

DEVELOPMENT OF RESISTANCE TO SF001, A NEXT GENERATION POLYENE ANTIFUNGAL

C. Pillar, A. Mendez, O. Walser, R. Jahanbakhsh, M. Thwaites, D. Hufnagel
Microbiologics, Kalamazoo, MI

ABSTRACT

Background: SF001, a next generation renal sparing polyene with long-acting, potent, broad spectrum fungicidal activity, is currently undergoing development. This project evaluated SF001 resistance development in *Candida* by assessing spontaneous mutation frequency (SMF) and change in MIC during serial passage.

Methods: SF001 and liposomal amphotericin B (L-AMB) SMF was determined with *C. albicans*, *C. glabrata*, *C. tropicalis*, *C. parapsilosis*, *C. krusei*, and *C. auris* (N=8). Mutants patched onto agar at the selection concentration were subjected to broth microdilution to confirm resistance.

SF001 and L-AMB resistance development was assessed by serial passage of 5 *Candida* spp. (same as above excluding *C. auris* and resistant *C. albicans*) on gradient agar plates. Growth at the leading edge of the plate was used to inoculate each passage for 20 passages. For every third passage, broth MIC values for SF001, L-AMB, and comparators were determined.

Results: SMF values for both SF001 and L-AMB were low (<10⁻⁹) for all isolates except for a single isolate of *C. auris*; in this instance, despite an SMF of 10⁻⁷-10⁻⁸, MIC values for mutants were at or within 2-fold of the parent isolate indicating that the mutants for this isolate were unstable. During serial passage, SF001 MIC values remained at or within 2-fold of that observed at the initial passage.

Conclusion: Overall, SF001 showed low propensity for resistance development in vitro for *Candida* spp. with low SMF and no increase in MIC values during serial passage.

BACKGROUND

- New and safer antifungals are needed to combat the growing number of serious fungal infections which are associated with a high degree of mortality and morbidity.^{1,2}
- Urea derivatives of AmB have been developed as less toxic alternatives to AmB based on selective binding of ergosterol without binding cholesterol; the non-selective binding of cholesterol by AmB is implicated in toxicity to the host.³
- Ergosterol is essential for yeast survival making it difficult for resistance to develop to polyenes that bind and extract sterols; in contrast, resistance to triazoles and echinocandins is readily selected and is a growing issue clinically.⁴
- Despite 50 years of use as a monotherapy, resistance to AmB is rare and when it does occur it negatively impacts fitness and virulence.⁴
- In this study, the development of resistance to SF001, a novel urea AmB derivative currently undergoing development, was evaluated in vitro for yeast by determining the spontaneous mutation frequency and assessing change in activity during serial passage.

METHODS

- Prior to SMF assays, the agar dilution MIC values of SF001 and L-AMB were determined in triplicate using Mueller-Hinton agar (MHA) in accordance with CLSI guidelines for testing of bacteria (M7) using an inoculum of 10⁴ CFU/spot.
- For SMF,⁵ 150 mm MHA plates with test agent at 4X, 8X, and 16X the MIC were inoculated in duplicate with approximately 0.5 to 1 x 10⁹ CFU. Isolates of *C. albicans* including fluconazole- (FLC) and caspofungin (CAS)-resistant isolates were evaluated alongside single isolates of *C. parapsilosis*, *C. krusei*, *C. tropicalis*, *C. glabrata*, and *C. auris*.
- After 48 hr of incubation, resulting mutants were counted for SMF calculation and determination of mutant prevention concentration (MPC), and were patched onto agar containing test article at the selection concentration prior to freezing; ID was confirmed by MALDI and broth microdilution susceptibility testing of yeast was conducted as described below.
- For serial passage,⁵ the same yeast evaluated for SMF excluding *C. auris* and resistant *C. albicans* were tested; MHA plates containing a gradient of test agent were inoculated using a standardized suspension prepared from the leading edge of growth from the prior passage, the inoculum was frozen for storage, and this process was repeated for 20 serial passages.
- At every third passage, the inoculum was plated for confirmatory ID and broth microdilution susceptibility testing as described below.
- Resulting SMF mutants and select serial passage inocula were subjected to broth microdilution susceptibility testing (CLSI M27 and M60) alongside the parent isolate for SF001 and comparators to confirm elevated MIC and to evaluate potential co-resistance.

RESULTS

Table 1. SMF and MPC of SF001 for yeast

Organism	Inoculum Size (CFU)	Agar MIC at 48 hr (µg/mL)	Selection - Fold-MIC	Count 1 (CFU)	Count 2 (CFU)	Average Count (CFU)	SMF	MPC (µg/mL)
<i>C. albicans</i> ATCC 90028	9.0 x 10 ⁸	1	4X	2	0	1	1.1 x 10 ⁻⁹	8
			8X	0	0	0	≤1.1 x 10 ⁻⁹	
			16X	0	0	0	≤1.1 x 10 ⁻⁹	
<i>C. albicans</i> ATCC MYA-573 (FLC-R)	7.0 x 10 ⁸	2	4X	0	0	0	≤1.4 x 10 ⁻⁹	≤8
			8X	0	0	0	≤1.4 x 10 ⁻⁹	
			16X	0	0	0	≤1.4 x 10 ⁻⁹	
<i>C. albicans</i> MMX 7424 (CAS-R)	6.8 x 10 ⁸	1	4X	0	0	0	≤1.5 x 10 ⁻⁹	≤4
			8X	0	0	0	≤1.5 x 10 ⁻⁹	
			16X	0	0	0	≤1.5 x 10 ⁻⁹	
<i>C. parapsilosis</i> ATCC 22019	1.3 x 10 ⁹	2	4X	0	0	0	≤7.7 x 10 ⁻¹⁰	≤8
			8X	0	0	0	≤7.7 x 10 ⁻¹⁰	
			16X	0	0	0	≤7.7 x 10 ⁻¹⁰	
<i>C. krusei</i> ATCC 6258	1.0 x 10 ⁹	4	4X	0	1	1	1.0 x 10 ⁻⁹	32
			8X	0	0	0	≤1.0 x 10 ⁻⁹	
			16X	0	0	0	≤1.0 x 10 ⁻⁹	
<i>C. tropicalis</i> ATCC 90874	4.6 x 10 ⁸	1	4X	15	20	18	4.0 x 10 ⁻⁸	8
			8X	0	0	0	≤2.2 x 10 ⁻⁹	
			16X	0	0	0	≤2.2 x 10 ⁻⁹	
<i>C. glabrata</i> ATCC 90030	2.0 x 10 ⁹	0.5	4X	17	8	13	6.5 x 10 ⁻⁹	>8
			8X	5	30	18	9.0 x 10 ⁻⁹	
			16X	14	15	15	7.5 x 10 ⁻⁹	
<i>C. auris</i> MMX 9862	2.0 x 10 ⁹	0.5	4X	1400	1300	1350	6.8 x 10 ⁻⁷	>8
			8X	220	210	215	1.1 x 10 ⁻⁷	
			16X	15	60	38	2.0 x 10 ⁻⁸	

Table 2. SMF and MPC of L-AMB for yeast

Organism	Inoculum Size (CFU)	Agar MIC at 48 hr (µg/mL)	Selection - Fold-MIC	Count 1 (CFU)	Count 2 (CFU)	Average Count (CFU)	SMF	MPC (µg/mL)
<i>C. albicans</i> ATCC 90028	9.0 x 10 ⁸	2	4X	0	0	0	≤1.1 x 10 ⁻⁹	32
			8X	1	1	1	1.1 x 10 ⁻⁹	
			16X	0	0	0	≤1.1 x 10 ⁻⁹	
<i>C. albicans</i> ATCC MYA-573 (FLC-R)	7.0 x 10 ⁸	2	4X	0	1	1	1.4 x 10 ⁻⁹	16
			8X	0	0	0	≤1.4 x 10 ⁻⁹	
			16X	0	0	0	≤1.4 x 10 ⁻⁹	
<i>C. albicans</i> MMX 7424 (CAS-R)	6.8 x 10 ⁸	2	4X	0	0	0	≤1.5 x 10 ⁻⁹	≤8
			8X	0	0	0	≤1.5 x 10 ⁻⁹	
			16X	0	0	0	≤1.5 x 10 ⁻⁹	
<i>C. parapsilosis</i> ATCC 22019	1.3 x 10 ⁹	8	4X	0	0	0	≤7.7 x 10 ⁻¹⁰	≤32
			8X	0	0	0	≤7.7 x 10 ⁻¹⁰	
			16X	0	0	0	≤7.7 x 10 ⁻¹⁰	
<i>C. krusei</i> ATCC 6258	1.0 x 10 ⁹	8	4X	0	0	0	≤2.2 x 10 ⁻⁹	≤32
			8X	0	0	0	≤1.0 x 10 ⁻⁹	
			16X	0	0	0	≤1.0 x 10 ⁻⁹	
<i>C. tropicalis</i> ATCC 90874	4.6 x 10 ⁸	2	4X	0	0	0	≤2.2 x 10 ⁻⁹	32
			8X	0	1	1	2.2 x 10 ⁻⁹	
			16X	0	0	0	≤2.2 x 10 ⁻⁹	
<i>C. glabrata</i> ATCC 90030	2.0 x 10 ⁹	2	4X	5	6	6	3.0 x 10 ⁻⁹	>32
			8X	10	5	8	4.0 x 10 ⁻⁹	
			16X	8	13	11	5.5 x 10 ⁻⁹	
<i>C. auris</i> MMX 9862	2.0 x 10 ⁹	2	4X	10	77	44	2.2 x 10 ⁻⁸	>32
			8X	15	14	15	7.5 x 10 ⁻⁹	
			16X	37	38	38	1.9 x 10 ⁻⁸	

RESULTS

Figure 1. Activity of SF001 and L-AMB during serial passage with yeast

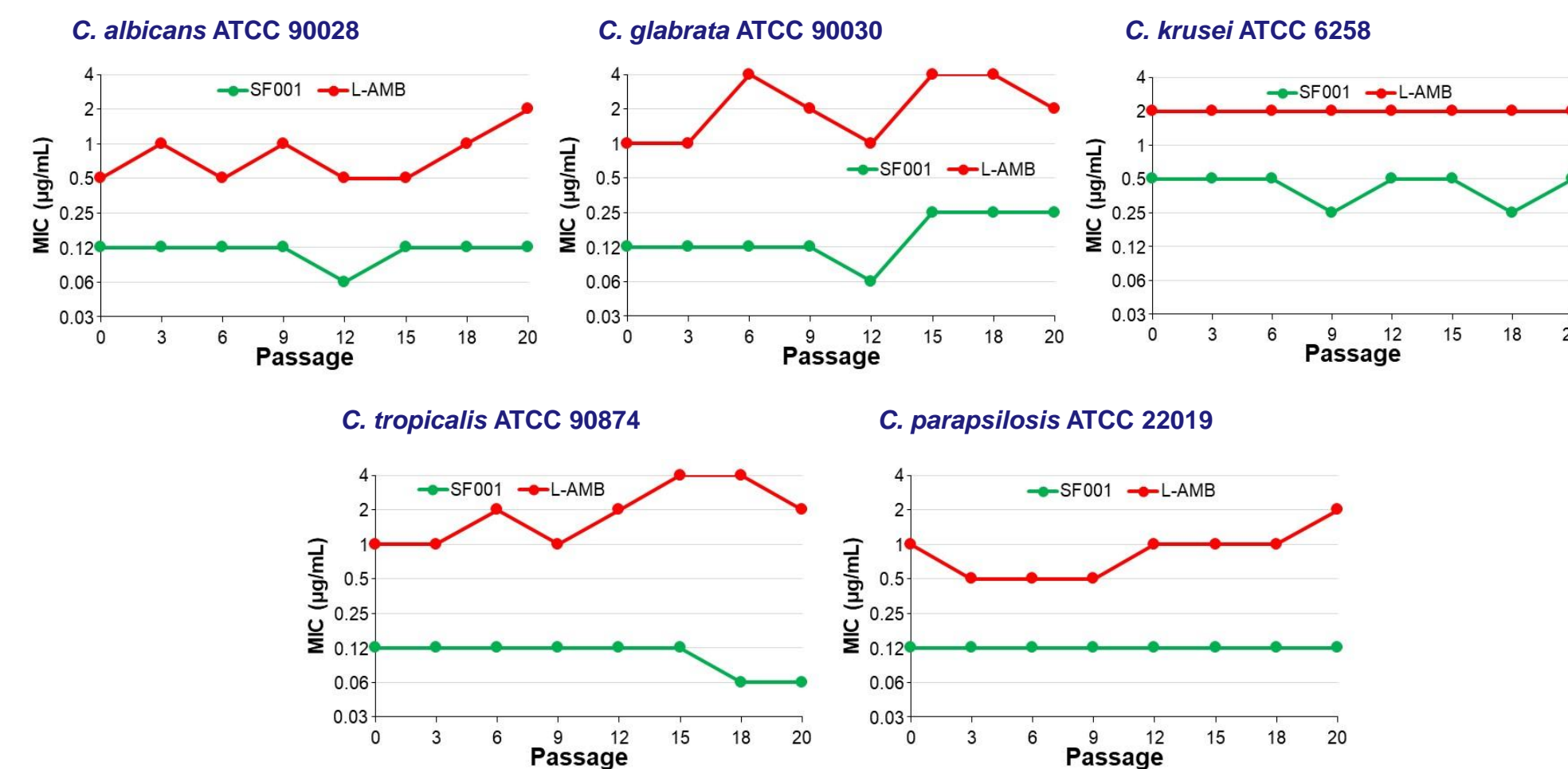


Table 3. Broth MIC values for SMF mutants and parent isolates

Test Organism	Selection Agent – Fold-MIC - Colony Number	MIC (µg/ml)				
		SF001	L-AMB	AmB	CAS	FLC
<i>C. albicans</i> ATCC 90028	parent	0.25	0.5	0.12	0.03	0.25
	SF001 - 4X - 1	0.25	0.5	0.5	0.06	>32
	SF001 - 4X - 2	0.25	0.5	0.12	0.03	>32
	L-AMB - 8X - 1	0.12	0.25	0.12	0.03	0.12
	L-AMB - 8X - 2	0.25	0.5	0.12	0.03	0.12
<i>C. albicans</i> ATCC MYA-573 (FLC-R)	parent	0.25	0.5	0.25	0.03	>32
	L-AMB - 4X - 1	0.25	0.5	0.5	0.03	>32
	parent	0.5	1	0.5	0.12	16
	SF001 - 4X - 1	0.12	1	0.25	0.03	0.5
	SF001 - 4X - 2	0.12	1	0.25	0.06	0.5
<i>C. tropicalis</i> ATCC 90874	parent	0.25	1	0.5	0.06	0.5
	L-AMB - 8X - 1	0.25	1	0.5	0.06	0.5
	parent	0.12	1	0.25	0.06	8
	SF001 - 4X - 1	1	2	0.5	0.06	8
	SF001 - 4X - 2	0.5	2	0.25	0.03	>32
<i>C. glabrata</i> ATCC 90030	SF001 - 8X - 1	1	4	0.5	0.06	1
	SF001 - 8X - 2	1	4	0.5	0.06	1
	SF001 - 16X - 1	1	4	0.5	0.06	8
	SF001 - 16X - 2	1	4	0.5	0.06	8
	L-AMB - 4X - 1	1	4	0.5	0.06	8
	L-AMB - 4X - 2	1	2	0.5	0.06	8
	L-AMB - 8X - 1	1	4	0.5	0.25	1
	L-AMB - 8X - 2	1	4	0.5	0.06	1
	L-AMB - 16X - 1	2	4	0.5	0.06	4
	L-AMB - 16X - 2	1	4	1	0.12	2
	9862-parent	0.12	0.5	0.12	0.03	2
	SF001 - 4X - 1	0.25	1	0.25	0.06	>32
SF001 - 4X - 2	0.25	1	0.25	0.03	>32	
SF001 - 16X - 1	0.5	1	0.25	0.06	>32	
<i>C. auris</i> MMX 9862	L-AMB - 4X - 1	0.12	0.5	0.12	0.06	2
	L-AMB - 4X - 2	0.12	0.5	0.12	0.06	2
	L-AMB - 8X - 1	0.12	0.5	0.12	0.03	2
	L-AMB - 8X - 2	0.12	0.5	0.12	0.03	2
	L-AMB - 16X - 1	0.12	0.5	0.12	0.03	2
	L-AMB - 16X - 2	0.12	0.5	0.12	0.03	2

Red shading highlights instances where at least a 4-fold increase in MIC relative to that of the parent was observed

CONCLUSIONS

- Overall, development of resistance in vitro among *Candida* spp. to SF001 was low, with low mutation frequencies and MPC values and little to no change in MIC values during serial passage.
- These findings are consistent with other polyenes, including L-AMB.

REFERENCES

- Bongomon F et al., *J Fungi* 2017;3(4):E57.
- Kainz K et al., *Microb Cell* 2020; 7(6):143.
- Davis SA et al., *Nat Chem Biol* 2015;11(7):481.
- Vincent BM et al., *PLoS Biol* 2013;11(10):e1001692.
- Locke JB et al., *Antimicrob Agents Chemother* 2016;60(10):6100.