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ORIGINAL ARTICLE

Antifungal susceptibilities of *Candida, Cryptococcus neoformans* and *Aspergillus fumigatus* from the Asia and Western Pacific region: data from the SENTRY antifungal surveillance program (2010–2012)

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The SENTRY Antifungal Surveillance Program monitors global susceptibility rates of newer and established antifungal agents. We report the *in vitro* activity of seven antifungal agents against 496 contemporary clinical isolates of yeasts and molds. The isolates were obtained from 20 laboratories in the Asia-Western Pacific (APAC) region during 2010 through 2012. Anidulafungin, caspofungin, micafungin, fluconazole, itraconazole, posaconazole and voriconazole were susceptibility tested using CLSI methods and species-specific interpretive criteria. Sequencing of *fks* hot spots was performed for echinocandin-resistant strains. Isolates included 13 species of *Candida* ($n=460$), 5 species of non-*Candida* yeasts (21), 5 species of *Aspergillus* (11) and 4 other molds. Echinocandin resistance was uncommon among eight species of *Candida* and was only detected in three isolates of *Candida glabrata*, two from Australia harboring mutations in *fks1* (F625S) and *fks2* (S663P). Resistance to the azoles was much more common and was observed among all species with the exception of *Candida dubliniensis*. Fluconazole resistance rates observed with *C. glabrata* (6.8%) was comparable to that seen with *Candida parapsilosis* (5.7%) and *Candida tropicalis* (3.6%). Cross resistance among the triazoles was seen with each of these three species. The mold-active azoles and the echinocandins were all active against isolates of *Aspergillus fumigatus*. Azole resistance was not detected among the isolates of *Cryptococcus neoformans*. Antifungal resistance is uncommon among isolates of fungi causing invasive fungal infections in the APAC region. As in other regions of the world, emerging resistance to the echinocandins among invasive isolates of *C. glabrata* bears close monitoring.
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INTRODUCTION

Surveillance programs devoted to tracking the occurrence of invasive fungal infections (IFI) have provided a wealth of information regarding emerging species and the frequency of antifungal resistance.[1–9] The increasing incidence of IFIs in the Asia and Western Pacific (APAC) regions have resulted in both small- and large-scale surveillance efforts in Australia,[10,11] India,[12,13] Japan,[14] Korea,[15–18] Malaysia,[19] Singapore,[20] Taiwan[21–27] and several other areas.[8,9] In contrast to data from North America and Europe,[1,7] *Candida glabrata* and *Candida krusei* are much less prominent as causes of IFI than either *Candida tropicalis* or *Candida parapsilosis* in several areas of the APAC region. Furthermore, resistance to fluconazole is much more common in isolates of *C. tropicalis* from Asia (11–15%) than in those from North America (2.7%) or Europe (1.1%).[7,22,27]

One of the limitations of the existing surveillance data from the APAC region is that most reports have not used the new species-specific interpretive criteria for the azoles and echinocandins against *Candida* and *Aspergillus*. In addition, many of these reports are limited to a single institution and most fail to compare results across several different countries or cities. The SENTRY Antimicrobial Surveillance Program is a survey that has been active since 1997 and has reported the frequency of pathogen occurrence and the susceptibilities to various antifungal agents on a global scale.[7,28] In the present study, we summarize the results of the APAC component of the SENTRY Program for the years 2010 through 2012, comparing the activities of three echinocandins and four triazoles tested against a collection of 496 isolates of *Candida* (460 isolates, 13 species), non-*Candida* yeasts (21 isolates, 3 species), *Aspergillus* (11 isolates, 5 species) and non-*Aspergillus* molds (4 isolates, 4 species). Data from institutions in China are summarized in a separate publication (Pfaller *et al.*,[29,30]). We have used molecular methods to confirm the identification of the less common species of *Candida*, as well as those of the non-*Candida* yeasts and all of the filamentous fungi. Furthermore, we applied the newly revised clinical breakpoints for the echinocandins, fluconazole and voriconazole to determine the resistance profiles of various *Candida* species[31] and the epidemiological cutoff values (ECVs) for these agents, as well as itraconazole and posaconazole, to detect emerging, resistance among less common species of *Candida*[31] and

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among isolates of *Aspergillus fumigatus*[32] and *Cryptococcus neoformans*.[33]

MATERIALS AND METHODS

Organisms

A total of 496 clinical isolates from patients with IFI were collected during 2010 through 2012 from 20 different laboratories in Australia (6 sites, 177 isolates), Hong Kong (1 site, 23 isolates), India (3 sites, 7 isolates), Korea (4 sites, 87 isolates), New Zealand (2 sites, 64 isolates), Singapore (1 site, 65 isolates), South Africa (1 site, 18 isolates), Taiwan (1 site, 3 isolates) and Thailand (1 site, 52 isolates) as part of the SENTRY Program (Table 1). In each case, collection was approved by the appropriate institutional review board. Each participating center recovered consecutive, non-duplicated isolates from patients with bloodstream infections, normally sterile body fluids, abscess, and tissue samples and respiratory tract infections (*Aspergillus* and other molds only). Isolates were identified at the participating institutions using methods routinely used at the submitting laboratory, including Vitek, MicroScan, API and Auxacolor, supplemented by classical methods for yeast and mold identification.[34,35] Isolates were submitted to JMI Laboratories (North Liberty, IA, USA), where the identification was confirmed by morphological, biochemical and molecular methods (all non-*Candida* yeasts and all molds) as described previously.[7,29]

Among the 460 isolates of Candida, there were 197 isolates of *C. albicans*, 88 of *C. glabrata*, 88 of *C. parapsilosis*, 55 of *C. tropicalis*, 8 of *Candida dubliniensis*, 7 of *Candida guilliermondii*, 5 of *C. krusei*, 5 of *Candida lusitaniae* and 6 of miscellaneous Candida spp. (2 *Candida* fabianii, 1 *Candida haemulonii*, 1 *Candida kefyr*, 1 *Candida metapsilosis*, and 1 *Candida orthopsilosis*). The collection also included *C. neoformans* (16 isolates), *Lodderomyces elongisporus* (1 isolate) *Rhodotorula mucilaginosa* (1 isolate), *Saccharomyces cerevisiae* (1 isolate) and *Trichosporon asahii* (2 isolates). Molds included *A. fumigatus* (six isolates), *A. clavatus* (one isolate), *Aspergillus foetidis* (one isolate), *Aspergillus niger* species complex (two isolates), *Aspergillus section Terrei* (one isolate), and four other molds (one *Lichtheimia ramosa*, one *Scedosporium aurantiacum*, one *Scedosporium prolificans* and one *Trichoderma longibrachiatum*).

Antifungal susceptibility testing

All yeasts were tested for *in vitro* susceptibility to the echinocandins (anidulafungin, caspofungin and micafungin) and the triazoles (fluconazole, posaconazole and voriconazole) using CLSI[36] broth microdilution methods. The Minimum inhibitory concentration (MIC) results for all agents were read following 24 h of incubation when the agents were tested against *Candida* spp., whereas MIC end points for the triazoles were read after 48 h when the drugs were tested against non-*Candida* yeasts.[36] In all instances, the MIC values were determined visually as the lowest concentration of drug that caused significant growth diminution levels ($\geqslant$ 50% inhibition relative to the growth control).[36,37]

In vitro susceptibility testing of *Aspergillus* spp. and other molds against the echinocandins and triazoles (itraconazole, posaconazole and voriconazole) was performed by broth microdilution as described in CLSI document M38-A2.[38] The triazole MICs and echinocandin minimum effective concentrations were determined as defined in the CLSI reference method.[38]

We used the revised CLSI clinical breakpoint values and epidemiological cutoff values to differentiate susceptible/wild-type strains from resistant/non-wild-type strains of each species.[31–33,36–40]

Quality control was performed as recommended in CLSI documents M27-A3[36] and M38-A2[38] using the strains *C. krusei* ATCC 6258 *C. parapsilosis* ATTC 22019, and *A. fumigatus* MYA-3626.

All isolates of *Candida* spp. that were resistant to one or more of the echinocandins were further characterized regarding the presence or absence of mutation in the hot spot regions of *fks1* and *fks2* (*C. glabrata* only) as described previously.[41,42]

Table 1 Geographic distribution of organisms collected during 2010 to 2012 from Asia-Western Pacific medical centers participating in the SENTRY Antifungal Surveillance Program

	No. of isolates in each geographic region									
Organism/organism group	*AUS*	*HK*	*IND*	*KOR*	*NZ*	*SNG*	*SA*	*TWN*	*TLD*	*Total*
Total, yeasts	177	23	7	87	64	65	18	3	52	496
All *Candida* spp.	159	23	7	85	61	65	15	3	42	460
C. albicans	69	11	3	39	28	25	6	3	13	197
C. glabrata	38	4		13	12	13	1		7	88
C. parapsilosis	30	5	2	13	14	7	8		9	88
C. tropicalis	4	2	2	16	1	17			13	55
C. dubliniensis	5				2	1				8
C. guilliermondii	6				1					7
C. krusei	3	1			1					5
C. lusitaniae	2			2	1					5
C. fabianii				2						2
C. haemulonii						1				1
C. kefyr	1									1
C. metapsilosis						1				1
C. orthopsilosis	1									1
Cryptococcus neoformans	5			1	1		3		6	16
Other yeasts[a]	2				2				1	5
Mold										
All *Aspergillus* spp.	8								3	11
A. fumigates	5								1	6
Other *Aspergillus* spp.[b]	3								2	5
Other molds[c]	3			1						4

Abbreviations: AUS, Australia; HK, Hong Kong; IND, India; KOR, Korea; NZ, New Zealand; SNG, Singapore; SA, South Africa; TWN, Taiwan; TLD, Thailand.
[a]Other yeasts include *Lodderomyces*, *elongisporus* (one strain), *Rhodotorula*, *mucilaginosa*, (one strain), *Saccharomyces cerevisiae*,(one strain) and *Trichosporon asahii* (two strains).
[b]Other *Aspergillus* spp. include *A. clavatus* (one strain), *A. foetidus* (one strain), *A. niger* species complex (two strains), and *Aspergillus* section *Terrei* (one strain).
[c]Other molds include *Lichtheimia ramosa* (one strain), *Scedosporium aurantiacum* (one strain), *Scedosporium prolificans* (ome strain) and *Trichoderma longibrachiatum* (one strain).

Table 2 Frequency of antifungal resistance among clinical isolates of *Candida* spp., *Cryptococcus neoformans* and *Aspergillus fumigatus* from the Asia-Western Pacific Region in the 2010–2012 SENTRY Surveillance Program

		MIC/MEC ($\mu g\,ml^{-1}$)			% by category [a,b]	
Species (no. tested)	*Antifungal agent*	*Range*	*50%*	*90%*	*S*	*R*
C. albicans (197)						
	Anidulafungin	⩽0.008–0.25	0.015	0.06	100.0	0.0
	Caspofungin	⩽0.008–0.12	0.03	0.06	100.0	0.0
	Micafungin	⩽0.008–0.12	0.015	0.03	100.0	0.0
	Fluconazole	⩽0.06–>128	0.12	0.25	99.5	0.5
	Posaconazole	⩽0.008–>8	0.03	0.06	93.4	6.6
	Voriconazole	⩽0.008–>8	⩽0.008	0.015	99.5	0.5
C. glabrata (88)						
	Anidulafungin	0.03–1	0.06	0.12	94.3	3.4
	Caspofungin	0.015–1	0.03	0.12	94.3	1.1
	Micafungin	⩽0.008–0.5	0.015	0.03	97.7	1.1
	Fluconazole	1–128	8	32	(93.2)	6.8
	Posaconazole	0.25–>8	1	2	97.7	2.3
	Voriconazole	0.03–4	0.25	0.5	90.9	9.1
C. parapsilosis (88)						
	Anidulafungin	0.25–4	2	4	88.6	0.0
	Caspofungin	0.12–2	0.25	0.05	100.0	0.0
	Micafungin	0.25–4	1	2	98.9	0.0
	Fluconazole	0.12–>128	0.5	4	87.5	5.7
	Posaconazole	0.015–0.5	0.06	0.25	98.9	1.1
	Voriconazole	⩽0.008–4	0.015	0.06	94.3	1.1
C. tropicalis (55)						
	Anidulafungin	⩽0.008–0.06	0.015	0.03	100.0	0.0
	Caspofungin	⩽0.008–0.06	0.03	0.06	100.0	0.0
	Micafungin	⩽0.008–0.06	0.03	0.03	100.0	0.0
	Fluconazole	0.12–64	0.25	1	94.6	3.6
	Posaconazole	⩽0.008–0.25	0.06	0.12	96.4	3.6
	Voriconazole	⩽0.008–2	0.03	0.03	94.6	3.6
C. dubliniensis (8)						
	Anidulafungin	0.015–0.06	0.03	—	100.0	0.0
	Caspofungin	⩽0.008–0.06	0.03	—	100.0	0.0
	Micafungin	⩽0.008–0.03	0.03	—	100.0	0.0
	Fluconazole	⩽0.06–0.25	0.12	—	100.0	0.0
	Posaconazole	0.015–0.06	0.03	—	100.0	0.0
	Voriconazole	⩽0.008	⩽0.008	—	100.0	0.0
C. guilliermondii (7)						
	Anidulafungin	0.25–2	2	—	100.0	0.0
	Caspofungin	0.06–0.5	0.25	—	100.0	0.0
	Micafungin	0.06–1	0.5	—	100.0	0.0
	Fluconazole	2–8	2	—	100.0	0.0
	Posaconazole	0.12–1	0.25	—	85.7	14.3
	Voriconazole	0.03–0.25	0.06	—	85.7	14.3
C. krusei (5)						
	Anidulafungin	0.03–0.12	0.06	—	100.0	0.0
	Caspofungin	0.06–0.25	0.06	—	100.0	0.0
	Micafungin	0.06–0.12	0.12	—	100.0	0.0
	Posaconazole	0.25–1	0.5	—	80.0	20.0
	Voriconazole	0.12–0.5	0.25	—	100.0	0.0

Table 2 (Continued)

Species (no. tested)	Antifungal agent	MIC/MEC (μg ml^{-1})			% by category [a,b]	
		Range	50%	90%	S	R
C. lusitaniae (5)						
	Anidulafungin	0.5–1	0.5	—	100.0	0.0
	Caspofungin	0.25–0.5	0.25	—	100.0	0.0
	Micafungin	0.25–0.5	0.25	—	100.0	0.0
	Fluconazole	0.12–2	0.25	—	80.0	20.0
	Posaconazole	0.06–0.12	0.06	—	80.0	20.0
	Voriconazole	⩽0.008–0.03	⩽0.008	—	100.0	0.0
Cryptococcus neoformans (16)						
	Fluconazole	0.5–8	2	8	100.0	0.0
	Posaconazole	0.015–0.5	0.12	0.25	100.0	0.0
	Voriconazole	⩽0.008–0.12	0.03	0.12	100.0	0.0
Aspergillus fumigatus (6)						
	Caspofungin	0.015–0.25	0.03	—	100.0	0.0
	Itraconazole	0.5–>8	1	—	83.3	16.7
	Posaconazole	0.5–>8	1	—	83.3	16.7
	Voriconazole	0.25–4	0.5	—	83.3	16.7

Abbreviations: MIC, minimum inhibitory concentration; MEC, minimum effective concentration; S, susceptible; R, resistant; 50 and 90%, MIC encompassing 50 and 90% of isolates tested, respectively.
Posaconazole ECVs were used to identify WT and non-WT isolates of *C. albicans* (ECV, 0.06 μg ml^{-1}), *C. glabrata* (ECV, 0.12 μg ml^{-1}), *C. parapsilosis* (ECV, 0.25 μg ml^{-1}), *C. tropicalis* (ECV, 0.12 μg ml^{-1}), and *C. krusei* (ECV, 0.5 μg ml^{-1}).[31]
The species-specific ECVs for all antifungal agents were used to classify isolates of *C. dubliniensis, C. guilliermondii, C. lusitaniae, C. neoformans* and *A. fumigatus*.[31–33,43]
[a]Susceptibility and resistance are defined as MICs of ⩽0.25 μg ml^{-1} and >0.5 μg ml^{-1}, respectively, for anidulafungin, caspofungin and micafungin against *C. albicans, C. tropicalis* and *C. krusei*, MICs of <0.12 μg ml^{-1} and >0.25 μg ml^{-1}, respectively, for anidulafungin and caspofungin and MICs of <0.06 μg ml^{-1} and >0.12 μg ml^{-1}, respectively, for micafungin against C. glabrata; MICs of <2 μg ml^{-1} and >4 μg ml^{-1}, respectively, for anidulafungin, caspofungin and micafungin and *C. parapsilosis* and *C. tropicalis*, MICs of ⩽32 μg ml^{-1} (susceptible dose dependent; SDD) and >32 μg ml^{-1}, respectively, against *C. glabrata*; MICs of ⩽0.12 μg ml^{-1} and >0.5 μg ml^{-1}, respectively, for voriconazole against *C. albicans, C. parapsilosis,* and *C. tropicalis*; and MICs of ⩽0.5 μg ml^{-1} and >1 μg ml^{-1}, respectively, against *C. krusei*.[31]
[b]*In lieu* of clinical breakpoints for voriconazole against *C. glabrata*, the epidemiological cutoff value (ECV) of 0.5 μg ml^{-1} was used to identify wild-type (WT; MIC<ECV) and non-WT (MIC>ECV) isolates.[31]

RESULTS AND DISCUSSION

Table 1 shows species distribution by country/geographic region of the fungi implicated in the IFIs in the APAC region during 2010–2012. As expected, *Candida* spp. accounted for the vast majority of infections in all countries. *Candida albicans* was most common in Taiwan (100.0%; only three isolates) and least common in Thailand (31.0%), whereas *C. glabrata* was most common in Australia (23.9%) and least common in India and Taiwan (0.0%). *C. parapsilosis* accounted for 53.3% of *Candida* infections in South Africa and was the second most common species in Hong Kong, India and New Zealand. *C. tropicalis* was most common in Thailand (31.0%) and was second in rank order in India, Korea and Singapore. Other miscellaneous species were largely contributed by sites in Australia and New Zealand, reflecting increased efforts at species-level identification in these countries.[11] The prominence of both *C. tropicalis* and *C. parapsilosis* over *C. glabrata* has been noted previously in other surveys in the APAC region.[12,14–18,20,21]

The non-*Candida* yeasts consisted largely of *C.* neoformans (76.2%), the majority of which were contributed by Australia and Thailand. Similarly, the vast majority of the molds were contributed by Australia and Thailand, most of which were *Aspergillus* spp.

The frequency of resistance to the azoles and the echinocandins for the eight most common species of *Candida, C. neoformans* and *A. fumigatus* are shown in Table 2. Resistance to the echinocandins was very uncommon among the eight species of *Candida* and was only detected in three isolates of *C. glabrata*, two of which were from Australia. Among the three echinocandin-resistant strains of *C. glabrata,* one was resistant to all three echinocandins (MIC ⩾0.5 μg ml^{-1}) and harbored a S663F mutation in *fks2*, one was resisitant to anidulafungin (MIC, 0.5 μg ml^{-1}), intermediate to both caspofungin (MIC, 0.25 μg ml^{-1}) and micafungin (MIC, 0.12 μg ml^{-1}) and harbored a F625S mutation in *fks1* and one was resistant to anidulafungin (MIC, 0.5 μg ml^{-1}) but susceptible to both caspofungin (MIC, ⩽0.12 μg ml^{-1}) and micafungin (MIC, ⩽0.06 μg ml^{-1}) and did not possess a mutation in either *fks1* or *fks2*.These results are similar to previous reports from the APAC region where echinocandin resistance has been very uncommon.[14,15,17,22,24,26] Whereas the earlier studies of echinocandin resistance among *Candida* spp. from APAC may have underestimated the frequency of resistance due to the use of old (higher) clinical breakpoint values, by using the new (lower) species-specific clinical breakpoints and epidemiological cutoff values we can confirm the lack of echinocandin resistance among most species from this region. The detection of echinocandin resistance among isolates of *C. glabrata*, yet susceptibility to this class among other species, is consistent with previous observations from North America, Europe and Latin America.[7]

In contrast to the echinocandins, resistance to the azoles was observed in all species of *Candida* with the exception of *C. dubliniensis* (Table 2). Cross resistance among the three triazoles was detected in all species with the exception of *C. dubliniensis, C. guilliermondii, C. krusei* and *C. lusitaniae.* Notably, the level of fluconazole resistance observed with *C. glabrata* (6.8%) was comparable to that seen with *C. parapsilosis* (5.7%) and *C. tropicalis* (3.6%). Most of the earlier studies of azole resistance in the APAC region are similar to that shown in Table 2; however, relatively high levels of fluconazole resistance have been reported among isolates of *C. glabrata* (11–12%) and *C. tropicalis* (11.6–15%) from Taiwan.[22,23,26,27]

Similar to that seen with the echinocandins, azole resistance among the *Candida* spp. from the APAC region was comparable to that observed among isolates from North America and Europe.[7]

Azole resistance was not detected among the isolates of *C. neoformans* and a single isolate of *A. fumigatus* from Australia was found to be non-wild type to itraconazole (MIC $>1\,\mu g\,ml^{-1}$), posaconazole (MIC $>0.5\,\mu g\,ml^{-1}$) and voriconazole (MIC $>1\,\mu g\,ml^{-1}$).

In summary, we provide additional information to the literature regarding the frequency of various fungal pathogens causing IFI and the accompanying antifungal resistance profiles of APAC isolates of *Candida, Cryptococcus* and *Aspergillus* spp. We acknowledge that a limitation of the survey is the relatively small number of isolates of both molds and non-*Candida* yeasts. Resistance to the echinocandins was rare and detected in *C. glabrata* isolates from Australia. This is consistent with data from other regions where *C. glabrata* is often the only species of *Candida* with resistance to this class of agents. Although azole resistance was apparent in most species of *Candida*, it was not as high as that reported from other surveys, most notably from Taiwan.[22,23,26] Likewise, resistance to the azole antifungals was not detected among *C. neoformans* isolates and was seen in only a single isolate of *A. fumigatus*. Whereas the rank order of *Candida* spp. for the entire collection is similar to that shown in other geographic surveys (*C. albicans*>*C. glabrata*=*C. parapsilosis*>*C. tropicalis*>*C. krusei* and so on), there were six different patterns of *Candida* species across the nine different countries, further demonstrating the importance of local epidemiological data. Antifungal resistance is relatively uncommon among isolates of fungi causing IFIs in the APAC region. As in other regions of the world emerging resistance to the echinocandins among invasive isolates of *C. glabrata* bears close monitoring.

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