologue of 3-hydroxy-3-methyl-glutaryl (HMG) coenzyme A reductase, a key enzyme involved in both ergosterol and siderophore biosynthesis.⁴⁷ Additionally, a mutation in the *cox10* gene, required for the normal biosynthesis of haem, was found to

directly contribute to triazole resistance. Haem is utilized by a number of ergosterol biosynthetic genes, including *cyp51A* and *cyp51B*, to catalyse enzymatic conversion of sterols. The mutation in *cox10* was observed to significantly reduce itraconazole susceptibility, yet voriconazole susceptibility was not clearly impacted.⁴⁸ Although the roles of these mutations in clinical triazole resistance is currently unknown, these mutations serve to demonstrate the potential impact of non-canonical mechanisms on the clinical utility of the triazole antifungals and future screening for such mutations among clinical isolates of *A. fumigatus* may be merited.

Epidemiology of triazole resistance

Triazole resistance among *A. fumigatus* isolates emerged as early as the 1980s in immunocompromised patients from the USA receiving itraconazole as an investigational agent. Subsequently, resistant isolates were recovered from patients in the 1990s in the Netherlands and France. However, in each of these instances, the identification and reporting of triazole resistance followed years after the original clinical cases in which the isolates were cultured.

Today, analysis of the prevalence of triazole resistance among *A. fumigatus* remains limited by challenges in isolating *Aspergillus* from clinical specimens, as well as the additional resources and expertise required to perform antifungal susceptibility testing of moulds.⁴⁹ However, triazole-resistant *A. fumigatus* isolates are nonetheless being reported around the world in increasing numbers.^{8,50–53} A recent study examining the genetic diversity of *A. fumigatus* isolates observed that 6% of the 2026 isolates obtained from 13 countries spanning four continents were triazole resistant.⁵⁴ This resistance appears to vary dramatically by both geographical region and patient population.

Both the increase in overall prevalence of triazole resistance and the variation in its distribution are perhaps best exemplified in epidemiology data from the Netherlands, where surveillance has been conducted since the first recovery of resistant clinical isolates. In the Netherlands, triazole resistance was originally observed to be present in 1.8% of *A. fumigatus* isolates collected from 1945 to 1998. Subsequently, examination of resistance rates across seven Dutch university medical centres from 2007 to 2009 found the average triazole resistance rate to have risen to 4.6%, with medical centre-specific rates ranging from 0.8% to 9.5%.^{7,51} No seasonal variation in resistance was observed and there was no clear geographical influence on the distribution of resistance. Importantly, the vast majority of triazole-resistant isolates were resistant to both itraconazole and voriconazole, and the case-fatality rate among patients with triazole-resistant disease was 88%. Triazole resistance has also been observed to be more prevalent among certain high-risk patient populations. When resistance rates were tracked specifically among haematology patients at two Dutch medical centres, the prevalence of triazole resistance was found to be dramatically greater than that of the general population, ranging from 17% to 30%.⁵⁵ Similar findings were also reported in a single-centre study of Dutch ICU patients with invasive aspergillosis, in which 26% of isolates were triazole resistant.⁵ Overall 90 day mortality of patients with triazole-resistant disease was 100% in this population.

In the USA, triazole resistance among *A. fumigatus* is now being reported in isolates of both clinical and environmental origin.^{6,8,56} However, only one large, passive, surveillance study of triazole resistance among US *A. fumigatus* isolates has been performed to date. In this study, 1026 isolates were screened and 51 (5%) were found to have an itraconazole MIC above the EUCAST breakpoint for susceptibility (CLSI has not published triazole breakpoints for *A. fumigatus*).⁵⁷ Voriconazole susceptibility was not reported. More recently, a study characterizing triazole-resistant isolates from the USA performed extensive antifungal susceptibility testing on isolates collected from 2001 to 2015. Of 26 triazole-resistant *A. fumigatus* clinical isolates tested, all were resistant to both itraconazole and posaconazole (applying EUCAST breakpoints) and only 7 and 8, respectively, were susceptible to isavuconazole

and voriconazole. Additionally, although the use of agricultural sterol-demethylase inhibitors is less common in the USA than other parts of the world, a study conducted by collecting environmental samples from peanut fields being treated with triazole agents found 56 of 200 recovered isolates (28%) were triazole resistant.⁶

The distribution of identifiable mechanisms contributing to triazole resistance also appears to vary dramatically by geographical region. In the Netherlands, as well as in other European and Asian countries such as the UK and India, the vast majority of triazole-resistant isolates share at least one common resistance mechanism, consisting of specific combinations of mutations in the sterol-demethylase gene *cyp51A* in conjunction with the formation of tandem repeats in the *cyp51A* promoter region (TR₃₄/L98H or TR₄₆/Y121F/T289A genotype).²⁷ While many of these isolates likely possess additional resistance mechanisms, as suggested by the varying degrees of agent-specific triazole resistance, the predominance of the TR₃₄/L98H and TR₄₆/Y121F/T289A genotypes among these resistant isolates cannot be overlooked. Conversely, in the USA only a small number of clinical isolates have been reported to possess this particular resistance mechanism. Of the 26 isolates in the aforementioned characterization of triazole-resistant *A. fumigatus* isolates, only 2 TR₃₄/L98H and 2 TR₄₆/Y121F/T289A genotype isolates were found, yet 16 isolates possessed other identifiable mutations in *cyp51A*. This discordance in prevalence of the TR₃₄/L98H and TR₄₆/Y121F/T289A genotypes has been linked to differences in the application of triazole-related agricultural fungicides. Supporting this link, the TR₃₄/L98H and TR₄₆/Y121F/T289A genotypes are most prevalent among clinical and environmental samples from regions with higher utilization of these fungicides and *in vitro* studies have demonstrated rapid acquisition of these and other *cyp51A* mutations upon exposure to agricultural triazoles. Notably, of the 56 triazole-resistant environmental samples from US peanut farms treated with triazoles, 21 (37.5%) were found to possess the TR₃₄/L98H genotype.⁶ This has led some experts to call for a reduction in the reliance on agricultural sterol-demethylase inhibitors in an effort to preserve the utility of the clinical agents.⁵⁸

While the identification and study of potential risk factors for triazole-resistant aspergillosis has been limited by the general paucity of susceptibility data available for clinical isolates, a small number of patient populations have been associated with higher prevalence of triazole-resistant aspergillosis. Multiple studies have observed rates of triazole-resistant *A. fumigatus* isolates from haematology-oncology patients in Europe, approximating and even exceeding 20%; however, it is important to note that rates more similar to the general prevalence of resistance have been observed at other institutions. Although prolonged use of prophylactic triazoles is not uncommon in this patient population, these studies have also noted recovery of triazole-resistant isolates from patients with no prior triazole exposure, suggesting other patient factors, possibly in conjunction with environmental acquisition of triazole-resistant isolates, may be involved.^{52,55,59,60} Additionally, two epidemiological studies examining the rates of triazole-resistant *A. fumigatus* among patients being treated in ICUs have also reported higher rates of resistance,^{5,59} though both of these studies examined patients from medical centres in the Netherlands, where environmental rates of triazole resistance are known to be high. It has been suggested that increased rates of triazole resistance among environmental isolates of *A. fumigatus*

may also increase the risk of triazole resistance among cases of aspergillosis.⁶¹ This concept of environmental acquisition of resistant isolates is supported by a report of a patient admitted to an ICU, with no prior history of antifungal drug exposure, suffering from proven invasive aspergillosis caused by an *A. fumigatus* isolate harbouring the TR₄₆/Y121F/T289A genotype. Environmental screening of the patient's home garden and household revealed multiple samples of a genetically indistinguishable and triazole-resistant *A. fumigatus*.⁶²

Conclusions

The mould-active triazoles are essential to both prophylaxis and treatment of invasive aspergillosis and few therapeutic alternatives exist. Although the overall prevalence of triazole resistance among clinical isolates of *A. fumigatus* is currently observed to range between 1% and 10%, these estimates are limited by a relative paucity of clinical samples with accompanying susceptibility data. Additionally, the recent dramatic increase in reports of resistant isolates from both clinical and environmental sources worldwide is of clear concern. Mutations in *cyp51A*, overexpression of *cyp51A* and overexpression of efflux pump genes are all known to contribute to resistance, yet much of the triazole resistance among *A. fumigatus* still remains unexplained. Also lacking is clinical experience with therapeutic options for the treatment of triazole-resistant *A. fumigatus* infections and mortality associated with these infections remains unacceptably high. Thus, further research is greatly needed to both better understand the emerging threat of triazole-resistant *A. fumigatus* and to develop novel therapeutic strategies to combat these resistant infections.

Transparency declarations

None to declare.

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