Impact of antifungal resistance in Australia

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The last two to three decades have seen a major increase in invasive fungal infections (IFIs), a small, but increasing proportion of which are caused by pathogens with partial or complete resistance to antifungal drugs. The increase in IFIs has largely been associated with the increase in immunocompromised and critically ill patients. Opportunistic infections with relatively drug-resistant environmental fungi account for much of the resistance. In addition, amongst the only fungal species to colonise humans, *Candida*, two species that are resistant *(C. krusei)* or relatively resistant *(C. glabrata)* to fluconazole have emerged. In part this is explained by the selection pressure exerted by widespread use of fluconazole. Together with the introduction of new antifungal drugs with selective and/or variable antifungal activity, these changes have stimulated interest in understanding mechanisms and origins of resistance, the identification of resistance in the laboratory and its relationship to clinical outcomes, and in surveillance of clinical isolates and populations at risk of IFIs.

Definitions of resistance

Primary drug resistance is that identified *in vitro* without previous exposure to an antifungal drug. Secondary resistance is stable resistance that is induced by exposure to an antifungal drug *in vivo* or *in vitro*. Most examples of resistance in fungal pathogens are primary in type. A notable exception is resistance of yeasts to flucytosine. This is seen frequently when flucytosine is used as a single agent to treat such infections.

Mechanisms of resistance

Most information is available for resistance of yeasts to azole drugs. There are three distinct types of mechanism: 1) Failure of accumulation of drug within the fungal cell due to increased efflux (via upregulation of multidrug efflux transporter genes), or reduced influx; 2) Reduced affinity for the P450-dependent enzyme target of azoles, Erg11p (lanosterol 14α-demethylase) or, in some cases, upregulation of the responsible gene *ERG11*; 3) Altered sterol composition. Resistance secondary to upregulation of the multidrug transporter genes is the most common mechanism [1].

Identification of resistance *in vitro* and correlation with clinical outcome

Susceptibility testing for yeasts and filamentous fungi has been standardised by the Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS) using broth dilution methods. These are still subject to inter- and intra-laboratory variability especially

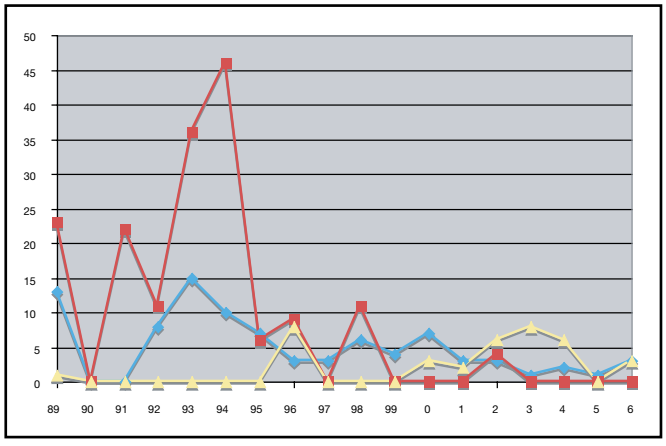
when testing filamentous fungi. As an example, breakpoints aimed at correlating susceptibility with clinical outcome have been established for fluconazole, itraconazole and voriconazole when tested against *Candida albicans,* but in limited clinical settings, e.g. oropharyngeal candidiasis in HIV-infected patients [3]. For some drugs, e.g. amphotericin B, testing on solid agar seems to be more reproducible.

Clinical outcomes are influenced not only by intrinsic susceptibility of the fungus, but also dose and pharmacokinetics of the drug, timing of initiation of therapy and host response factors. Thus, knowledge of the minimal inhibitory concentration (MIC) of drug can only be a guide to antifungal selection even in settings where it has been correlated with clinical outcome. In general, MIC predicts clinical resistance more accurately than susceptibility. It has been suggested that with *Candida,* a MIC indicative of susceptibility will correlate with clinical responsiveness 60% of the time and a MIC predictive of resistance will correlate with clinical responsiveness 40% of the time [2].

Surveillance for resistance

MICs are useful in identifying trends in antifungal susceptibility (or resistance) in common and emerging pathogens over time. Data from the Australian Mycology Reference Laboratory (Women's and Children's Hospital, Adelaide) of the rates of resistance of selected pathogens, recorded from 1989–2006, are shown in Figure 1. Fluconazole resistance in *C. albicans* and *Cryptococcus neoformans* peaked around 1993–1994 at the height of the HIV/AIDS epidemic in Australia. However, since the introduction of HAART in late 1996, resistance rates have dropped dramatically (Figure 1). Resistance to itraconazole among *Aspergillus fumigatus* is uncommon and appeared to peak around 2003, but has since declined with the introduction of voriconazole.

Figure 1. Percentage resistance (y axis) over time (1989–2006; x axis) for *Candida albicans* against fluconazole (blue line), *Cryptococcus neoformans* against fluconazole (red line) and *Aspergillus fumigatus* against itraconazole (yellow line).



The rates of resistance to antifungal drugs vary with the fungal species and antifungal agent for a given species (Tables 1 and 2). Among *Candida species* recovered from blood, resistance to amphotericin B and flucytosine remain rare. *Candida krusei* was predictably resistant to fluconazole but all isolates were susceptible to voriconazole and posconazole. Azole resistance among *C. glabrata* was substantial (Table 1) with 10% of isolates cross-resistant to all azoles, including voricoanzole and posaconazole. Of note, nearly 10% of *Candida parapsilosis*, *Candida guilliermondi* and *C. krusei* had MICs to caspofungin that were considered 'resistant' (Table 1). Table 2 shows that although there are no break points established for voriconazole susceptibility for moulds, resistance to voriconazole among *Aspergillus* species has not been encountered in Australia. The survey of *Scedosporium* and other mould isolates indicates that *in vitro* resistance is common for most antifungal agents but *Scedosrpoium apiospermum* is susceptible to voriconazole and posconazole (Table 2).

Resistance to flucytosine

There is a high prevalence of flucytosine resistance in yeasts in the US (up to 8% of *C. albicans* blood stream isolates in a recent report) [4]. This contrasts with figures of 1.6% in Australia and 0.2% in Europe (current article) [5]. The major problem with flucytosine is the frequency and speed at which isolates develop resistance during therapy. Since this has been identified as a major cause of treatment failure, monotherapy with flucytosine is not recommended. Combination therapy with AMB significantly reduces clinical resistance and flucytosine is now mainly used with AMB for treatment of neuro- or disseminated cryptococcosis.

Resistance to polyenes

With most fungal species the range of MICs of amphotericin B determined by CLSI-based methods is too small to discriminate effectively between 'resistant' and 'susceptible'. Although E-tests and other solid agar based methods appear promising, to date, no solution to this problem has been agreed upon. Temporal trends of amphotericin B susceptibility among *Candida* species in Australia indicate that MICs of most species remain low at < 1.0 mg/L with the exception of *C. krusei* (MIC_{90} 1.0 mg/L).

It is recognised that certain *Candida* species (specifically *C. lusitaniae*, *C. lipolytica*, *C. krusei*, *C. guillermondii* and some *C. albicans)* are intrinsically resistant to, or have a propensity to

develop resistance to, AMB [6] but it has been difficult to estimate this prevalence with certainty. Whilst resistance to AMB can be readily induced *in vitro*, the rate at which secondary resistance develops during therapy cannot be effectively quantified. However, the consensus of opinion leaders is that it is low [1], a view supported by our data (Table 1). Failure of AMB therapy including breakthrough candidemia, attributable to development of AMB resistance, has also remained uncommon [1] with only isolated cases of acquired resistance among *Candida* species described, especially in cancer patients [7]. In cancer patients there is some evidence of a correlation between failure of AMB therapy and *in vitro* resistance and clinicians should remain vigilant for treatment failure. The emergence of *Candida* and *C. neoformans* strains with cross-resistance to azoles and polyenes has been occasionally reported in HIV infected patients after prolonged azole exposure [1].

Primary resistance to AMB has emerged in parallel with an increase in infections due to uncommon environmental fungi. In Australia, a substantial (70–>95%) of *Scedosporium* and *Fusarium* isolates are resistant to amphotericin B (Table 2) consistent with that in the US [8]. Primary resistance to AMB has been encountered in non-*fumigatus Aspergillus* species, particularly *A. terreus,* the recently described species *A. lentulus* and other species within the Clade *fumigatus* [9]. Although our results indicate that resistance to AMB is uncommon in Australia, given differences in susceptibility, species level identification of *Aspergillus* isolated from clinically important body sites is recommended.

Resistance to triazole antifungal agents

The clinical consequences of triazole resistance are most obvious in *Candida* infections, particularly those associated with AIDS. There is unequivocal evidence that the use of azoles particularly, but not exclusively, fluconazole is a risk factor for secondary azole resistance arising after long-term treatment of oesophageal candidiasis as reviewed by Sanglard and Odds [1]. Fluconazole resistance rates of up to 41% were demonstrated [10]. Widespread azole use is also believed to explain the appearance of *C. dubliniensis* in this population – a species notable for its ability to rapidly develop stable resistance to fluconazole *in vitro* [11]. Since the fall in oesophageal candidiasis following the success of HAART for HIV infection, azole-resistant isolates are now rare (Figure 1). This epidemiological curve suggests that the emergence and disappearance of fluconazole resistance was a function of selective pressure exerted on *Candida* by the prolonged duration and level of drug exposure. Prolonged maintenance of such selective pressure would be expected induce stable resistance and lead to therapeutic failure.

Although the widespread use of azoles as monotherapy in patients with disseminated candidiasis and other deep-seated infections, could create a serious problem [12] there is little evidence of large-scale emergence of resistant Candida strains or species in the

Table 1. Candida resistance* rates for blood stream isolates (Australian Candidemia Study).

Species (no.)	Antifungal agent (frequency [%] resistance to)						
	AMB	**5FC**	**FLU**	**ITR**	**VOR**	**POS**	**CAS**
C. albicans (447)	0	1.6	0	0	0	0	0
C. parapsilosis (182)	0	0.5	0.5	1	0	0	0
C. glabrata (167)	0	0	23	86	10†	10	0
C. krusei (46)	0	0	91	9	0	0	0
C. tropicalis (46)	0	0	0	0	0	0	0
C. dubliniensis (22)	0	4	0	0	0	0	0
C. guilliermondii (11)	0	0	0	36	0	0	0
C. lusitaniae (8)	0	0	0	0	0	0	0

*Resistance is defined as the following MICs (ug/ml): AMB >4; 5FC >32; FLU >64; ITR >1; VOR >4; POS >2; CAS >2 (no breakpoints have been established for posaconazole or caspofungin). † All voriconazole-resistant strains showed cross resistance to all other azoles, but were susceptible to amphotericin B and caspofungin.

clinical setting [1]. In many epidemiological studies of candidemia, decreases in the prevalence of *C. albicans* and increases in non-albicans *Candida* species (especially *C. glabrata*) have been reported. This is important as candidemia due to non-albicans *Candida* species has been associated with increased attributable mortality [13]. MIC data from Australia (Table 1) and elsewhere confirm that *C. glabrata* in particular is less susceptible to fluconazole and itraconazole than *C. albicans,* and *C. krusei* is intrinsically resistant. Selection pressure from the routine use of fluconazole prophylaxis has resulted in the emergence of *C. krusei* and *C. glabrata* infections, including candidemia, in patients with haematological stem cell transplantations. However, examination of published MIC results does not reveal a temporal increase in the prevalence of resistance among blood isolates of *Candida* [1]; a finding supported by our Australian data. Furthermore, although there is evidence that rates of azole-resistant *Candida* isolates vary between geographical regions and institutions [14], there is no evidence that resistant strains predominate in any particular institution. A review of the susceptibility of recently tested isolates in North America and Europe also showed low rates of fluconazole and itraconazole resistance in *C. albicans* [1].

Since the introduction of voriconazole in 2002, similar concerns regarding emergence of resistant strains have arisen. Our data

Table 2. Aspergillus, Scedosporium, Fusarium and Rhizopus resistance rates.

Species (n)	Frequency (%) of resistance to:			
	AMB	**ITR**	**VOR**	**POS***
A. flavus (28)	32	0	0	0
A. fumigatus (191)	6	2	0	0
A. niger (26)	0	0	0	0
A. terreus (29)	48	0	0	0
S. apiospermum (87)	70	26	0	0
S. prolificans (134)	93	100	81	100
F. oxysporium (14)	78	56	33	nd
F. solani (21)	86	100	14	nd
Rhizopus sp. (9)	0	71	100	0

*For the purposes of this article resistance is defined as the following MICs (ug/ml): AMB >4; ITR >1; VOR >4; POS >2 (No breakpoints have been established for moulds; nd = not done).

Table 3. Synergy testing results for S. prolificans against terbinafine in combination with voriconazole or itraconazole.

Drug combination	∑FIC < 0.5 (S)	∑FIC > 0.5-4 (NS)	∑FIC > 4 (A)	
Voriconazole/Terbinafine (n=109)		94 (86.2%)	15 (13.8%)	0
Itraconazole/Terbinafine (n=93)		56 (60.2%)	37 (39.8%)	0

The summation of the fractional inhibitory concentration (ΣFIC) was calculated as follows: (MIC agent A in combination/MIC agent A alone) + (MIC agent B in combination/MIC agent B alone). Synergy was defined as a ΣFIC of ≤ 0.5; No synergy >0.5 - 4; Antagonism > 4.

indicate almost all *Candida* species are very susceptible to voriconazole. A small subset (10%) of *C. glabrata* isolates were resistant (MICs ≥4); these exhibited cross-resistance to all azoles including posaconazole (Table 1). Resistance to multiple triazole drugs including itraconazole, voriconazole and posaconazole was reported recently in *A. fumigatus* in the Netherlands [16]. MICs of other filamentous fungi are not always predictable. For example, voriconazole exhibits relatively high MICs against *Scedosporium prolificans* compared with *S. apiospermum* and these are often associated with therapeutic failure (Table 2). *Fusarium* isolates show variable MICs against voriconazole and posaconazole and the only azole with activity against zygomycetes is posaconazole. For some intrinsically resistant moulds, like *S. prolificans, in vitro* synergy studies for the combination of voriconazole and terbinafine may prove useful (Table 3). Terbinafine appears to be synergistic with voriconazole and itraconazole against most isolates of *S. prolificans*. Anecdotal experience indicates that combination therapy results in clinical benefit.

Resistance to echinocandins

Because the echinocandins have been in clinical use only since 2001, the clinical significance of strains which test 'resistant' *in vitro* is unclear. As seen in Table 1, caspofungin is highly active against all *Candida* species including *C. parapsilosis* in Australia (MIC_{90}1.0 mg/L). Based on MIC determinations for blood stream isolates, MICs of caspofungin and other echinocandins are higher for *C. parapsilosis* than for other common *Candida species* (this article, [16]). This difference has raised concern that *C. parapsilosis* might respond less well than other *Candida* or require higher doses of drug. However, in clinical trials there has been no evidence of reduced responsiveness of small numbers of *C. parapsilosis* infections to echinocandin therapy [17]. In another analysis, MIC results of caspofungin for *Candida* species did not correlate with clinical outcome [18]. On the basis of these limited data, *in vitro* susceptibility results may not be clinically useful. Cross-resistance between echinocandins is usually complete.

We found no increased MICs ('resistance') to caspofungin among Australian isolates of *Aspergillus* species At the time of writing, the drug is only licensed for the salvage treatment of invasive aspergillosis (IA). However, there are numerous case reports detailing its efficacy in the primary therapy of IA. Other moulds including *Scedosporium*, *Fusarium* and the zygomycetes are resistant to all echinocandins.

Conclusion

The selection of an antifungal agent is dependent on both the causative pathogen and clinical setting. Resistance to currently available antifungal drugs is uncommon among *Candida* species with two main exceptions – *C. krusei* is resistant to fluconazole and up to 30% of *C. glabrata* isolates may be azole-resistant, including 10% that are resistant to voriconazole and posaconazole. Azole resistance in *Aspergillus* species is rare. Many non-*Aspergillus* moulds are less susceptible or resistant to most currently available antifungal agents. Antifungal susceptibility testing for clinically significant fungal isolates is recommended.

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