

# The emergence of azole resistance in *Aspergillus fumigatus* complex in New Zealand

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## ABSTRACT

**BACKGROUND:** Azole resistance in *Aspergillus fumigatus* (*A. fumigatus*) is increasing globally. A pan-azole-resistant isolate prompted genetic analysis of local azole-resistant isolates to determine resistance genotypes.

**METHODS:** All *A. fumigatus* complex isolates were tested by the broth colorimetric micro-dilution method, Sensititre® YeastOne® (SYO) (TREK Diagnostic Systems, West Sussex, England). Epidemiological cutoff values derived from the Clinical & Laboratory Standards Institute method were used to determine the proportion of non-wild-type (non-WT) isolates (ie, those with an increased likelihood to harbour acquired mechanisms of resistance). Non-WT isolates were identified by  $\beta$ -tubulin gene sequencing and the genotype for azole resistance was determined. The history of the patient with the first pan-resistant isolate was reviewed along with the treatment history of patients with azole-resistant strains.

**RESULTS:** From January 2001 to August 2020, antifungal susceptibility testing was performed on 260 *A. fumigatus* complex isolates: six isolates were non-WT for one or more azole agent, two *A. fumigatus sensu stricto* and four other members within the species complex: two *A. fischeri* and two *A. lentulus*. There were three non-WT isolates for amphotericin B, three for itraconazole, five for posaconazole and five for voriconazole. All six non-WT strains were isolated in the past nine years ( $P < 0.01$ ), and four in the past three years. Azole-resistance genotyping for the *A. fumigatus sensu stricto* isolates detected amino acid changes at hot spots in the *cyp51A* gene: one at G54E and one at G138C. All six isolates were WT for caspofungin. Five of the six patients with azole-resistant strains had previous azole treatment, and the patient with the pan-azole-resistant strain had been on continuous azole treatment for 42 months preceding strain isolation.

**CONCLUSIONS:** New Zealand can be added to the growing list of countries with azole-resistant *A. fumigatus* complex isolates, including pan-azole resistance in *A. fumigatus sensu stricto*. While uncommon and mostly found in cryptic species within the complex, azole resistance is increasing. The results provide a baseline for monitoring this emerging antifungal resistance trend in *A. fumigatus* in New Zealand.

**A** *Aspergillus* spp. are the most common cause of invasive mould infections globally.<sup>1,2</sup> *Aspergillus fumigatus* (*A. fumigatus*) complex is the most frequently encountered group and molecular methods show it is comprised of more than 60 species, including *A. fumigatus* itself (*A. fumigatus sensu stricto*), *A. fischeri* and *A. lentulus*.<sup>3,4</sup> Species within the complex have varying susceptibility profiles.<sup>4-8</sup>

Triazoles are the cornerstone of recommended treatments for invasive aspergillosis (IA).<sup>9</sup> Azoles target an essential step in fungal

ergosterol synthesis encoded by the *cyp51A* gene. Major mechanisms of azole resistance in *A. fumigatus* include mutations at hot spots in the *cyp51A* gene (target modification), alone or in combination with tandem repeats (TRs) in the gene's promotor region (over expression), or the upregulation of efflux pumps.<sup>10,11</sup> Hot-spot mutations are described in patients who have received courses of azole treatment, whereas TR-associated resistance has been linked to acquisition of environmental strains that have been exposed to azoles used in agriculture.<sup>10, 12-15</sup>

Although there are no interpretive criteria for susceptibility or resistance for moulds, except with respect to voriconazole and *A. fumigatus sensu stricto*,<sup>16</sup> epidemiological cutoff values (ECVs) have been established for a limited number of species complex-antifungal agent pairings. An ECV defines the upper minimal inhibitory concentration (MIC) limit of wild-type (WT) isolates, without acquired resistance mechanisms, and non-wild-type (non-WT) isolates likely to harbour acquired resistance mechanisms.<sup>17,18</sup> ECVs do not group isolates into susceptible, likely to respond to treatment, resistant or unlikely to respond to treatment, but can be used when no interpretive criteria exist for identifying strains more likely to have acquired mechanisms of resistance and, by inference, less likely to respond to treatment.

We recently reviewed the antifungal susceptibility results of mould isolates performed in Auckland from 2001–2019.<sup>19</sup> Over the 19 years there were four *A. fumigatus* complex isolates that were non-WT for azole agents, but because molecular analysis of the *cyp51A* gene was not performed, their mechanisms of resistance was not known.<sup>19</sup> The 2020 isolation of a pan-azole-resistant *A. fumigatus sensu stricto* isolate prompted us to undertake genotype testing on local *A. fumigatus* complex isolates non-WT for azole agents to determine their mechanism of resistance.

## Materials and methods

### Isolates

All isolates tested were from Auckland City Hospital, or referred from other New Zealand laboratories, for the period January 2001–August 2020. The laboratory information system was interrogated to obtain a file of all *A. fumigatus* complex isolates that had anti-fungal susceptibility testing performed (detailed methods are reported elsewhere).<sup>19</sup> Isolates were initially identified by morphology.

### Antifungal susceptibility testing

All isolates were tested by the broth colorimetric micro-dilution method, Sensititre® YeastOne® (SYO) (TREK Diagnostic Systems, West Sussex, England), following the manufacturer instructions.

Endpoint interpretations followed Clinical & Laboratory Standards Institute (CLSI) methods.<sup>16,20</sup> The minimal inhibitory concentration (MIC) endpoint was defined as the lowest concentration producing complete inhibition of growth of amphotericin B (AMB), itraconazole (ITC), posaconazole (POS) and voriconazole (VCZ).<sup>20</sup> The minimal effective concentration (MEC) for the echinocandins (caspofungin, micafungin and anidulafungin) was defined as the lowest concentration producing small, rounded, compact hyphal forms compared to the hyphal growth of the growth control.<sup>20</sup> Readings were made at 24 hours for echinocandins and 48 hours for other agents. AMB and ITC were tested throughout the period. Other agents were tested as they were incorporated into the SYO assay, meaning not all agents were tested on each isolate.

To determine the proportion of non-WT isolates, CLSI ECVs for AMB ( $\leq 2$ mg/L), ITC ( $\leq 1$ mg/L) and VOR ( $\leq 2$ mg/L) were used.<sup>18</sup> CLSI has not published an ECV for POS, so the CLSI-based ECV published by Buil et al,  $\leq 0.25$ mg/L, was used.<sup>21</sup> VOR susceptibility was defined as  $\leq 0.5$ mg/L.<sup>16</sup>

### Identification and resistance genotyping

All non-WT azole isolates were recovered from the culture collection water stocks and identified by  $\beta$ -tubulin gene sequencing.<sup>3,22</sup> Isolates were retested to confirm the MICs/MECs and their azole-resistance mechanism was determined by *cyp51A* gene sequencing.<sup>23</sup>

### Case history and antifungal exposure

The notes of the patient with the first pan-azole resistant isolate of *A. fumigatus sensu stricto* were summarised and the electronic medical records of patients with the other non-WT azole isolates were reviewed to record azole exposure.

The two other New Zealand laboratories performing mould antifungal susceptibility testing were contacted and asked whether they had encountered any isolates with non-WT azole MICs.

### Ethics

Cases were reviewed as part of the Australasian Society for Infectious Diseases Mycology Special Interest Group's Case Registry of *Aspergillus* infections. Health and

Disability Ethics Committee approval 20/NTB/35 and Auckland District Health Board institutional approval A+8799.

## Results

### Isolates

Over the 19.7-year period, 260 initial isolates of *A. fumigatus* complex isolates were tested and there were six non-WT isolates. Eighty one percent were from Auckland, with 65% from Auckland City Hospital. Table 1 shows the number of isolates tested for each antifungal, the identity of the six non-WT isolates (as determined by  $\beta$ -tubulin gene sequencing), their years of isolation, their susceptibility results and the proportion of non-WT strains..

During 2001–2010, no non-WT isolates were detected (143 tested). Six (5%) were detected 2011 to 2020 (117 tested), with a p-value of <0.01. Four of the six non-WT isolates were encountered in the past three years. All isolates were WT for caspofungin (MECs  $\leq 0.5\text{mg/L}$ ).<sup>18</sup>

The colonial appearance of both *Aspergillus fumigatus sensu stricto* isolates was typically blue-green with a suede-like surface, whereas the colony appearance of the other species varied from pale blue-green in *A. lentulus* to white floccose colonies in *A. fischeri*. The difference in colonial appearance on Sabouraud dextrose agar aided in recognising mixed cultures. Of the six isolates reported here (see Table 1), one *A. lentulus* (Isolate 4) and the two *A.*

*fumigatus sensu stricto* were isolated in pure culture; one *A. lentulus* (Isolate 3) and one *A. fischeri* (Isolate 2) were isolated in mixed culture with a WT *A. fumigatus*; and one *A. fischeri* (Isolate 1) was isolated in mixed culture with a WT *A. fumigatus* and *Purpureocillium lilacinum*.

### Genotypes

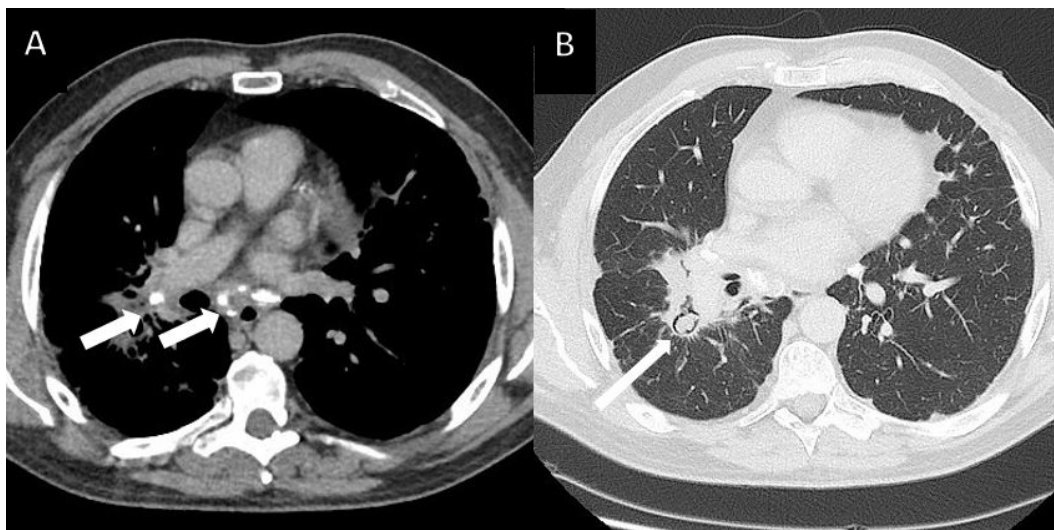
For the two *sensu stricto* isolates, different hot-spot mutations in the *cyp51A* gene were detected that resulted in amino acid changes at either Gly54Glu (G54E) or Gly138Cys (G138C) (Table 1). Neither isolate had TR mutations present. For *A. lentulus* and *A. fischeri* there was no amplification of the *cyp51A* gene, presumably due to mismatches in the primer binding regions.

Neither of the other laboratories performing susceptibility testing on *A. fumigatus* had encountered pan-azole resistant strains (J. Creighton and L. Sanders, personal communication). One had tested an isolate from a patient reported here (Isolate 5) and obtained the same results (J. Creighton, personal communication). The other laboratory had encountered a single isolate that was non-WT for POS, MIC=0.5mg/L (L. Sanders, personal communication).

### Clinical history for pan-azole resistant isolate

Case 6 (Table 1) was a 61-year-old male who presented in December 2016 with six months of cough and breathlessness and an abnormal chest radiograph. He had been previously well, was a regular cyclist and a

**Figure 1:** (A) Calcified nodes (arrows). (B) Intracavity body (arrow).



**Table 1:** Antifungal susceptibility results (mg/L) for amphotericin B and azoles against *Aspergillus fumigatus* complex isolates: January 2001–August 2020.

Antifungal agent			Amphotericin B	Itraconazole	Posaconazole	Voriconazole	
Interpretive criteria for wild-type (WT) isolates (mg/L) <sup>1</sup>			≤2	≤1	≤0.25	≤1	
Isolates tested			260	260	215	238	
Non-WT isolates <sup>2</sup> (year isolated)	Specimen type	Clinical background					Resistance genotype
1. <i>A. fischeri</i> <sup>3</sup> (2018)	B. wash <sup>4</sup>	Lung transplant	1	0.5	0.25	2	Not determined
2. <i>A. fischeri</i> <sup>3</sup> (2020)	B. wash	Lung transplant	2	0.5	0.5	2	Not determined
3. <i>A. lentulus</i> (2015)	B. wash	ALL and BMT <sup>5</sup>	8	1	0.5	4	Not determined
4. <i>A. lentulus</i> (2011)	B. wash	AML and BMT <sup>5</sup>	8	2	1	8	Not determined
5. <i>A. fumigatus sensu stricto</i> (2018)	Sputum	Cystic fibrosis	2	>16	2	0.25	Gly54Glu(G54E) <sup>7</sup>
6. <i>A. fumigatus sensu stricto</i> (2020)	Lung cavity	Sarcoidosis	4	>16	2	>8 <sup>6</sup>	Gly138Cys(G138C) <sup>7</sup>
Total non-WT (%)			3 (1.2%)	3 (1.2%)	5 (2.3%)	5 (2.1%)	

<sup>1</sup> Clinical Laboratory Standards Institute (CLSI) criteria used for amphotericin B, itraconazole and voriconazole.<sup>18</sup> Posaconazole criterion from CLSI based methods.<sup>21</sup>

<sup>2</sup> All non-WT isolates identified by  $\beta$ -tubulin gene sequencing.

<sup>3</sup> Synonyms: *Aspergillus thermomutatus* (anamorph) and *Neosartorya pseudofischeri* (teleomorph).

<sup>4</sup> Bronchial washing/alveolar lavage specimen.

<sup>5</sup> ALL, acute lymphocytic leukaemia; AML, acute myeloid leukaemia; BMT, bone marrow transplant.

<sup>6</sup> For *A. fumigatus sensu stricto* the interpretive criteria for voriconazole susceptibility is ≤0.5 mg/L.<sup>16</sup>

<sup>7</sup> No *cyp51A* gene tandem repeat mutations detected.



lifelong non-smoker. Medications included anti-inflammatory agents and amitriptyline for back pain; he had required a laminectomy 25 years before.

CT chest showed multiple calcified mediastinal nodes (Figure 1A) and bilateral cavities with one containing an intracavitary body, a presumed fungal ball (aspergilloma), (Figure 1B).

Endo-bronchial ultrasound guided fine needle aspiration of a mediastinal node revealed non-necrotising granulomas. Bronchial washings grew an *Aspergillus fumigatus* complex isolate, which was identified by morphology, with negative molecular and culture tests for *Mycobacterium tuberculosis* (TB). Susceptibility testing was not performed on this isolate. QuantiFERON-TB Gold and anti-neutrophil cytoplasmic antibodies were negative. The angiotensin converting enzyme level was normal. *Aspergillus* serology was positive with *Aspergillus* RAST of 3+ and *Aspergillus* specific IgG elevated at 48mg/L (expected <40mg/L). The static and dynamic lung functions were within normal limits.

He was diagnosed with chronic cavitary pulmonary aspergillosis on background sarcoidosis and in early 2017 was commenced on itraconazole 200mg twice daily. This was changed to voriconazole 200mg twice daily in April 2018 when six month's funding became available. When funding expired, he reverted to itraconazole in October 2018 for two months before recommencing voriconazole in December 2018. This was self-funded from an overseas supplier from December 2018, although he did occasionally take itraconazole depending on his supply of voriconazole and to reduce cost.

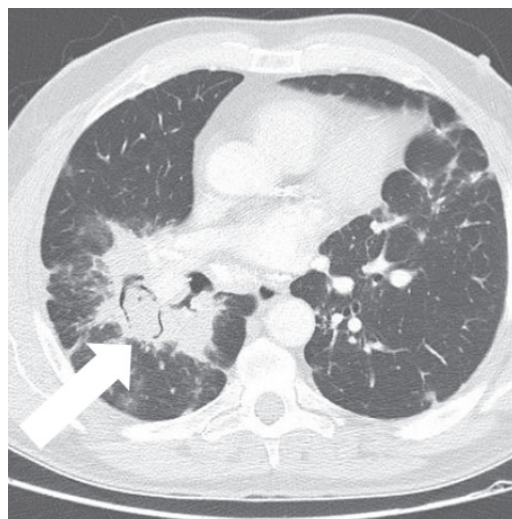
In March 2017 a cardiology opinion was sought in view of the symptoms of breathlessness in the presence of normal lung-function tests. A coronary angiogram confirmed obstructive coronary disease requiring a complex multi-vessel coronary revascularisation followed by dual antiplatelet therapy with aspirin and clopidogrel. A cardiac MRI showed a mildly dilated left ventricle with ejection fraction of 45% and no evidence of cardiac sarcoidosis.

In April 2018, while on dual antiplatelet therapy and itraconazole, he developed moderate volume haemoptysis. There was

no accessible vessel amenable for bronchial artery embolisation. The haemoptysis resolved with cessation of clopidogrel and the commencement of voriconazole.

During the subsequent 16 months he continued to have episodes of mild, intermittent haemoptysis, which led to a large volume haemoptysis in August 2020. The voriconazole level obtained at this time was therapeutic at 2.6mg/L (goal 1–5 mg/L). A CT chest showed enlarging bilateral lung cavities and a presumed aspergilloma in the right lung (Figure 2).

**Figure 2:** Chest CT after 42 months of continuous azole treatment. Lung cavities had enlarged and a larger intracavitary body is present in right lung (arrow).



A bronchial artery embolisation was attempted but was unsuccessful, so he underwent a right pneumonectomy. The resected lung showed a large lung cavity with a fungal ball. Histopathology revealed the features of sarcoidosis with multiple non-necrotising granulomas in both the lung and mediastinal nodes. The necrotic material in the centre of the cavity was an aspergilloma consisting of a dense mass of septate acute-angled branching hyphae.

Samples of the viscous cavity contents were obtained for mycology. Direct examination showed large amounts of dichotomously branching hyphae, and culture recovered *Aspergillus fumigatus sensu stricto*. Antifungal susceptibility results and azole resistance genotype are shown in Table 1 (Case 6).

## Azole exposure before isolation of non-WT isolates

Case 1 had no previous azole treatment. Case 2 had three previous six-month courses of either voriconazole, in 2015 and 2016, or posaconazole, in 2017. The isolate was obtained two and a half years after last azole treatment. Case 3 had four months of fluconazole prophylaxis following bone-marrow transplant and was on fluconazole at the time of isolation. Case 4 had past posaconazole prophylaxis when undergoing remission induction chemotherapy for acute myeloid leukaemia and voriconazole for fungal lung infection eight months earlier. They were also treated with amphotericin B and were off azole treatment at the time of isolation. Case 5 had itraconazole treatment of unknown duration(s) for allergic bronchopulmonary aspergillosis, which was stopped in early 2014. Itraconazole was recommenced for four and a half months, stopping mid-April 2018. The sputum isolate was obtained late May 2018 when the patient was off azole treatment.

## Discussion

*A. fumigatus* complex contains subspecies (eg, *A. fumigatus sensu stricto* and many other 'cryptic' species) that are difficult to distinguish by conventional testing methods and require molecular methods for correct identification.<sup>3,4</sup> This study establishes the low rate of azole resistance in *A. fumigatus* complex in New Zealand and the current rarity of pan-azole-resistant *A. fumigatus sensu stricto*. All non-WT isolates were encountered in the past 10 years, and most in the last three. Most non-WT isolates were cryptic species within the complex. Three of the non-WT isolates were in mixed culture with WT *A. fumigatus* isolates. Mixed cultures have been reported before and underscore the need to carefully inspect cultures to detect colonial variants.<sup>14</sup> The AMB MIC for both *A. lentulus* isolates (8mg/L) appears higher than that reported for five Australian isolates (all  $\leq 2$ mg/L). However, the small numbers, as well as biological and inter-laboratory variability, limit comparison between our countries.<sup>3,19</sup> Others have reported the high MICs we observed for amphotericin in *A. lentulus* isolates.<sup>6,24</sup>

Azole resistance in *A. fumigatus* was first found in isolates obtained in the late 1980s. Resistance is now encountered in many countries (3.2% prevalence, range 0–26%).<sup>25</sup> Resistance may vary within a country<sup>12</sup> and can increase significantly within five years.<sup>26</sup> In Spain 7.4% of 847 *A. fumigatus* clinical isolates from 2019 were azole resistant, with a significant subspecies difference (5.5% *A. fumigatus sensu stricto*, n=828, vs 95% in cryptic species, n=19).<sup>27</sup> Importantly, cross-resistance within the class is common, which limits treatment options.<sup>26,28</sup> Resistant isolates are encountered in azole-naïve patients and the mortality rate for azole-resistant invasive aspergillosis is often very high.<sup>12,14</sup>

Azole resistance is uncommon in Australia (approximately 2%).<sup>29,30</sup> It is reassuring that the non-WT for azole agents was low in New Zealand (1.2–2.1% of isolates tested), and complete azole cross-resistance was only observed in one *A. lentulus* and one *A. fumigatus sensu stricto*, which was also categorised as VOR resistant by the recently published CLSI interpretive criterion.<sup>16</sup>

We did not attempt TR genotyping on the non-*sensu stricto* isolates, because the primers for *A. fumigatus sensu stricto* do not amplify the region in cryptic species in the complex. The proportion resistance due to TR and hot-spot mutations varies significantly between countries. In the Netherlands, for isolates known to have *cyp51A* mutations, 90% and 10% were due to TR and hot-spot mutations, respectively, with 35% having neither type of mutation detected.<sup>31</sup> In a UK series, however, where most isolates were from patients with chronic pulmonary aspergillosis (CPA) and previous azole treatment, hot-spot mutations dominated (>90%) and TR mutations were uncommon (<5%).<sup>26</sup> In Australia the proportion of hot-spot, TR or no-*cyp51A* mutations detected in 12 isolates were 50%, 25% and 25%, respectively.<sup>29,30</sup> Although we did not detect TR mutations in *A. fumigatus sensu stricto*, their future occurrence in New Zealand is possible because, even though they comprise only a small proportion of all fungicide use, triazole fungicides are used in agriculture in New Zealand.<sup>32</sup> However, information on the use of triazole in agriculture is more than 15 years old, and we know of no contemporary data on the extent

of environmental use of triazoles on our crops. We support the call for better surveillance on pesticide use in New Zealand.<sup>32</sup> The isolation of a non-WT azole isolate in an azole-naïve patient should arouse suspicion of TR mutations, and resistance genotyping should be performed.

Currently there is a limited understanding of the resistance mechanisms operating in the cryptic species within the complex, some of which are described as having intrinsic azole resistance.<sup>4,5,6,11,24</sup> The *cyp51A* gene and its promotor region that detect either hot-spot or TR mutations are different for *A. fumigatus sensu stricto* and cryptic species such as *A. lentulus* and *A. fischeri*.<sup>7,33</sup> The non-amplification of *cyp51A* we encountered reflects this. More sequence data are required to understand the resistance mechanisms in these species.

Triazoles are not known to be mutagenic and therefore resistance is selected when genetic variation occurs in the progeny of *Aspergillus* species.<sup>34</sup> The most recognised resistance mechanisms occur either in triazole-treated patients, hot-spot mutations (found in Case 5 and Case 6) or in isolates from environments that have been exposed to triazole fungicides, TR mutations. The former is more likely to occur in patients with CPA on azole treatment due to pulmonary cavities where *A. fumigatus* can undergo asexual sporulation, which is more prone to mutations.<sup>34</sup> The clinical impact of azole resistance has been unclear, as many earlier studies with small patient numbers have not shown outcome differences. In a recent report of a large multicentre retrospective cohort, mortality at both 42 days and 90 days was statistically higher (21% and 25%, respectively) in aspergillosis due to triazole-resistant versus triazole-susceptible strains.<sup>35</sup>

The interplay of the human immune system and *Aspergillus* largely determines the pattern of clinical illness, which can range from IA seen predominantly in haematopoietic, solid-organ transplant and neutropenic patients, to allergic bronchopulmonary aspergillosis seen predominantly in immunocompetent people with a hyper-immune response.<sup>2</sup> CPA represents a mid-spectrum seen mostly in patients with underlying lung disease and who are not overtly immunocompromised. CPA can

occur in those with previously treated TB, non-tuberculous mycobacteria or bronchiectasis. Aspergillosis was reported in 2% of a cohort of sarcoidosis patients.<sup>36</sup> CPA usually affects middle-aged men and presents in an indolent fashion with constitutional symptoms (malaise, anorexia, sweats), chronic cough with occasional haemoptysis and shortness of breath. Slow and evolving lung cavities, usually with thick walls and either with or without a fungus ball (aspergilloma), are typical of CPA and were observed in Case 6.<sup>2</sup> Treatment of CPA is challenging due to a high relapse/recurrence rate of up to 25% despite prolonged treatment (>12 weeks). The anti-fungal treatment for CPA is suppressive rather than curative and those with progressive disease require lifelong antifungal therapy.<sup>2</sup> CPA is associated with high mortality, with 27% over a mean follow-up duration of 30 months and 50% over five years in separate studies.<sup>37,38</sup> In the face of worsening symptoms, or progressive disease, strong consideration should be given to obtaining deep respiratory samples to determine antifungal susceptibility of the patient's isolate(s) to enable treatment optimisation. In addition, therapeutic drug monitoring is recommended for voriconazole because genetic polymorphisms in cytochrome P450 are responsible for significant inter-patient pharmacokinetic variability resulting in phenotypes ranging from poor to super metabolisers, both of which may require dose adjustments to prevent toxicity or improve efficacy, respectively.<sup>39</sup>

While both European and US guidelines recommend susceptibility testing for epidemiological purposes, they differ in recommendations for routine testing. The European guideline recommends susceptibility testing unless the patient is azole-naïve and from a region without resistance found in surveillance programmes.<sup>40</sup> Given the low rate of resistance, the US guideline does not recommend routine testing unless the isolate has atypical growth or there are clinical concerns for resistance.<sup>41</sup> We consider it prudent to perform susceptibility testing for clinically significant isolates from a lower respiratory tract specimen, or from an immunocompromised host with invasive infection or an immunocompetent host with cavitary lung infection. If a prolonged (>12

week) treatment course is planned, initial susceptibility testing should be performed, as it would be before initiating long-term anti-bacterial or anti-mycobacterial treatment.

Currently, funding for voriconazole is available only for six months for immunocompromised patients with proven, probable or possible invasive aspergillosis. The better bioavailability of voriconazole and probably higher mortality with resistant strains has shifted treatment choice in favour of voriconazole, especially in those with extensive CPA.<sup>2</sup> We suggest the indications for voriconazole should be extended to include patients with CPA requiring long-term treatment.

Our study has strengths and limitations. Most isolates came from Auckland, a city with the country's largest population and concentration of tertiary/quaternary clinical services. The testing was performed by a small number of staff using the same method in one laboratory. All isolates had their MICs/MECs confirmed by repeat testing and the susceptibility profiles were consistent with other reports<sup>4,6,24</sup> Molecular methods determined the identification of all the non-WT isolates. However, this

report has limitations. Although our data are not a complete summary of testing in the country, as two other laboratories also perform mould susceptibility testing, neither of these laboratories have encountered pan-azole resistance. The isolates were deemed relevant enough to have susceptibility testing performed, but we do not know the proportions reflecting proven or likely infection or colonisation. In addition, isolates are not always obtained from patients treated for suspected aspergillosis, meaning the denominator data underestimate the burden of *Aspergillus* infection in New Zealand. CLSI methods do not always result in high MICs for all azoles in isolates with known resistance mechanisms and may not detect reduced susceptibility for all mutations (ie, our testing may have missed some isolates with *cyp51A* mutations).<sup>31</sup>

In conclusion, New Zealand can be added to the growing list of countries with azole-resistant *A. fumigatus* complex isolates, including pan-azole resistance in *A. fumigatus sensu stricto*. Although uncommon and mostly found in cryptic species within the complex, azole resistance is increasing. Our results provide a baseline for monitoring this emerging antifungal resistance trend in *A. fumigatus* in New Zealand.



**Competing interests:**

Nil.

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