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SUSCEPTIBILITY

APX001A *In Vitro* Activity against Contemporary Blood Isolates and *Candida auris* Determined by the EUCAST Reference Method

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ABSTRACT APX001A is the active moiety of the first-in-class drug candidate APX001. So far, most susceptibility testing studies have examined ≤30 isolates/species, and only one used the EUCAST method. Here, we investigated the *in vitro* activity of APX001A and five comparators against 540 candidemia and 122 *C. auris* isolates. Isolates (17 *Candida* and 3 yeast species) were identified using CHROMagar, matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF) and, when needed, internal transcribed space (ITS) sequencing. EUCAST E.Def 7.3.1 susceptibility testing included APX001A, amphotericin B, anidulafungin, micafungin, fluconazole, and voriconazole. Wild-type upper limits (WT-UL) were established following the EUCAST principles for epidemiological cutoff value setting for APX001A, allowing classification as wild type (WT) or non-WT. APX001A MIC_{50} values (mg/liter) were as follows: *Candida albicans*, *Candida dubliniensis*, and *Candida tropicalis*, 0.004 to 0.008; *Candida parapsilosis* and *Candida auris*, 0.016; *Candida glabrata*, 0.06; and *Candida krusei*, >0.5. APX001A MICs against the rare species varied from ≤0.0005 (*C. pelliculosa*) to >0.5 (*Candida norvegensis*). APX001A was equally or more active *in vitro* than the comparators against all species except *C. krusei* and *C. norvegensis*. Four isolates were APX001A non-WT; all were fluconazole resistant. A correlation was observed between APX001A and fluconazole MICs across all species except *Candida guilliermondii* and *C. auris*, and when comparing high and low fluconazole MIC isolates of *C. albicans*, *C. dubliniensis*, *C. glabrata*, *C. tropicalis*, and *C. auris*. APX001A showed promising *in vitro* activity against most *Candida* and other yeast species, including *C. auris*, compared to five comparators. WT-UL were suggested for the common species, and a new and unexplained correlation to fluconazole susceptibility was observed.

KEYWORDS APX001A, EUCAST, *Aspergillus*, *Candida*, *Candida auris*, amphotericin B, antifungal susceptibility testing, azoles, candidemia, echinocandins

APX001A (formerly E1210) is the active moiety of the first-in-class small-molecule drug candidate APX001, currently in phase 1 clinical trials. It has broad-spectrum activity that includes *Candida*, *Aspergillus*, and rare molds. APX001A inhibits the conserved fungal inositol acyltransferase enzyme GWT1, thereby preventing glycosylphosphatidylinositol (GPI)-anchored protein maturation and compromising fungal growth.

In vitro activity against *Candida* spp. has been investigated using the Clinical and Laboratory Standards Institute (CLSI) M27A-3 methodology.1 CLSI MIC_{90} values of <0.008 to 0.06 mg/liter were observed across the different species, except for *Candida krusei* (CLSI MICs of 2 to >32 mg/liter) (2, 3). APX001A retained activity against isolates with acquired resistance to fluconazole and echinocandins (2). Moreover, *in vitro*

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activity was recently demonstrated against 16 *Candida auris* isolates (4). APX001A has been investigated in murine models of oropharyngeal and invasive candidiasis and demonstrates *in vivo* activity against *Candida albicans*, *Candida tropicalis*, and *C. auris* (4–6). The area under the concentration-time curve over 24 h in the steady state for the free, unbound fraction divided by the MIC (fAUC$_{0-24}$/MIC ratio) has been proposed to be the pharmacokinetic/pharmacodynamic (PK/PD) index that best correlates with efficacy (7). A lower stasis fAUC/MIC target was found for *Candida glabrata* (1.31 ± 0.27) compared to those for *C. albicans* (20.60 ± 6.50) and *C. auris* (14.67 ± 8.30), suggesting that clinical breakpoints should be species specific.

APX001A *in vitro* susceptibility evaluations utilizing the European Committee on Antimicrobial Susceptibility Testing (EUCAST) E.Def 7.3.1 method are very scarce and are limited to a study investigating 12 to 25 *Candida* isolates from each of the 5 most common species (*C. albicans*, *C. glabrata*, *C. krusei*, *Candida parapsilosis*, and *C. tropicalis*) (3). We therefore included APX001A in our prospective EUCAST antifungal susceptibility testing of bloodstream isolates referred as part of the nationwide fungemia surveillance program in Denmark to generate population-based contemporary EUCAST MIC data for this compound and for future epidemiologic cutoff (ECOFF) and clinical breakpoint setting. In parallel, we investigated the susceptibility pattern of 122 Indian clinical *C. auris* isolates to APX001A and comparator drugs by the EUCAST method to also generate MIC data for this multidrug-resistant yeast that is rapidly emerging as a significant cause of nosocomial infections (references 8 and 9; see also https://www.cdc.gov/fungal/diseases/candidiasis/candida-auris.html). We found that APX001A was highly active against both yeast bloodstream isolates and *C. auris* isolates, but also demonstrated an unexplained correlation between APX001A and fluconazole MICs across and within the yeast species.

RESULTS

APX001A against quality control strains. *C. parapsilosis* ATCC 22019 and *C. krusei* ATCC 6258 were tested 46 and 84 times, respectively, during the study period. The MIC results (mg/liter) for *C. parapsilosis* ATCC 22019 were as follows: modal MIC, 0.03; MIC$_{50}$, 0.016; range, 0.008 to 0.03. A total of 44/46 (95.7%) MICs were within the range of 0.016 to 0.03 mg/liter. The MICs against *C. krusei* ATCC 6258 were 0.5 mg/liter on one occasion and >0.5 mg/liter (outside the tested concentration range) for the rest (n = 83).

APX001A activity against contemporary Danish blood isolates and *C. auris*. APX001A displayed *in vitro* activity with MICs of <0.5 mg/liter against all *Candida* bloodstream isolates except those of *C. krusei* and *Candida norvegensis* (Table 1). *C. albicans*, *Candida dubliniensis* and *C. tropicalis* were the most susceptible species among the 6 most common species, with MIC$_{50}$ values of 0.004 to 0.008 mg/liter, followed by *C. parapsilosis* (MIC$_{50}$, 0.016 mg/liter) and *C. glabrata* (MIC$_{50}$, 0.06 mg/liter). APX001A MICs varied considerably against the rare *Candida* and yeast species, with *Candida pelliculosa* being the most susceptible organism (MIC, ≤0.0005 mg/liter), followed by *Candida fermentati*, *Candida guilliermondii*, *Candida lusitaniae*, *Candida metapsilosis*, *Candida utilis*, *Candida orthopsilosis*, *Candida nivariensis*, and *Saccharomyces cerevisiae*, with MICs in the range of 0.004 to 0.03 mg/liter; *Magnusiomyces capitatus*, *Candida kefyr*, and *Cryptococcus neoformans*, with MICs in the range of 0.125 to 0.5 mg/liter; and *C. norvegensis*, with MICs of >0.5 mg/liter. Finally, APX001A also displayed *in vitro* activity against Indian clinical *C. auris* isolates, with MIC$_{50}$ and modal MIC values of 0.016 mg/liter and a MIC range of 0.001 to 0.125 mg/liter (Table 1).

APX001A wild-type upper limit (WT-UL) values were determined visually and statistically using the EUCAST ECOFFinder program for the species for which the MIC distributions were not truncated (Table 1). The WT-UL were as follows: *C. albicans*, 0.03 mg/liter; *C. dubliniensis*, 0.016 mg/liter; *C. glabrata*, 0.125 mg/liter; and *C. tropicalis*, 0.03 mg/liter, irrespective of which method was used for determination. For *C. parapsilosis*, a visual WT-UL was set at 0.06 mg/liter, but a statistical WT-UL could not be determined because there were no MICs higher than the modal MIC. Applying these WT-UL values

TABLE 1 APX001A MICs (mg/liter) for contemporary Danish bloodstream isolates and *C. auris* isolates

Species	*N*	MIC (mg/liter)[b]												Range	MIC_{50} (mg/liter)	MIC_{90} (mg/liter)	WT-UL[a]		
																		Statistical	
		≤0.0005	0.001	0.002	0.004	0.008	0.016	0.03	0.06	0.125	0.25	0.5	>0.5				Visual	97.50%	99%
Danish blood isolates																			
Candida albicans	218		1	9	24	108	69	7						0.001–0.03	0.008	0.016	0.03	0.03	0.03
Candida dubliniensis	29			5	14	9		**1**						0.002–0.03	0.004	0.008	0.016	0.016	0.016
Candida glabrata	179					1	6	52	102	16	**2**			0.008–0.25	0.06	0.06	0.125	0.125	0.125
Candida krusei	31											1	30	0.5–>0.5	>0.5	>0.5	ND	ND	ND
Candida parapsilosis	19					2	8	9						0.008–0.03	0.016	0.03	0.06	NP	NP
Candida tropicalis	33				10	13	9			**1**				0.004–0.125	0.008	0.016	0.03	0.03	0.03
Other *Candida* spp.[c]	25	1			4	10	1	5		1	2		1	≤0.0005–>0.5	0.008	0.25	ND	ND	ND
Other yeast spp.[d]	6							1		1	3	1		0.3–0.5	0.25	0.25	ND	ND	ND
Indian clinical isolate																			
Candida auris	122		4	6	11	16	41	38	5	1				0.001–0.125	0.016	0.03	0.06	0.125	0.125

[a]WT-UL, wild-type upper limit determined visually and statistically using the ECOFFinder program, including 97.5% and 99% of the isolates, respectively. The ECOFFinder program requires at least one isolate with MIC at least one step above the modal MIC to calculate an upper limit. NP, not provided; ND, not done.

[b]MIC values above the WT-UL are highlighted in bold and underlined.

[c]Other *Candida* spp. included *Candida fermentati* (*n* = 2), *Candida guilliermondii* (*n* = 6), *Candida kefyr* (*n* = 3), *Candida lusitaniae* (*n* = 7), *Candida metapsilosis* (*n* = 1), *Candida nivariensis* (*n* = 1), *Candida norvegensis* (*n* = 1), *Candida orthopsilosis* (*n* = 2), *Candida pelliculosa* (*n* = 1), and *Candida utilis* (*n* = 1).

[d]Other yeast spp. included *Cryptococcus neoformans* (*n* = 4), *Magnusiomyces capitatus* (*n* = 1), and *Saccharomces cerevisiae* (*n* = 1).

for classification of the blood isolates into WT and non-WT isolates, the following isolates were classified as non-WT for APX001A: *C. dubliniensis*, 1/29 (3.4%; MIC, 0.03 mg/liter); *C. glabrata*, 2/179 (1.1%; both with MIC of 0.25 mg/liter); and *C. tropicalis* 1/33 (3.0%; MIC, 0.125 mg/liter). Overall, 4/540 (0.74%) of yeast blood isolates were classified as non-WT.

For *C. auris*, the visual (0.06 mg/liter) or statistical (0.125 mg/liter) WT-UL differed by one dilution. Depending on which WT-UL was adopted, 0.8% or none of the *C. auris* isolates displayed MICs above the WT-UL.

APX001A *in vitro* activity compared to comparators. The *in vitro* susceptibility to APX001A was compared to that for the five other antifungal agents, adopting modal MICs no more than two dilutions apart as the criterion for equal *in vitro* activity (see Table 2). APX001A was equally or more active than amphotericin B, anidulafungin, micafungin, fluconazole, and voriconazole on a mg/liter basis against all species except *C. krusei* and *C. norvegensis*, against which the two echinocandins were superior. Non-WT susceptibility was found for 0 to 1% of the isolates, depending on the species, for APX001A, compared to 0% for amphotericin B, 0 to 1.1% for anidulafungin, 0 to 3.2% for micafungin, 0 to 15.2% for fluconazole, and 0 to 18.2% for voriconazole.

For *C. auris* specifically, the modal MIC was 0.016 mg/liter for APX001A, compared to 1 mg/liter, 0.06 mg/liter, 0.125 mg/liter, >256 mg/liter, and 1 mg/liter for amphotericin B, anidulafungin, micafungin, fluconazole, and voriconazole, respectively. Non-WT susceptibility was observed more often for the echinocandin and azole comparators. Thus, 10/122 (8.2%) and 8/122 (6.6%) of the *C. auris* isolates were non-WT to anidulafungin and micafungin, respectively, and 121/122 (99.2%) isolates were resistant to fluconazole, adopting the non-species-specific breakpoint of >4 mg/liter for resistance (Table 2 and Table S10 in the supplemental material).

Comparison of APX001A and fluconazole MICs. Four of four bloodstream isolates that were non-WT for APX001A were also fluconazole resistant (*C. dubliniensis* fluconazole MIC, 32 mg/liter; *C. glabrata* isolate fluconazole MICs, >32 mg/liter; and *C. tropicalis* fluconazole MIC, ≥16 mg/liter), suggesting a possible correlation between fluconazole and APX001A MICs. Therefore, the APX001A and fluconazole modal MICs were compared across species represented with at least four isolates (Fig. 1). A linear correlation was observed across *Candida* and *Cryptococcus* species, with the exception of *C. guilliermondii* and *C. auris*. Similarly, when APX001A MICs were examined for *C. albicans*, *C. dubliniensis*, *C. glabrata*, *C. tropicalis*, and *C. auris* isolates grouped according to fluconazole MICs, respectively, a correlation was again observed (Table 3).

DISCUSSION

APX001A *in vitro* activity overall. This study confirms previous reports that APX001A is a highly active compound on a mg/liter basis against all *Candida* species except *C. krusei* and the phylogenetically related species *C. norvegensis* (2, 3, 5, 10). The MIC_{50} values for *C. albicans* and *C. glabrata* were identical to those presented in the only other study presenting APX001A EUCAST MICs and a single dilution below those for *C. parapsilosis* and *C. tropicalis*. This indicates that EUCAST testing of APX001A may be associated with limited interlaboratory variation (3). APX001A also demonstrated activity against a range of rare *Candida* and other yeast species, with only *Magnusiomyces capitatus*, *C. kefyr*, and *Cryptococcus neoformans* being less susceptible than *C. glabrata* on a mg/liter basis. Of note, this included *C. auris*, an organism that is often drug or multidrug resistant, and which has emerged as a significant cause of nosocomial infections over the past 6 years in Asia, South Africa, Latin America, the United States, and in Europe (with cases in Spain, the United Kingdom, Germany, France, Belgium, Norway, and Austria) (8, 11, 12). This extends the findings of two recent reports studying a smaller number of *C. auris* isolates but confirming the *in vitro* activity *in vivo* using immunocompromised murine models of disseminated candidiasis (4, 7).

Single-center WT-UL were suggested for *C. albicans*, *C. dubliniensis*, *C. glabrata*, *C. parapsilosis* and *C. tropicalis* using visual inspection of the MIC distributions and statistically using the EUCAST ECOFFinder program. Overall, the WT-UL were in close

TABLE 2 Comparison of APX001A MICs (mg/liter) to those for other licensed antifungal agents for contemporary Danish bloodstream isolates and *C. auris*

Species	APX001A			AMB			ANF			MFG			FLC			VRC		
	MIC range	Modal MIC	% > ECOFF	MIC range	Modal MIC	% > ECOFF	MIC range	Modal MIC	% > ECOFF	MIC range	Modal MIC	% > ECOFF	MIC range	Modal MIC	% > ECOFF	MIC range	Modal MIC	% > ECOFF
Danish blood isolates																		
C. albicans	0.001–0.03	0.008	0	0.06–0.5	0.25	0	≤0.004–0.016	< = 0.004	0	≤0.004–0.03	0.008	1.8	0.03–4	0.25	0.5	≤0.004–0.06	≤0.004	0.5
C. dubliniensis	0.002–0.03	0.004	3.4	0.03–0.125	0.06	0	≤0.004–0.016	0.008	0	≤0.004–0.03	0.016	ND	0.06–32	0.125	3.4	≤0.004–0.125	0.008	3.4
C. glabrata	0.008–0.25	0.06	1	0.06–0.5	0.5	0	0.008–0.25	0.016	1.1	≤0.004–0.125	0.016	2.2	0.5–>32	4	9.5	0.016–>4	0.06	9.5
C. krusei	0.5–>0.5	>0.5	ND[a]	0.5–1	1	0	0.016–0.06	0.03	0	0.06–0.5	0.125	3.2	8–>32	32	ND	0.125–2	0.25	ND
C. parapsilosis	0.008–0.03	0.03	0	0.25–1	0.5	0	0.5–2	0.5	0	0.5–2	2	0	0.5–2	0.5/1	0	0.008–0.03	0.016	0
C. tropicalis	0.004–0.125	0.008	3.4	0.25–0.5	0.5	0	≤0.004–0.03	0.016	0	≤0.004–0.06	0.03	0	0.25–32	0.25/0.5	15.2	0.008–>4	0.016	18.2
Other *Candida* spp.	≤0.0005–>0.5	0.008	ND	0.125–1	0.25	ND	≤0.004–2	0.03	ND	0.016–0.5	0.06	ND	0.125–32	0.5	ND	0.008–0.25	0.008	ND
Other yeast spp.	0.3–0.5	0.25	ND	0.25–1	0.25	ND	0.06–>4	>4	ND	0.125–>4	>4	ND	2–16	16	ND	0.016–0.5	0.125	ND
Indian clinical isolate																		
Candida auris	0.001–0.125	0.016	0	0.5–1	1	0	1–>32	0.06	8.2	0.03–>32	0.125	6.6%	1–>256	>256	ND	0.004–4	1	ND

[a]ND, not done.

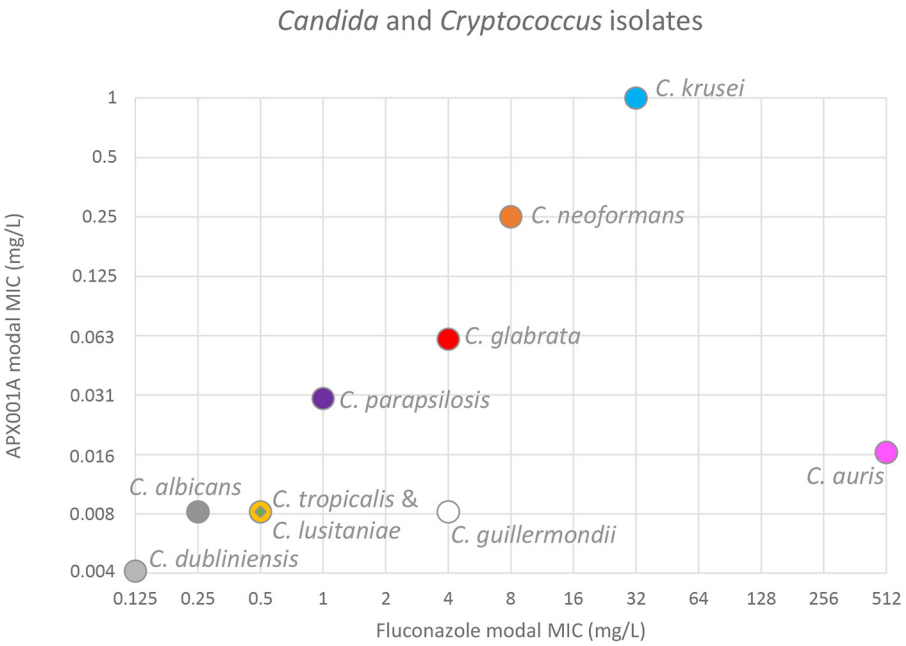

FIG 1 Correlation between APX001A and fluconazole modal MICs for bloodstream isolates represented by at least four isolates. *C. albicans* (dark gray circle), *C. auris* (pink), *C. dubliniensis* (light gray circle), *C. glabrata* (red circle), *C. guilliermondii* (white circle), *C. lusitaniae* (green diamond), *C. krusei* (turquoise circle), *C. parapsilosis* (purple circle), *C. tropicalis* (yellow circle), and *Cryptococcus neoformans* (orange circle).

agreement across the methods for determination and criteria used (97.5 or 99% of the isolates included), although the visual WT-UL was one dilution more restrictive than the statistical one for *C. auris*. While establishment of formal epidemiologic cutoff (ECOFF) values awaits the generation of multicenter data meeting the EUCAST criteria for aggregation, we believe that the WT-UL estimated here may serve for differentiating non-WT from WT organisms, provided that agreement with the quality control (QC) strain ranges and species-specific modal MIC values is ensured.

APX001A *in vitro* activity compared to that of licensed compounds. APX001A was equally or more efficacious *in vitro* than amphotericin B, anidulafungin, micafungin, fluconazole, and voriconazole on a mg/liter basis. Moreover, only amphotericin B was

TABLE 3 APX001A MICs for isolates with low and high fluconazole MICs

Species or fluconazole susceptibility	APX001A MIC (mg/liter)										
	≤0.0005	0.001	0.002	0.004	0.008	0.016	0.03	0.06	0.125	0.25	0.5
C. albicans											
Fluconazole MIC, ≤1 mg/liter		1	9	24	108	69	6				
Fluconazole MIC, 4 mg/liter							1				
C. dubliniensis											
Fluconazole MIC, ≤0.5 mg/liter			5	14	9						
Fluconazole MIC, 32 mg/liter							1				
C. glabrata											
Fluconazole MIC, ≤16 mg/liter					1	6	52	92	7		
Fluconazole MIC, ≥32 mg/liter								10	9	2	
C. tropicalis											
Fluconazole MIC, ≤8				10	13	8					
Fluconazole MIC, ≥16 mg/liter						1			1		
C. auris											
Fluconazole MIC, ≤64 mg/liter		4	6	9	2						
Fluconazole MIC, >64 mg/liter				2	14	41	38	5	1		

associated with fewer non-WT organisms than APX001A. This renders APX001A a promising agent, particularly in light of (i) the increasing proportion of bloodstream infections due to *C. glabrata*, which is intrinsically fluconazole nonsusceptible, (ii) the increasing number of outbreaks involving *C. auris*, and (iii) the increasing acquired echinocandin resistance in *C. glabrata*, and at some centers of fluconazole resistance in *C. tropicalis* (11, 13–16).

APX001A PK/PD targets have recently been explored for *C. albicans*, *C. glabrata*, and *C. auris* for the CLSI method. Pfaller and colleagues performed a head to head comparison of EUCAST and CLSI MICs for *C. albicans* ($n = 21$) and *C. glabrata* ($n = 20$) isolates and found the EUCAST MIC_{50} to be one step lower for *C. albicans* and one step higher for *C. glabrata*. Subsequently, CLSI APX001A MIC_{50} values of 0.004 to 0.008 mg/liter have been reported, which are 1 to 2 dilutions lower than the EUCAST MIC_{50} reported here (4, 7). Translating the proposed CLSI PK/PD targets to EUCAST targets, the doses required to achieve stasis *f*AUC/MIC targets would be as follows: 41.20 (*C. albicans*), 0.66 (*C. glabrata*), 7.34 (*C. auris*). However, these EUCAST targets need proper validation.

APX001A-fluconazole MIC correlation. We observed a yet-unrecognized correlation between fluconazole and APX001A susceptibility. Fluconazole inhibits the lanosterol 14-α-demethylase enzyme, thereby inhibiting ergosterol formation and incorporation into the cell membrane. Azole resistance has been linked to target gene mutations, increased expression of the target enzyme, and active efflux mediated by multidrug transporters (17). APX001A, on the other hand, targets fungal inositol acyltransferase enzyme GWT1, thereby preventing GPI-anchored protein maturation and compromising fungal growth. Thus, although the drug targets differ from one another, both are associated with the fungal membrane. We did not find any isolates with elevated APX001A MICs (compared to the WT APX001A population) in the absence of elevated fluconazole MICs. In contrast, we observed isolates with fluconazole resistance but WT APX001A MICs. This suggests that some, but not all, fluconazole resistance mechanisms affect APX001A susceptibility. Further research into the mechanisms of cross-resistance is warranted, as well as exploration of APX001 optimal dosing regimens to determine if non-WT isolates with elevated MICs can be successfully treated.

The MIC distribution for APX001A against *C. auris* spanned eight dilutions, a range that is broader than that for any of the other *Candida* species. MIC distributions for WT populations typically span five dilution steps. Broader ranges may reflect technical issues compromising reproducibility of the MIC testing, inclusion of isolates with incorrect species identification, or isolates that are non-WT. The narrower MIC ranges observed for the other species and compliance with the previously published EUCAST MIC data suggest that technical issues are less likely to be the explanation. Broad and multimodal MIC distributions were recently reported for azoles against this *C. auris* strain collection, and whole-genome sequencing data of *C. auris* has demonstrated that a considerable proportion of *C. auris* isolates harbored *ERG11* target gene alterations (16, 18). Analysis of *ERG11* target gene alterations of Indian *C. auris* isolates detected the amino acid substitutions Y132 and K143 in 77% ($n = 34/44$) of strains that were fluconazole resistant, whereas WT genotypes, i.e., without substitutions at these positions, were observed in isolates with low fluconazole MICs (1 to 2 mg/liter), suggesting that these substitutions confer a phenotype of resistance to fluconazole similar to that described for *C. albicans* (19). We speculate that the presence of azole resistance mechanisms affects APX001A susceptibility and thus explains the broad MIC distributions for both APX001A and the azoles.

In conclusion, we provide here the largest MIC data set so far for APX001A. APX001A showed promising *in vitro* activity against *Candida* and other yeasts, including *C. auris*, on a mg/liter basis and compared to five comparators, with the exception of *C. krusei* and *C. norvegensis*. WT-UL were suggested for the common species, and a new and unexplained correlation to fluconazole susceptibility was observed.

MATERIALS AND METHODS

Danish bloodstream isolates. A total of 540 yeast bloodstream isolates collected during a 15-month study period (1 October 2016 to 31 December 2017) was included. The species distribution (number of isolates) was as follows: *C. albicans* (218), *C. dubliniensis* (29), *C. glabrata* (179), *C. krusei* (31), *C. parapsilosis* (19), *C. tropicalis* (33), other *Candida* spp. (25), and other yeast spp. (6). The isolates were obtained as part of the nationwide Danish surveillance program and thus represent a contemporary national and population-based isolate collection. During the study period, one *Fusarium* bloodstream isolate was also collected but not included.

Indian *C. auris* isolates. A total of 122 clinical isolates of *C. auris* were collected from individual patients in 6 tertiary care hospitals in India from 2010 to 2015. The isolates were mainly from patients with candidemia (blood; $n = 100$), and other specimens ($n = 22$) from invasive *Candida* infections included tissue, pleural fluid, and a single isolate from pus.

Susceptibility testing. EUCAST MICs were determined following E.Def 7.3.1 methodology (20). APX001A (Amplyx Pharmaceuticals, San Diego, CA) pure substance was stored in aliquots at −80°C and stock solutions prepared in dimethyl sulfoxide (DMSO, 5000 mg/liter; Sigma-Aldrich, Brøndby, Denmark). The final drug concentration ranges studied were 0.001 to 0.5 mg/liter for yeast blood isolates and 0.001 to 0.5 mg/liter for *C. auris* isolates. The following comparator compounds were also investigated (final concentration range and source of compound in parentheses): anidulafungin (0.004 to 4 mg/liter; Pfizer A/S, Ballerup, Denmark), micafungin (0.004 to 4 mg/liter; Astellas Pharma Inc., Tokyo, Japan), amphotericin B (0.004 to 4 mg/liter; Sigma-Aldrich), fluconazole (0.03 to 32 mg/liter for bloodstream isolates and 0.5 to 256 mg/liter for *C. auris*; Sigma-Aldrich), and voriconazole (0.004 to 4 mg/liter; Pfizer A/S, Ballerup, Denmark). Cell culture-treated microtiter plates (Nunc MicroWell 96-well microplates, catalog no. 167008; Thermo Fisher Scientific) were used throughout. Microtiter plates were prepared with 2-fold drug dilutions in double-concentration medium according to the EUCAST methodology and frozen at −80°C prior to use. The EUCAST quality control (QC) strains *C. parapsilosis* ATCC 22019 and *C. krusei* ATCC 6258 were tested in parallel.

Data management. MIC ranges, modal MIC (the most common MIC), MIC_{50}, and MIC_{90} values were calculated. Wild-type upper limits (WT-UL), defined as the upper MIC value where the wild-type distribution ends, were determined following principles for setting EUCAST ECOFFs. However, as the values reported here are not formally accepted EUCAST APX001A ECOFFs, we used the term "WT-UL" to avoid confusion. The conventional method for determining ECOFF is a visual inspection of histograms of the MICs for single species (the eyeball method) (21). Additionally, WT-UL were determined statistically using 97.5% and 99% endpoints and the EUCAST ECOFF finder program (21).

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/AAC.01225-18.

SUPPLEMENTAL FILE 1, PDF file, 0.3 MB.

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