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MICROBIOLOGY

Antifungal susceptibility of clinical mould isolates in New Zealand, 2001–2019

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Summary

The objective of this study was to review the antifungal susceptibility of clinical mould isolates performed by the New Zealand Mycology Reference Laboratory.

Isolates were either local or referred for testing from other New Zealand laboratories. All isolates were tested by the broth colorimetric microdilution method, Sensititre YeastOne (SYO). Epidemiological cut-off values (ECVs) derived from either the Clinical and Laboratory Standards Institute (CLSI) method or SYO were used to determine the proportion of non-wild type (non-WT) isolates, i.e., those with an increased likelihood to harbour acquired mechanisms of resistance.

A total of 614 isolates were tested. Most isolates (55%) were from the respiratory tract followed by musculoskeletal tissue (17%), eye (10%) and abdomen (5%). The azoles had similar activity except for voriconazole which was less active against the *Mucorales*. The echinocandins had good activity against *Aspergillus* spp., other hyaline moulds and dematiaceous isolates but were inactive against *Fusarium* spp., *Lomentospora prolificans* and the *Mucorales*. Amphotericin B had best activity against the *Mucorales*. The two least susceptible groups were *Fusarium* spp. and *L. prolificans* isolates. Three *Aspergillus* isolates were non-WT for amphotericin B, and four non-WT for azoles. Non-WT were not encountered for caspofungin. Non-*Aspergillus* isolates in New Zealand have susceptibility patterns similar to those reported elsewhere. In contrast to a growing number of other countries, azole resistance was rare in *A. fumigatus sensu stricto*. Non-WT isolates were uncommon. The results provide a baseline for monitoring emerging antifungal resistance in New Zealand and support current Australasian treatment guidelines for invasive fungal infections.

Key words: Antifungals; susceptibility; *Aspergillus*; fungi; human; ECV; wild-type.

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INTRODUCTION

Filamentous fungi, moulds, cause serious infections which are difficult to treat and have high mortality.[1] While interpretive criteria exist for common bacteria and yeasts to allow reporting of susceptible, likely to respond to the antimicrobial, or resistant, unlikely to respond, such criteria do not exist for moulds. For many mould species, complexes or groups, high minimum inhibitory concentrations (MICs) indicate little or no chance of clinical utility of a given antifungal agent or class. Some moulds, e.g., many *Fusarium* spp. isolates, can be considered 'resistant' to all available antifungal agents.[2]

Apart from voriconazole for *Aspergillus fumigatus sensu stricto* there are no interpretive criteria for moulds. However epidemiological cut-off values (ECVs) have been established for a limited number of species/complex antifungal agent pairings. An ECV defines the upper MIC, or for the echinocandins the minimum effective concentration (MEC), limit of wild-type (WT) isolates, without acquired resistance mechanisms, and non-wild-type (non-WT) isolates likely to harbour acquired resistance mechanisms.[3,4] While WT does not indicate an isolate is treatable with a given antifungal, ECVs help identify those isolates far more likely to have acquired molecular resistance mechanisms.[4] A species/complex's intrinsic susceptibility to an antifungal may result in an ECV which would imply no clinical utility for treatment, e.g., an amphotericin B ECV of 8 mg/L.

There are no published data on the susceptibility of clinical isolates of filamentous fungi in New Zealand. The aims of this study were to analyse the susceptibility results of mould isolates tested at the New Zealand Mycology Reference Laboratory from 2001–2019, apply ECVs to determine the frequency of non-WT isolates, and compare New Zealand susceptibility patterns to those reported from other countries, particularly Australia.[5–8]

MATERIALS AND METHODS

All isolates tested from Auckland City Hospital, or referred from other New Zealand laboratories, for the 19 year period 2001–2019 were included in the study. Susceptibility testing was performed in the National Mycology Reference Laboratory, Auckland City Hospital, New Zealand. Susceptibility results are entered into the laboratory information system which was scanned for all entries for the 19 year period. Extracted data were analysed using Excel.

Isolates

Only the first isolate of any species was included for each patient. If there were multiple isolates of the same species recovered from a patient at the same time the more resistant isolate was included for analysis. If susceptibility results were the same, the most invasive isolate was included. Isolates were identified by morphology with only a few isolates identified by molecular methods, which for *Aspergillus* spp. was based on β-tubulin gene sequencing.[6]

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Antifungal susceptibility testing

All isolates were tested by the broth colorimetric microdilution method, Sensititre YeastOne (SYO) (TREK Diagnostic Systems, England) following the manufacturer's instructions. Endpoint interpretations followed Clinical and Laboratory Standards Institute (CLSI) methods.[9–11] The MIC endpoint was defined as the lowest concentration producing complete inhibition of growth for amphotericin B (AMB), itraconazole (ITC), posaconazole (POS) and voriconazole (VCZ).[10] The MEC for the echinocandins, caspofungin (CAS), micafungin (MIF), and anidulafungin (AND) was defined as the lowest concentration producing small, rounded, compact hyphal forms compared to the hyphal growth of the growth control.[10] Panels were usually incubated for 24–72 h. For *Aspergillus* spp. readings were made at 24 h for echinocandins and 48 h for other agents. For slower growing moulds the MIC/MEC reading was made when the growth control well showed adequate growth. This meant for some isolates, e.g., *Phialophora* spp., the reading was performed after 4–5 days incubation. AMB and ITC were tested throughout the 19 year period, with other agents tested as they were incorporated into the SYO assay: VCZ, CAS and POS in late 2002, mid-2005 and mid-2006, respectively; AND and MIF in 2012.

To determine the typical value, i.e., the central tendency of a set of MIC/MEC values, the geometric mean (GeoM) was calculated. When the MIC/MEC values were low (<) or high (>) off scale values the concentration was converted to the next lowest and highest two-fold dilution concentration, respectively.

All *Aspergillus* isolates non-WT for azoles were recovered from the culture collection water stocks and retested to confirm the MIC and identified by β-tubulin gene sequencing.

The control organisms *Pichia kudriavzevii* [previously *Candida krusei*; American Type Culture Collection (ATCC) 6258] and *Candida parapsilosis* (ATCC 22019) were used to quality control each delivery of SYO plates, which was approximately 2 monthly. An isolate's results were only recorded and reported when the results for the control organisms were within the acceptable range.

To determine the proportion of non-WT isolates published ECVs were used. Most were CLSI method derived ECVs for *Aspergillus* spp.,[4,12] *Mucorales*,[13] *Fusarium* spp.,[14] and *Scedosporium* spp.[15] As ECVs are method related, recent SYO generated ECVs for four *Aspergillus* spp. were also applied.[16]

The two other New Zealand laboratories which perform antifungal susceptibility testing on moulds were contacted and asked when their testing started how many tests were performed in 2019.

RESULTS

Isolates

Over the 19 year period 614 initial isolates were tested. Most of the isolates (81%) were from Auckland patients, with 65% from Auckland City Hospital; 14% were from the other North Island laboratories, and 5% from South Island laboratories.

More than half (55%) of the isolates were from the respiratory tract, followed by musculoskeletal tissue (17%), eye (10%), and abdomen (5%) (Table 1). Overall, 391 isolates (64%) were from sterile sites or bronchoalveolar lavage/wash specimens.

Aspergillus spp. were the most common (303/614, 49%), with *A. fumigatus* complex isolates the most frequent (231 isolates; 76% of all aspergilli and 38% of all isolates). The other five groups were *Fusarium* spp. (72, 12%), *Scedosporium apiospermum* complex (54, 9%), *Lomentospora prolificans* (16, 3%), other hyaline moulds (44, 7%), dematiaceous moulds (80, 13%), and the *Mucorales* (45, 7%) (Table 2).

Susceptibility results

A small number of isolates did not have results recorded for all antifungal agents.

Table 1 Sources of mould isolates having susceptibility testing performed 2001–2019

Site	*n*	%
Respiratory[a]	337	55
Musculoskeletal tissue[b]	107	17
Eye	59	10
Abdomen[c]	31	5
Ear	15	2
Skin	15	2
Aspirate	11	2
Other[d]	39	6
Total	614	

[a] Bronchial wash/lavage 164 (49%), sputum 83 (25%), sinus aspirate/wash/tissue 42 (12%), lung tissue 17 (5%), chest aspirate/drain fluid 14 (4%), tracheal aspirate 12 (4%), tissue other 5.
[b] Finger 23 (21%), arm/shoulder 14 (13%), spine 1, hip 4 (4%), leg/thigh 27 (25%), foot 35 (33%), other 3.
[c] CAPD fluid 24 (77%), tissue/pus 6 (19%), other 1.
[d] Wound (8), central nervous system (8), blood (7), nail (5), tissue (5), mouth (2), urine (1), not recorded (3).

AMB had modest activity against most groups tested apart from *Fusarium* spp., *Scedosporium apiospermum* complex and *Lomentspora prolificans* (Table 2). AMB had the most potent activity against the *Mucorales* with its lowest GeoM of 0.34 mg/L. The AMB MICs for most *A. nidulans* (7/10) and all *A. terreus* isolates were ≥2 mg/L. The echinocandins had good activity for aspergilli, other hyaline and dematiaceous moulds. They had no useful activity against any *Fusarium* spp., *L. prolificans* or *Mucorales* isolates (Table 2). MIF tended to have lower GeoMs than CAS or AND, and the $MIC_{50/90}$ values for AND and MIF were lower than CAS. Amongst the azoles ITC, POS and VCZ all had activity for groups other than *Fusarium* spp. and *L. prolificans* (Table 2). POS tended to have the lowest GeoM amongst ITC, POS and VCZ. VCZ had higher GeoM against the *Mucorales* (Table 2).

Only AMB had useful activity among *Fusarium* spp. The next least susceptible group was *L. prolificans* (Table 2). For the aspergilli, other hyaline moulds and dematiaceous moulds both the echinocandins and azoles had low *in vitro* $MIC_{50/90}$ and GeoM values. The azoles had low GeoMs for the *Mucorales* for which AMB was also active. For the *S. apiospermum* complex amongst the azoles VCZ had the lowest $MIC_{50/90}$ and GeoM values followed by POS and ITC. There were six *S. aurantiacum* isolates and for five the AMB MICs were ≥8 mg/L, and VCZ had lower MICs than POS or ITR. Amongst the dematiaceous isolates: for *Alternaria* spp. (*n*=9) all AMB MICs were ≤1 mg/L and azole MICs were similar; for *Exophiala dermatitidis* (*n*=20) and *Exophiala jeanselmei* complex (*n*=9) all AMB MICs were ≤1 mg/L and VCZ and ITR MICs were similar; for *Pleurostomophora richardsiae* (*n*=10) the AMB MICs for six isolates were ≥2 mg/L and azoles had equivalent MICs.

CLSI have recently published interpretive criteria for VOR and *A. fumigatus sensu stricto*: susceptible ≤0.5 mg/L, intermediate 1 mg/L, and resistant ≥2 mg/L.[11] The three isolates where the MIC was ≥2 mg/L were all non-*sensu stricto* isolates (Supplementary Table 5, Appendix A). Three isolates met the intermediate criterion but were not identified by molecular methods and may contain non-*sensu stricto* isolates. At least 204 isolates (97%) were susceptible.

Table 2 Susceptibility of mould isolates to eight antifungal agents: 2001–2019

Group	Descriptor	AMB	CAS	MIF	AND	ITC	POS	VCZ
Aspergillus spp.[a]	*n*	303	243	158	163	301	247	282
	MIC range	0.125–>8	≤0.008–1	<0.008–0.015	≤0.015–0.12	0.016–>16	≤0.008–2	0.03–8
	MIC $_{50/90}$	1/2	0.03/0.06	<0.008/0.015	≤0.015/0.03	0.25/0.5	0.12/0.25	0.12–0.5
	GeoM	1.4	0.06	0.015	0.03	0.72	0.18	0.35
Fusarium spp.[b]	*n*	69	48	15	15	69	49	64
	MIC range	0.5–8	8–>16	>8	>8	1–>16	1–>8	0.5–>8
	MIC $_{50/90}$	2/4	>16/>16	>8/>8	>8/>8	>16/>16	>8/>8	4/8
	GeoM	2.0	32	16	16	5.7	16	2.8
Scedosporium apiospermum complex[c]	*n*	53	36	33	323	52	42	49
	MIC range	0.25–>8	0.25–>8	0.12–>8	0.25–>8	0.06–>16	0.12–2	0.06–1
	MIC $_{50/90}$	4/>8	2/>8	0.25/8	2/8	0.5/1	0.5/1	0.12/0.5
	GeoM	2.8	2	1.4	2	1.4	0.49	0.24
Lomentospora prolificans	*n*	16	10	6	6	16	11	14
	MIC range	1–>16	8–>16	>8	4–>8	0.5/>16	0.5–>8	0.125–>16
	MIC $_{50/90}$	4/>8	>8/>8	–	–	>16/>16	>8/>8	2/8
	GeoM	5.7	16	16	8	4	4	2
Other hyaline moulds[d]	*n*	39	28	19	19	40	26	35
	MIC range	0.06–>16	<0.008–>16	<0.008–>8	≤0.015–>8	0.03–>16	0.015–>8	0.015–>16
	MIC $_{50/90}$	2/>8	4/>16	0.25/>8	2/>8	1/>16	0.5/2	0.5/>8
	GeoM	1.4	0.36	0.25	0.36	0.98	0.49	0.69
Dematiaceous moulds[e]	*n*	80	43	15	15	79	51	67
	MIC range	0.25–8	0.12–≥16	0.015–>8	<0.015–>8	<0.015–>16	0.015–2	0.015–2
	MIC $_{50/90}$	0.5/2	8/>16	0.25/>8	0.25/8	0.25/0.5	0.12/0.5	0.12/1
	GeoM	1.4	1.9	0.49	0.35	0.51	0.11	0.17
Mucorales isolates[f]	*n*	44	34	14	14	44	33	40
	MIC range	≤0.12–2	16–>16	>8	>8	0.06–>16	0.12–>8	2–16
	MIC $_{50/90}$	0.5/1	>16/>16	>8/>8	>8/>8	1/8	0.5/2	16/>16
	GeoM	0.34	23	16	16	1.5	1.4	8

AMB, amphotericin B; AND, anidulafungin; CAS, caspofungin; ITC, itraconazole; MIF, micafungin; POS, posaconazole; VCZ, voriconazole.
MIC $_{50/90}$, minimal inhibitory concentration for 50% and 90% of strains respectively. Represents minimal effective concentrations (MECs) for CAS, MIF and AND. GeoM, geometric mean MIC/MEC. MIC/MEC values in mg/L.
[a] *Aspergillus flavus* complex (n=22), *A. fumigatus* complex (232), *A. nidulans* complex (11), *A. niger* complex (20), *A. terreus* complex (15), *Aspergillus* spp. (3).
[b] *Fusarium oxysporum* complex (10), *F. solani* complex (45), *Fusarium* spp.(17).
[c] *Scedosporium apiospermum* (40), *S. boydii* (1), *S. dehoogii* (6), *S. aurantiacum* (6), *Scedosporium* sp. (1).
[d] *Acremonium kiliense* (2), *Acremonium strictum* (1), *Arthrographis kalrae* (4), *Beauveria bassiana* (1), *Gliocladium* sp. (1), *Coniochaeta mutabilis (1), Paecilomyces variotii* (5), *Penicillium* spp. (4), *Phialemoniopsis curvata* (1), *Plectosporium tabacinum* (1), *Purpureocillium lilacinum* (15), *Rasamsonia argillacea* (2), *Scopulariopsis* sp. (1), *Trichoderma harzianum* (1), *Trichoderma pseudokoningii* (1), *Trichoderma* spp. (2), other (1).
[e] *Acrophialophora fusipora* (2), *Alternaria* spp. (9), *Aureobasidium pullulans* (1), *Bipolaris australiensis* (1), *Curvularia* spp. (3), *Exophiala dermatitidis* (20), *Exophiala jeanselmei* complex (9), *Exophiala* spp. (7), *Fonsecaea pedrosoi* (3), *Neoscytalidium dimidiatum* (4), *Verruconis gallopava* (2), *Phaeoacremonium* spp. (6), *Phialemonium dimorphosporum* (1), *Phialophora* sp. (1), *Pleurostomophora richardsiae* (10), *Rhinocladiella atrovirens* (1).
[f] *Cunninghamella bertholletiae* (1), *Lichtheimia corymbifera* (12), *Lichtheimia* sp. (1), *Mucor* spp. (14), *Rhizopus microsporus* (11), *Rhizopus* spp. (5), *Syncephalastrum racemosum* (1).

Non-wild type isolates

For CLSI derived ECVs there were few non-WT isolates (Table 3; Supplementary Tables 1–5, Appendix A). For *Aspergillus fumigatus* complex there four non-WT isolates, all identified by β-tubulin gene sequencing: an isolate of *A. lentulus* non-WT for AMB (MIC 8 mg/L), ITC (MIC 2 mg/L), POS (MIC 1 mg/L) and VCZ (MIC 8 mg/L); another *A. lentulus* non-WT for AMB (MIC 8 mg/L), and VCZ (MIC 4 mg/L); an isolate of *Neosartorya pseudofischeri* (*A. thermomutatus*) was non-WT for VCZ (MIC 2 mg/L) but WT for both ITC and POS (MICs 0.5 and 0.25 mg/L respectively); an *A. fumigatus sensu stricto* isolate was non-WT for both ITC and POS (MICs of 16 and 2 mg/L, respectively) but WT for VCZ (MIC 0.25 mg/L). All *A. flavus* complex, *A. nidulans* complex, A. *niger* complex and *A. terreus* complex were WT. All *Aspergillus* isolates were WT for CAS (Supplementary Table 2. Appendix A). There was one non-WT *Mucorales* isolate, a *Rhizopus microsporus* isolate, non-WT for POS. For *Fusarium solani* it was not possible to determine the proportion of non-WT for POS and VCZ because many MICs were >8 mg/L and the ECV is 32 mg/L. There were two *F. oxysporum* isolates non-WT for POS, both of which were WT for VCZ. For *Scedosporium apiospermum* itself, identified phenotypically, there were two, four, and one non-WT isolates for CAS, MIF, and AND, respectively. All were WT for ITC, POS and VCZ (Table 3).

Recently published SYO derived ECVs for VCZ for *A. flavus* complex, and *A. terreus* complex and ITC for *A. niger,* are all 1 mg/L vs 2–4 mg/L for CLSI derived ECVs.[4,16] The VCZ ECV for *A. fumigatus* complex is 1 mg/L for both methods.[4,16] The SYO method specific ECVs identified one non-WT isolate for VCZ for *A. flavus* (Table 3; Supplementary Table 5, Appendix A).

The two other laboratories performing mould susceptibility testing had been doing so from at least 2010. In 2019 there were 93 moulds tested in the country; 77 (83%) here and 16 (17%) in the other two laboratories (L. Sanders and J. Creighton, personal communication). We estimate that for 2001–2019 our testing volume represented 85–90% of all mould susceptibility tests undertaken in New Zealand.

Table 3 Epidemiological cut-off values (mg/L) for moulds and the proportion of wild-type mould isolates in New Zealand: 2001–2019

Species/complex	AMB		CAS		MIF		AND		ITC		POS		VCZ		Assay reference, ECV reference
	ECV	≤ ECV *n* (%)	ECV	≤ ECV *n* (%)	ECV	≤ ECV *n* (%)	ECV	≤ ECV *n* (%)	ECV	≤ ECV *n* (%)	ECV	≤ ECV *n* (%)	ECV	≤ ECV *n* (%)	
Aspergillus flavus	4	22 (100)	0.5	14 (100)					1	21 (100)	0.5	19 (100)	2	20 (100)	10, 4
Aspergillus fumigatus	2	229 (98.7)	0.5	187 (100)					1	230 (99.1)			1	207 (98.6)	10, 4
Aspergillus fumigatus											0.5	187 (98.9)			9, 12
Aspergillus nidulans									1	11 (100)	1	8 (100)	2	10 (100)	9, 12
Aspergillus niger	2	20 (100)	0.3	17 (100)					4	20 (100)	2	16 (100)	2	17 (100)	10, 4
Aspergillus terreus	4	15 (100)	0.1	10 (100)					2	15 (100)	1	12 (100)	2	15 (100)	10, 4
Aspergillus flavus													1	19 (95)	16, 16
Aspergillus fumigatus													1	207 (98.6)	16, 16
Aspergillus niger									1	20 (100)					16, 16
Aspergillus terreus													1	15 (100)	16, 16
Fusarium oxysporum	8	10 (100)							32	All >16	8	8 (80)[a]	16	10 (100)	9, 14
Fusarium solani	8	45 (100)							32	All >16	32	4 (13)[b]	32	34 (85)[c]	9, 14
Scedosporium apiospermum	16	32 (80)[d]	8	25 (93)	1	27 (87)	8	30 (97)	16	39 (100)	2	28 (100)	4	35 (100)	9, 15
Scedosporium boydii	16	1 (100)	8	–	1	–	8	–	16	1 (100)	4	1 (100)	2	1 (100)	9, 15
Lichtheimia corymbifera	2	12 (100)									2	8 (100)			9, 13
Rhizopus arrhizus	4	1 (100)							2	1 (100)	2	1 (100)			9, 13
Rhizopus microsporus	2	11 (100)									2	9 (90)			9, 13

[a] Two were >8 mg/L.
[b] 27 were >8 mg/L.
[c] Six were >8 mg/L.
[d] Eight were >8 mg/L.

DISCUSSION

This study establishes significant baseline data for the fungal susceptibility of New Zealand moulds from clinical specimens.

High AMB MICs were only observed in *Aspergillus* spp. among *A. fumigatus* complex isolates. The AMB MIC for both *A. lentulus* isolates (8 mg/L) appears higher than that reported for five Australian isolates (all ≤2 mg/L) but the small numbers, as well as biological and inter-laboratory variability, limit comparison between countries.[6] The AMB MICs for both *A. nidulans* and *A. terreus* support the poor clinical responses reported for these clinically important species complexes.[17,18]

Azole resistance in *A. fumigatus* was first found in isolates obtained in the late 1980s. Resistance is now encountered in many countries, with 3.2% prevalence (range 0–26%).[19] Azole resistance may vary within a country, and is a rapidly evolving problem.[20] Importantly cross resistance within the class is common, limiting treatment options.[21] Resistant isolates are encountered in azole naive patients and the mortality rate for azole resistant invasive aspergillosis is very high.[20,21] Azole resistance is uncommon in Australia. Retrospective review of clinical *A. fumigatus* isolates tested 2000–2013 at the Australian National Mycology Reference Centre found that the MICs for nine of 418 (2%) isolates were above the ECVs.[5] More recent surveillance also reported a 2% rate of resistance in clinical isolates but none in 185 environmental isolates.[8] It is reassuring that the non-WT for azole agents was low in New Zealand, only four *Aspergillus* isolates (0.7–1.1% of isolates for which ECVs exist), and complete 'cross-resistance' within the class was only observed in one *A. lentulus* isolate. At least 97% of *A. fumigatus* isolates were susceptible by the recent interpretive criterion for VOR.[11] When SYO derived ECVs were applied, one *A. flavus* isolate was non-WT for VCZ.

We did not encounter CAS non-WT isolates in local *Aspergillus* isolates and the MICs for AND and MIF for *Aspergillus* spp. were low. However, this *in vitro* activity should not be taken as indicating clinical efficacy. The lack of adequate clinical data means that the echinocandins have only a low grade recommendation as second line agents, after VOR and a lipid form of AMB, for invasive aspergillosis.[22] Non-WT *A. fumigatus* isolates for CAS make up ~3% in Australian isolates and clinical resistance is rare.[6,23] Echinocandin MEC values should not be used to drive treatment decisions. We use selective reporting of antifungal agents as part of local antimicrobial stewardship and do not report echinocandin results for *Aspergillus* isolates. It would be useful to have more SYO generated ECVs for common moulds.

The results for *Fusarium* spp. are in line with those of others including Australian isolates.[2,6,14,24–26] The echinocandins were inactive. Amphotericin B MICs for a reasonable proportion of *F. solani* isolates were ≤1 mg/L as reported by others.[6,14,25,26] The better activity of voriconazole compared to other azoles was also observed.[2,6,14,24] The *F. oxysporum* isolates were all WT for AMB and VCZ. For other antifungal *Fusarium* spp. pairings it was not possible to determine the complete WT proportion because the highest dilution in the SYO assay did not cover the relevant published ECVs.[14]

The MIC distributions of most antifungal agents against *Scedosporium* spp. do not show a normal distribution, making species based predictions of susceptibility results difficult and leading to the call that testing of all isolates is essential for guiding antifungal treatment.[27] New Zealand *S. apiospermum* complex isolate results closely mirror Australian results, namely modest echinocandin activity,

AMB MICs mostly ≥1 mg/L, and best activity seen in the azoles, particularly POS and VCZ.[6] For *L. prolificans* (previously *S. prolificans*), the echinocandins were inactive, AMB MICs were mostly ≥2, and azoles were effectively inactive, with VCZ having the lowest MICs and for our isolates the lowest GeoM.[6] Australasian results reflect global susceptibility patterns.[27,28] The new antifungal agent, olorofim, has been reported to have low MICs for *Scedosporium apiospermum* and *L. prolificans*, with a MIC_{90} value of 0.25 mg/L for Australian isolates of both species.[28] It is hoped that the current salvage treatment study using olorofim will show efficacy for what are currently practically impossible to treat *L. prolificans* infections. The susceptibility of the *S. aurantiacum* isolates matched a previous report, with AMB MICs being ≥8 mg/L and with VCZ having the lowest MICs/GeoM.[27] ECVs for *S. apiospermum* should be regarded as preliminary because although they meet most CLSI requirements for generating ECVs, e.g., MICs for ≥100 isolates from ≥ three laboratories and isolate identification by molecular methods, they have not been formally published,[15] although the data on which they are based has.[27] Isolates were all WT for ITC, POS and VCZ, and most were WT for AMB and CAS.

For other hyaline moulds all the echinocandins and azoles were active. The results closely resemble the susceptibility of Australian isolates for the main species tested, namely *Paecilomyces variotii* and *Purpureocillium lilacinum*.[6] For the dematiaceous moulds the AMB MICs and relative azole activities reflect those reported for Australian isolates, apart from local *E. jeanselmei* complex where the azole MIC distributions were similar, whereas the VCZ MICs tended to be higher in Australian isolates; however, the isolate antifungal pairings available for comparison were small, 7–10 and 6–9, respectively.[6]

For *Mucorales*, mainly *Lichtheimia corymbifera*, *Rhizopus microsporus* and *Mucor* spp., the AMB MICs were ≤1 mg/L for most isolates as reported in Australia and elsewhere.[6,13,29] As expected the echinocandins were inactive. The GeoM of ITC and POS indicated greater activity than VCZ.

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 in one laboratory, using the same method by a small number of staff (mainly KR, and WPM). However, there are limitations to the study. Our data are not a complete summary of testing in the country as two other laboratories also perform susceptibility testing; nevertheless, our report covers 85–90% of tests performed during the 19 year study period. Our referred isolates may have been more likely to have been sent because of clinical relapse/failure after or during antifungal therapy. While this may have increased the number of isolates exposed to antifungal agents and therefore more likely to be non-WT, these isolates were rare. Neither clinical nor treatment data were available for analysis and only a few isolates were identified by molecular methods. 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.[30] This report shares the limitations of similar summaries on mould susceptibilities including: differences in patient mix; prevalence of different resistance mechanisms; different end points between antifungal agents; and ability to detect tolerance to antifungal agents. As such the results reflect a best case estimate of susceptibility given the inherent limitations of testing methods for moulds. All our testing is based on CLSI methods and another well validated method exists, i.e., the European Committee on Antimicrobial Susceptibility Testing (EUCAST) method. While not currently used locally for mould susceptibility testing this is likely to change, as it has recently for bacterial susceptibility testing. However, there are important method differences between CLSI and EUCAST and results generated by one method must not be interpreted with criteria used by the other.[31] Lastly, we have not had any molecular testing performed for acquired mechanisms of azole resistance. This would be informative given the association between non-WT MICs and the frequent presence of known resistant mechanisms.[5,8,23,32,33]

In conclusion, while acknowledging the limitations listed above, local mould isolates had broadly similar susceptibility profiles to those reported elsewhere, particularly Australia. Non-WT strains for azoles were rare and three of the four non-WT isolates were in cryptic species within the *A. fumigatus* complex. The results add to the global surveillance of antifungal resistance. Importantly our local susceptibility profiles provide further regional data supporting current Australasian treatment guidelines.[7,22]

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APPENDIX A. SUPPLEMENTARY DATA

Supplementary data to this article can be found online at https://doi.org/10.1016/j.pathol.2020.09.030.

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