***In Vitro* Antifungal Susceptibility of the Emerging Multidrug-Resistant Pathogen *Candida auris* to Miltefosine Alone and in Combination with Amphotericin B**

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Candida auris, a globally emerging human fungal pathogen, has arisen as a public health concern worldwide because of its ability to cause nosocomial outbreaks and its resistance to multiple antifungal drugs (1, 2). *C. auris* is resistant to fluconazole in up to 90% of isolates and exhibits reduced susceptibility to other azoles (3, 4); resistance to amphotericin B and 5-flucytosine has also been reported (5–7). Echinocandin resistance is relatively rare, and this drug class has been chosen as the first line of choice to treat *C. auris* infections; however, reduced susceptibility to echinocandins has been increasingly reported (6, 8, 9). Thus, in the absence of new antifungal drugs currently available to treat the disease, alternative antifungal regimens are urgently sought. Miltefosine, a type of alkyl-phospholipid analogue, is a clinically licensed antileishmanial drug. The drug was found to possess *in vitro* antifungal activity against a pan-antifungal drug-resistant fungus, *Lomentospora prolificans*, but demonstrated different *in vitro* and *in vivo* activities against *Cryptococcus neoformans* (10–12). In this study, we examined the *in vitro* antifungal susceptibility of miltefosine alone or in combination with fluconazole or amphotericin B against 12 *C. auris* clinical isolates, which included 10 from the CDC and FDA Antibiotic Resistance (AR) Bank and 2 from the Johns Hopkins Hospital (representing 4 different geographic clades).

The MICs were determined using the broth microdilution method or the broth checkerboard method from the CLSI M27-A4 document (13). The endpoints were defined as at least 50% inhibition of growth for azoles alone and in combination with miltefosine and as 100% inhibition of growth for amphotericin B alone and in combination with miltefosine. The endpoint for miltefosine alone was read at 50% and 100% inhibition, respectively. Drug synergy testing (repeated three times) was assessed by calculating the fractional inhibitory concentration index (FICI) as follows: $FICI \leq 0.5$ for synergism, $0.5 < FICI < 4$ for indifference, and $FICI > 4$ for antagonism (14). The minimum fungicidal concentrations (MFCs) were defined as the lowest concentration that eliminated 99.9% of the colonies formed on the plates as previously described (15). Strains of *Candida parapsilosis* ATCC 22019 and *Candida krusei* ATCC 6258 were used as controls.

Eleven isolates (11/12) were fluconazole resistant (MIC₉₀, 256 µg/ml), and seven isolates (7/12) had a high MIC for voriconazole (MIC₉₀, 4 µg/ml) (Table 1), according to the tentative MIC breakpoints recommended by the U.S. Centers for Disease Control and Prevention (<https://www.cdc.gov/fungal/candida-auris/c-auris-antifungal.html>). Three isolates (3/12) were resistant to amphotericin B (2 µg/ml) and fluconazole (128 to 256 µg/ml). All isolates were susceptible to micafungin (data not

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TABLE 1 *In vitro* antifungal susceptibility testing of *C. auris* isolates

Isolate no.	Antifungal ^a			MIL (μg/ml) at:		
	AMB (μg/ml)	FLU (μg/ml)	VOR (μg/ml)	MIL (μg/ml) at:		
				MIL-1	MIL-2	MFC
1	1	256	8	2	4	4
2	1	256	2	2	2	2
3	2	256	2	2	2	2
4	1	256	4	2	2	2
5	1	256	4	2	2	2
6	0.5	2	0.03	2	2	2
7	1	256	2	2	2	2
8	2	256	4	2	4	4
9	1	256	1	2	2	2
10	2	128	0.5	2	2	2
11	1	128	0.5	2	2	2
12	1	128	0.5	2	2	2

^aAMB, amphotericin B; FLU, fluconazole; VOR, voriconazole; MIL, miltefosine; MIL-1, the minimum concentration of MIL that inhibited 50% of fungal growth; MIL-2, the minimum concentration of MIL that inhibited 100% of fungal growth; MFC, minimum fungicidal concentration.

shown here). All isolates showed a miltefosine MIC at 2 μg/ml using endpoint reading at either 50% or 100% inhibition (with an exception for two isolates showing an MIC at 4 μg/ml read at 100% inhibition). The drug showed fungicidal activity against *C. auris* (Table 1). Although there are no established breakpoints for miltefosine, the pharmacokinetics of miltefosine in children and adults treated with leishmaniasis showed high miltefosine plasma concentrations (i.e., 17.2 to 42.4 μg/ml), and no serious adverse events were reported (16, 17). Therefore, as it has fungicidal activity at an MIC of 2 to 4 μg/ml, miltefosine may be a good alternative drug of choice to treat *C. auris*; however, more pharmacokinetics and pharmacodynamics studies are needed.

The synergistic effect of miltefosine with amphotericin B was observed for three isolates (3/12) (Table 2). The MIC of amphotericin B was reduced 8-fold for 3 isolates and 4-fold for 5 isolates. This finding was consistent with previous studies that showed a major reduction in amphotericin B susceptibility when it was used in combination with miltefosine against clinical molds (18, 19). However, miltefosine and fluconazole combinations showed indifferent interaction for all isolates. To the best of our knowledge, this is the first study examining the activity of miltefosine against *C. auris*. Although we only tested a dozen *C. auris* isolates, our data showed that miltefosine has potential activities against *C. auris* either alone or in synergy with amphotericin B.

TABLE 2 Antifungal synergy testing of *C. auris* isolates

Isolate	MIC (μg/ml) for:				MIC (μg/ml) for:			
	AMB	MIL	AMB/MIL ^b	FICI ^a	FLU	MIL	FLU/MIL ^c	FICI ^a
1	1	4	0.25/2	0.75	>128	2	>128/>1	2
2	1	2	0.25/0.5	0.5	>128	2	>128/>1	2
3	2	2	0.5/0.5	0.5	>128	2	>128/>1	2
4	1	2	0.125/1	0.625	>128	2	>128/>1	2
5	1	2	0.125/1	0.625	>128	2	>128/>1	2
6	0.5	2	0.125/1	0.75	2	2	>1/>1	2
7	1	2	0.25/1	0.75	>128	2	>128/>1	2
8	2	2	0.25/1	0.625	>128	2	>128/>1	2
9	1	2	0.25/1	0.75	>128	2	>128/>1	2
10	2	2	0.5/0.5	0.5	128	2	>64/>1	2
11	1	2	0.5/1	1	128	2	>64/>1	2
12	1	2	0.25/1	0.75	128	2	>64/>1	2

^aFICI (fractional inhibitory concentration index) values in bold indicate synergy.

^bThe 100% inhibition endpoint was used for this combination.

^cThe 50% or more inhibition endpoint was used for this combination.

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