Efficacy of antifungal drug combinations against widespread primary resistance, cross-resistance, and multidrug-resistance in *Candida auris* from New York Hospitals and Healthcare Facilities

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Abstract

Background: Since 2016, New York hospitals and healthcare facilities have faced an unprecedented outbreak of pathogenic yeast *Candida auris*. Despite enhanced surveillance and control measures, the outbreak continues unabated and, at the end of 2018, the case counts are over 300 patients. We used CLSI broth microdilution method (BMD) to test over one thousand *C. auris* isolates from 150 affected facilities and found high-resistance to fluconazole (FZ, MIC_{50} >256 mg/L), and variable resistance to other broad-spectrum antifungal drugs. Therefore, we evaluated if two-drug combinations are efficacious against drug-resistant *C. auris*.

Methods: Fifteen *C. auris isolates* with variable resistance to a number of antifungals were selected for the combination testing. BMD plates were custom-designed, and quality controlled by TREK Diagnostic System. The tests involved microtiter wells with the initial 144 two-drug combinations and their two-fold dilutions (1/2 -1/32) to get 864 two-drug combinations finally. We utilized MIC_{100} endpoints for the drug combination readings as reported earlier for the intra- and inter-laboratory agreements obtained against *Candida* species and *Aspergillus fumigatus* (Antimicrob Agents Chemother. 2015. 59:1759 –1766). Additionally, minimum fungicidal concentrations (MFC) were determined for the test isolates.

Results: The study results were derived from 12,960 MIC_{100} readings, and MFC readings for fifteen *C. auris* isolates tested against 864 two-drug antifungal combinations for nine antifungal drugs. Flucytosine (FLC) at 2.0 mg/L potentiated most successful

combinations with other drugs. Micafungin (MFG), Anidulafungin (AFG), Caspofungin (CAS) at individual concentrations of 0.25 mg/L combined well with FLC (2.0 mg/L) to yield MIC_{100} for 14, 13, and 12 of 15 *C. auris* isolates tested, respectively. MFG/FLC combination was also fungicidal for 4 of 15 isolates. AMB / FLC (0.25/1.0 mg/L) yielded MIC_{100} for 13 isolates and MFC for three test isolates. Posaconazole (POS), and Isavuconazole (ISA) and Voriconazole (VRC) also combined well with FLC (0.25/2.0 mg/L) to yield MIC100 for 12, 13, and 13 isolates, respectively. POS/FLC combination was fungicidal for three isolates. None of the combinations were effective for *C. auris* 18-1, which tested resistant against FZ and FLC, except POS/FC (0.12/1.0 mg/L).
Conclusions: A limited set of two drug-combinations of antifungals were efficacious *in vitro* against fifteen *C. auris* strains with primary resistance, cross-resistance, and multidrug-resistance. The simplified format for two-drug combination susceptibility test will assist clinical laboratories and clinicians in making appropriate treatment choices for *C. auris* candidemia.

Introduction

Candida auris is an emerging multidrug-resistant yeast, causing invasive healthcare-associated infection with high mortality worldwide [1]. It was described in 2009 as a new yeast species from the ear discharge of a Japanese patient[2]. In the ensuing years, many reports of multidrug-resistant *C. auris* infections came from South Asia, South Africa, South America as well as travel-related cases from other parts of the world [3-6]. The largest, localized, uninterrupted *C. auris* outbreak in the US healthcare facilities continues to afflict the New York metro areas (2013-2019); *C. auris* caused 23 deaths among first 51 clinical case-patients in New York [7]. In response, the New York State Department of Health (NYSDOH) laboratory scientists and epidemiologists developed enhanced protocols undertook unprecedented surveillance and testing for *C. auris* [8]. Till date, over 15,000 clinical samples from 150 facilities were processed in the NYSDOH laboratory. The CLSI broth microdilution method (BMD) was used to test nearly one thousand *C. auris* isolates, which revealed high-resistance to fluconazole (MIC_{50} >256 mg/L), and variable resistance to other broad-spectrum antifungal drugs (Chaturvedi et al. unpublished data). Therefore, we investigated the efficacy of two-drug combinations against a representative set of fifteen drug-resistant *C. auris*.

Materials and Methods

Isolates – *C. auris* isolates were initially processed on Sabouraud dulcitol agar at 40℃, and single colonies used to confirm identify with MALDI-TOF-MS and ITS -D1/D2 sequencing as described earlier. The isolates were stored at -80℃ in 20% sterile glycerol.
Drug combinations – Fluconazole (FZ) was not tested in combinations due to wide-spread resistance in our isolate collection. Nine drugs were selected for the combination testing: amphotericin B (AMB), anidulafungin (AFG), caspofungin (CAS), micafungin

(MFG), flucytosine (FLC), isavuconazole (ISA), posaconazole (POS), voriconazole (VRC) and itraconazole (ITC). An advisory from the Centers for Diseases Control and Prevention was used for C. auris antifungal susceptibility testing and interpretation (https://www.cdc.gov/fungal/candida-auris/c-auris-antifungal.html).
Combination testing – Broadly, we followed a method described earlier from a multilaboratory evaluation of antifungal combination testing [9]. Two fixed concentrations of each drug were tested, a value conforming to Cmax in the product insert, and a concentration considered to be resistant for a majority of pathogenic yeasts. The drug combinations were dispensed in a total volume of 100 µl. The 96-well microtiter plates were custom-manufactured and tested for sterility by TREK Diagnostic System, Part of Thermo Fisher Scientific, Oakwood Village, OH, USA. They were transferred under dry ice to NYSDOH Mycology Laboratory and stored at -80℃until used. The schematic of plate design is provided in supplementary file 2. We also prepared two-fold dilutions of each drug combination by adding 100 µl sterile RPMI 1640 to the initial well and transferring 100 µl of 200 µl mix into microwell of a new 96-well microtiter plate. This dilution scheme provided 1/2, 1/4, 1/8, 1/16, and 1/32 concentrations of each drug in the initial well. The inoculation, incubation, and MIC_{100} end-point reading were as published earlier from a multilaboratory evaluation of antifungal combination testing [9]. Briefly, *C. auris* cultures were streaked on Sabouraud dextrose agar for 24 hours at 35℃, cells suspended in sterile water to absorbance (A_{530}) 0.08-0.1 absorbance as measured with a Mettler Toledo UV5 bio spectrophotometer; 20 µl of the cell suspension was added to the 11 ml of RPMI 1640 broth tube. One-hundred-microliter of the suspension in RPMI 1640 was added to each well of the 96-well microtiter plate with the drug combinations. The plates were incubated at 35℃ for 48-hrs, and MIC_{100} reading was obtained with an illuminated convex mirror plate reader. Twenty microliters of the drug-fungal suspension from each well, after $MIC1_{00}$ determination, was transferred to the well of a fresh 96-well microtiter plate that contained 180 µl of sterile RPMI-1640 to set-up the sterility testing. The sterility plates were incubated at 35℃ for 48-hrs, and minimum fungicidal concentration (MFC) determined from each well with no visible growth.

Results

Among the test group, *C. auris* 16-1 was susceptible to all antifungals tested while the remaining fourteen isolates showed primary resistance to FZ (Table 1). Only *C. auris* 18-1 and 18-13 showed primary resistance to FLC. The distribution of resistance in remaining *C. auris* was triazole resistance (2 isolates), echinocandin resistance (6 isolates), and AMB resistance (4 isolates) (Table 2). Except for primary echinocandin-resistance in one isolate, most *C. auris* isolates revealed cross-resistance and multidrug-resistance as detailed in Table 1. The combination test results were derived from 12,960 MIC_{100} readings, and MFC readings for fifteen *C. auris* isolates tested against 864 two-

drug antifungal combinations for nine antifungal drugs (Supplementary Table 1). Flucytosine (FC) at 2.0 mg/L potentiated most successful combinations with other drugs (Table 2). MFG, AFG, and CAS at individual concentrations of 0.25 mg/L combined well with FLC (2.0 mg/L) to yield MIC_{100} for 14, 13, and 12 out of 15 *C. auris* isolates tested. MFG/FLC combination was also fungicidal at the same concentration for 4 of 15 isolates. AMB / FLC (0.25/1.0 mg/L) yielded MIC_{100} for 13 isolates and MFC for three test isolates. Azoles POS, ISA and VRC also combined well with FLC (0.25/2.0 mg/L) to yield MIC_{100} for 12, 13, and 13 isolates, respectively. POS/FLC combination was fungicidal for three isolates (Table 2). The summary results for the remaining combinations are presented in supplementary table 2. The relative performances of the eight promising drug combinations against all test isolates are detailed in Table 3. Remarkably, MIC_{100} readings were obtained for all selected combinations, but there were very few corresponding MFC readings. *C. auris* 18-1, resistant to FZ and FLC initially, also tested resistant to all antifungal combinations except POS/FLC (0.12/1.0 mg/L) (Table 3).

Discussion

The clinical and public health microbiology community is at high alert due to the alarming rise in the incidence and spread of drug-resistant *C. auris* globally [10]. An aggressive therapeutic intervention is indicated for invasive *C. auris* infections associated with high mortality[7, 11]. The results of the current study fill an essential gap in the published literature as there is no report on the efficacy of antifungal combinations against *C. auris*. Our findings suggested that *C. auris* with various resistance patterns are susceptible to low dose combinations of existing drugs. Furthermore, several of the effective combinations of antifungals exerted fungicidal activities, which was an additional indicator of drug combination efficacy. The earlier multi-laboratory evaluation indicated that MIC_{100} endpoints provide a reliable method for antifungal combination testing instead of FIC (fractional inhibitory concentration) index [9]. We further optimized the method by using fixed drug concentrations instead of the checkerboard dilutions as shown in earlier publications [12, 13]. Additionally, we showed that a more robust outcome could be obtained by determining MIC_{100} and MFC for the same drug combination concurrently. As the test isolates belonged to the South Asian clade, it remains to be determined if the resistant *C. auris* strains from South Africa and South America clades respond similarly to the antifungal combinations. Further clinical relevance of the findings reported in the study could be evaluated by pharmacokinetic / pharmacodynamic studies and testing in the appropriate animal models [14, 15].

Conclusions

A limited set of two drug-combinations of antifungals were efficacious *in vitro* against fifteen *C. auris* strains with primary resistance, cross-resistance, and multidrug-

resistance. The simplified format of the two-drug combination susceptibility test will allow the clinical laboratories to help clinicians in making appropriate treatment choices for *C. auris* candidemia.

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Transparency declaration

B. O'Brien performed the experiments and tabulated and analyzed the data, S. Chaturvedi supervised single drug testing and interpretation, helped in the interpretation of combination testing and critiqued draft manuscript, and V. Chaturvedi conceived and designed the study, interpreted data, and wrote the manuscript.

Table 1. Broth microdilution determination of antifungal MIC_{50} (mg/L) and interpretative criteria (S, I, R) for the test isolates of *Candida auris*

C. auris	Source	Antifungal drug									
Susceptible		FZ	VOR	ITC	ISA	POS	AFG	CAS	MFG	AMB	FLC
16-1	Ear	8	0.25	0.5	0.25	0.25	0.12	0.12	0.12	0.5	0.5
		S	S	S	S	S	S	S	S	S	S
Primary resistance Cross- resistance*											
17-12*	Urine	32	0.5	0.06	0.015	0.03	8	8	8	1	0.032
		S	S	S	S	S	R	R	R	S	S
18-14	NA	>256	1	0.25	0.5	0.03	0.06	0.015	0.06	1	0.047
		R	S	S	S	S	S	S	S	S	S
18-15	Blood	>256	1	0.25	0.5	0.03	0.12	0.03	0.06	1.5	0.064
		R	S	S	S	S	S	S	S	S	S
Multidrug resistance											
17-1	Blood	>256	2	0.5	0.5	0.25	4	4	4	1	0.094
		R	S	S	S	S	R	R	R	S	S
17-13	Urine	>256	1	0.25	0.5	0.03	0.06	0.03	0.06	3	0.032
		R	S	S	S	S	S	S	S	R	S
17-14	Groin swab	>256	2	0.5	0.5	0.12	4	2	4	0.75	0.032
		R	S	S	S	S	R	R	R	S	S
17-15	Skin rash swab	>256	2	0.5	0.5	0.25	4	2	4	0.75	0.032
		R	S	S	S	S	R	R	R	S	S
17-16	Urine	>256	2	0.5	0.5	0.12	4	2	4	1	0.023
		R	S	S	S	S	R	R	R	S	S
17-17	Nares swab	>256	4	0.5	0.5	0.06	0.25	0.03	0.06	3	0.064
		R	R	S	S	S	S	S	S	R	S
17-18	Nares swab	>256	2	0.5	1	0.12	0.25	0.06	0.06	3	0.064
		R	S	S	S	S	S	S	S	R	S
18-1	Wound swab	128	0.5	0.12	0.12	0.03	0.25	0.03	0.12	1	>32
		R	S	S	S	S	S	S	S	S	R
18-2	Ascites fluid	>256	2	1	1	0.25	8	2	4	1.5	0.064
		R	S	R	R?	S	R	R	R	S	S
18-5	Urine	>256	2	0.25	0.5	0.06	0.12	0.03	0.12	2	0.094
		R	S	S	S	S	S	S	S	R	S
18-13	Rectal swab	256	0.5	0.12	0.12	0.03	0.12	0.03	0.12	1	>32
		R	S	S	S	S	S	S	S	S	R

* cross-resistance to all echinocandins
NA not available

Table 2. Most effective antifungal combinations against *Candida auris*

Antifungal combinations	MIC_{100} mg/L (No. positive / No. tested)	MFC mg/L (No. positive / No. tested)
MFG + FLC	0.25/2.0 (14/15)	0.25/2.0 (4/15)
AMB + FLC	0.25/1.0 (13/15)	0.5/2.0 (3/15)
AFG + FLC	0.25/2.0 (13/15)	0.25/2.0 (1/15)
CAS + FLC	0.25/2.0 (12/15)	0.25/2.0 (2/15)
POS + FLC	0.25/2.0 (12/15)	0.25/2.0 (3/15)
ISA + FLC	0.25/2.0 (13/15)	0.25/2.0 (1/15)
ITC + FLC	0.12/2.0 (12/15)	0.12/2.0 (1/15)
VOR + FLC	0.5/2.0 (13/15)	NA (0/15)

Table 3. Most promising antifungal combinations for *Candida auris*

Combi-nation	End-reading	*Candida auris*														
		16-1	17-1	17-12	17-13	17-14	17-15	17-16	17-17	17-18	18-1	18-2	18-5	18-13	18-14	18-15
MFG + FLC	MIC_{100}	0.12* / 1.0	0.12 / 1.0	0.06/ 0.5	0.12 / 1.0	0.12 / 1.0	0.12 / 1.0	0.12 / 1.0	0.12 / 1.0	0.25 / 2.0	NA	0.12 / 1.0	0.12 / 1.0	0.12 / 1.0	0.12 / 1.0	0.12 / 1.0
	MFC	NA	NA	0.12/ 1.0	NA	0.25 / 2.0	NA	NA	0.12 / 1.0	NA	NA	NA	NA	NA	NA	0.25 / 2.0
AMB + FLC	MIC_{100}	0.25/ 1.0	0.25 / 1.0	0.25/ 1.0	0.25 / 1.0	0.25 / 1.0	0.25 / 1.0	0.25 / 1.0	0.25 / 1.0	0.25 / 1.0	NA	0.25 / 1.0	0.25 / 1.0	NA	0.25 / 1.0	0.25 / 1.0
	MFC	NA	0.25 / 1.0	NA	NA	NA	NA	NA	0.5/ 2.0	NA	NA	NA	NA	NA	0.25 / 1.0	NA
AFG + FLC	MIC_{100}	0.12/ 1.0	0.12 / 1.0	0.12/ 1.0	0.12 / 1.0	0.12 / 1.0	0.25 / 2.0	0.12 / 1.0	0.12 / 1.0	0.25 / 2.0	NA	0.12 / 1.0	0.12 / 1.0	NA	0.12 / 1.0	0.12 / 1.0
	MFC	NA	NA	0.25/ 2.0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
CAS + FLC	MIC_{100}	0.12/ 1.0	0.12 / 1.0	0.12/ 1.0	0.12 / 1.0	0.12 / 1.0	0.12 / 1.0	0.25 / 2.0	0.12 / 1.0	NA	NA	0.12 / 1.0	0.25 / 2.0	NA	0.12 / 1.0	0.12 / 1.0
	MFC	NA	NA	NA	0.12 / 1.0	NA	NA	NA	0.25 / 2.0	NA	NA	NA	NA	NA	NA	NA
POS + FLC	MIC_{100}	0.12/ 1.0	0.12 / 1.0	0.12/ 1.0	NA	NA	0.12 / 1.0	0.12 / 1.0	0.12 / 1.0	0.12 / 1.0	0.12 / 1.0	0.12 / 1.0	0.12 / 1.0	NA	0.12 / 1.0	0.25 / 2.0
	MFC	0.12/ 1.0	NA	0.25/ 2.0	NA	NA	NA	NA	NA	NA	NA	0.25 / 2.0	NA	NA	NA	NA
ISA + FLC	MIC_{100}	0.12/ 1.0	0.12 / 1.0	0.12/ 1.0	0.12 / 1.0	0.12 / 1.0	0.12 / 1.0	0.12 / 1.0	0.12 / 1.0	0.12 / 1.0	NA	0.12 / 1.0	0.12 / 1.0	NA	0.12 / 1.0	0.25 / 2.0
	MFC	NA	NA	NA	NA	NA	NA	NA	0.25 / 2.0	NA	NA	NA	NA	NA	NA	NA
ITC + FLC	MIC_{100}	0.03/ 0.5	0.06 / 1.0	0.06/ 1.0	0.06 / 1.0	NA	0.06 / 1.0	0.06 / 1.0	0.06 / 1.0	0.06 / 1.0	NA	0.06 / 1.0	0.06 / 1.0	NA	0.06 / 1.0	0.12 / 2.0
	MFC	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	0.12 / 2.0	NA	NA	NA	NA
VOR + FC	MIC_{100}	0.25/ 1.0	0.25 / 1.0	0.125 / 0.5	0.25 / 1.0	0.25 / 1.0	0.25 / 1.0	0.25 / 1.0	0.25 / 1.0	0.25 / 1.0	NA	0.25 / 1.0	0.25 / 1.0	NA	0.25 / 1.0	0.5/ 2.0
	MFC	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

* mg/L
NA Not applicable as MFC was not obtained

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