

Inoculum Density has an Impact on SF001 Fungicidal Activity against *Candida albicans*

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AMENDED ABSTRACT

Background: AM-2-19 is a novel polyene that selectively targets ergosterol over cholesterol, minimizing toxicity associated with this class while maintaining potency against yeast and mould. AM-2-19, like other polyenes, is fungicidal. The drug product SF001 is a novel micellar formulation of AM-2-19. In this study, the time-kill kinetic (TK) was evaluated for SF001 and liposomal amphotericin-B (LAmB) against *Candida albicans*.

Methods: TK was assessed at multiples of the MIC (4X, 8X, and 16X) for both test isolates at high ($\sim 5 \times 10^5$ CFU/mL), medium ($\sim 5 \times 10^4$ CFU/mL), and low ($\sim 5 \times 10^3$ CFU/mL) inoculum density and at fixed concentrations of 4, 8, and 16 mg/L for ATCC 90028 and of 8, 16, and 32 mg/L for ATCC MYA-2732 (high inoculum density). Colony counts were determined at 2, 4, 6, 24, and 48 hr.

Results: SF001 was 4-fold more potent than LAmB by MIC. For ATCC 90028 at the high inoculum density, 3-log killing was observed by 6 hr for both SF001 and LAmB; regrowth was noted for SF001 at the 24 and 48 hr timepoints. Regrowth was less apparent with LAmB where higher LAmB concentrations were tested due to higher LAmB MICs. As the test concentration of SF001 was increased (to match that of LAmB), sustained 3-log kill was observed for SF001. Decreasing the inoculum density while testing SF001 at multiples of the MIC also resulted in less regrowth at later timepoints, with minimal regrowth at 8X and 16X the MIC at low inoculum density. Similar results were observed with MYA-2732. Overall, apart from the regrowth, the kinetics of SF001 were similar to LAmB.

Conclusion: SF001 and LAmB exhibited fungicidal activity against *C. albicans*. Regrowth observed with SF001 at later timepoints was diminished either by increasing the test concentration to match that of LAmB or by decreasing inoculum density. These results suggest that the regrowth observed is likely due to a stoichiometric imbalance between target (ergosterol) and available SF001.

BACKGROUND

- Significant mortality and morbidity result from fungal infections with around 1 billion infections globally per year and 1.7 million deaths annually.^{1,2}
- SF001 is a micellar formulation of a novel analog of amphotericin B (AmB) with potentially less toxicity than AmB due to its binding specificity for ergosterol over cholesterol, the latter of which is associated with toxicity.^{3,4}
- Polyenes exert antifungal activity via a sterol sponge mechanism by which they self-assemble in fungal membranes and rapidly extract ergosterol from lipid bilayers; activity is concentration-dependent and requires achieving an appropriate stoichiometric balance between drug and target. There also may be alternate mechanisms where polyenes form pores in the membrane.^{5,6}
- In this study, the impact of concentration and inoculum density on fungicidal activity observed with SF001 relative to liposomal amphotericin-B (LAmB) was evaluated against *Candida albicans*.

METHODS

- SF001, LAmB, and AmB were tested by broth microdilution (CLSI M27 & M60).⁷
- SF001 and LAmB were tested at multiples of the MIC (4X, 8X, 16X) and viable yeast were assessed at 2, 4, 6, 24, and 48 hr by serial dilution and plating.
- SF001 was also tested at higher concentrations based on the LAmB MIC (4, 8, and 16 $\mu\text{g}/\text{mL}$ for *C. albicans* ATCC 90028, and 8, 16, and 32 $\mu\text{g}/\text{mL}$ for ATCC MYA-2732).
- Killing of yeast by SF001 and LAmB was evaluated by time-kill⁸ using three different inoculum densities against ATCC 90028 and ATCC MYA-2732 (approximately 5×10^5 , 5×10^4 , and 5×10^3 CFU/mL).

RESULTS

- The SF001 MIC is generally 4-fold lower than the LAmB MIC when tested against *C. albicans*; results are based on multiple independent replicates at a standard inoculum density (**Table 1**).
- When tested at high inoculum densities, a 3-log kill was observed by 6 hr for both SF001 and LAmB against ATCC 90028, and ATCC MYA-2732 exhibited similar growth kinetics but SF001 did not reach 3-log kill (**Figure 1**).
- Growth kinetics for SF001 were largely similar to LAmB, except that regrowth at 24 and 48 hr was observed with SF001 when evaluated at high inoculum densities (**Figure 1**).
- As the initial inoculum density was decreased, killing with SF001 to levels at or below the limit of detection (LOD) was more apparent. Regrowth was either delayed or no longer apparent, particularly at 16X the MIC (**Figure 1**).
- As the test concentration of SF001 was increased (to match that of LAmB), sustained 3-log kill was observed for SF001 (**Figure 2**).

Table 1. Activity of SF001 & comparators against *C. albicans*

Organism	Median MIC (mg/L) n=3		
	SF001	LAmB	AmB
ATCC 90028	0.12	0.5	0.25
ATCC MYA-2732	0.25	1	0.25

LAmB: liposomal amphotericin-B; AmB: amphotericin-B; n=number of replicates for each isolate

Figure 1. Kill-curves for SF001 and LAmB based on multiples of the MIC against ATCC 90028 (A) and ATCC MYA-2732 (B) using high, medium, and low inoculum densities

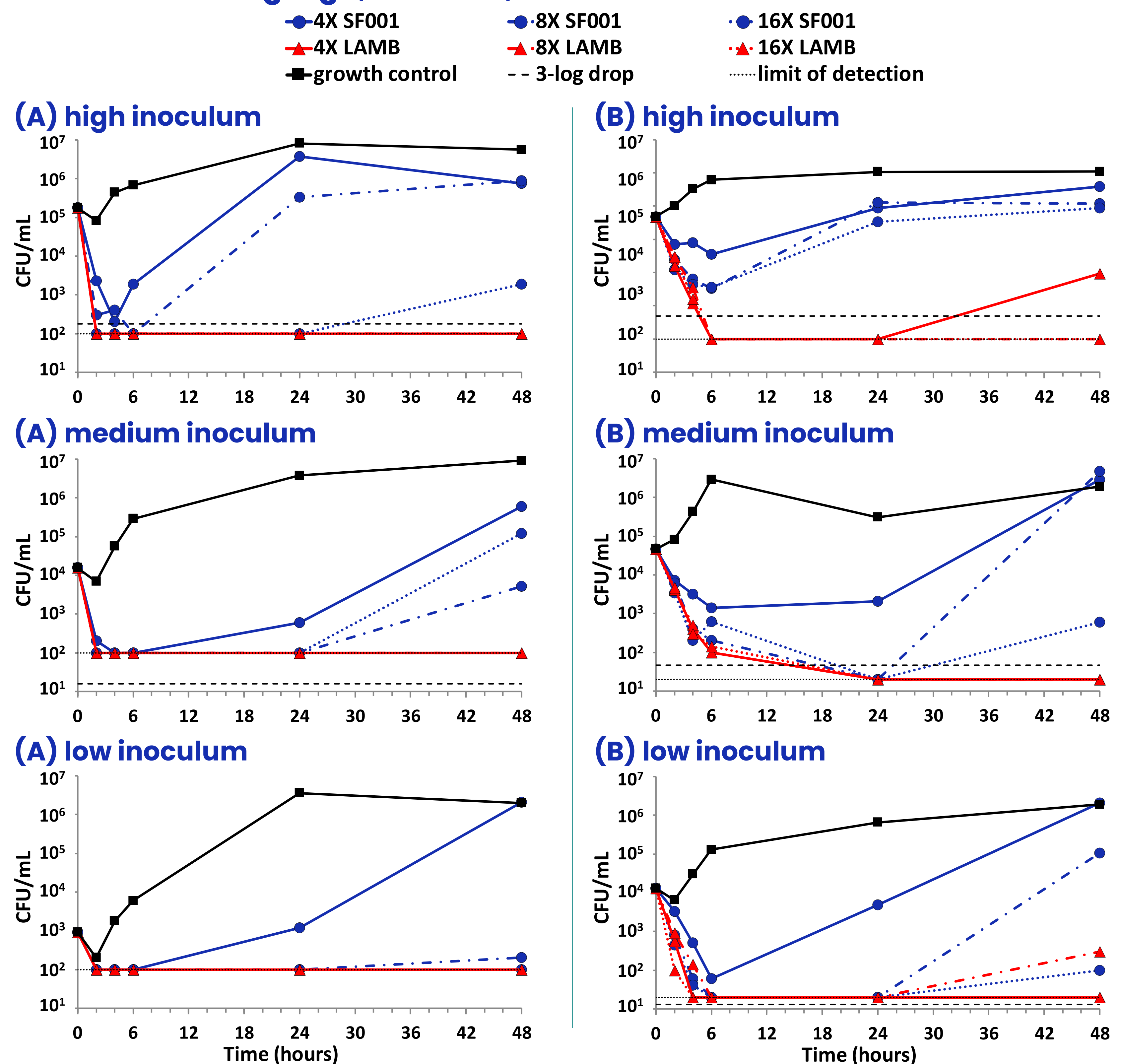
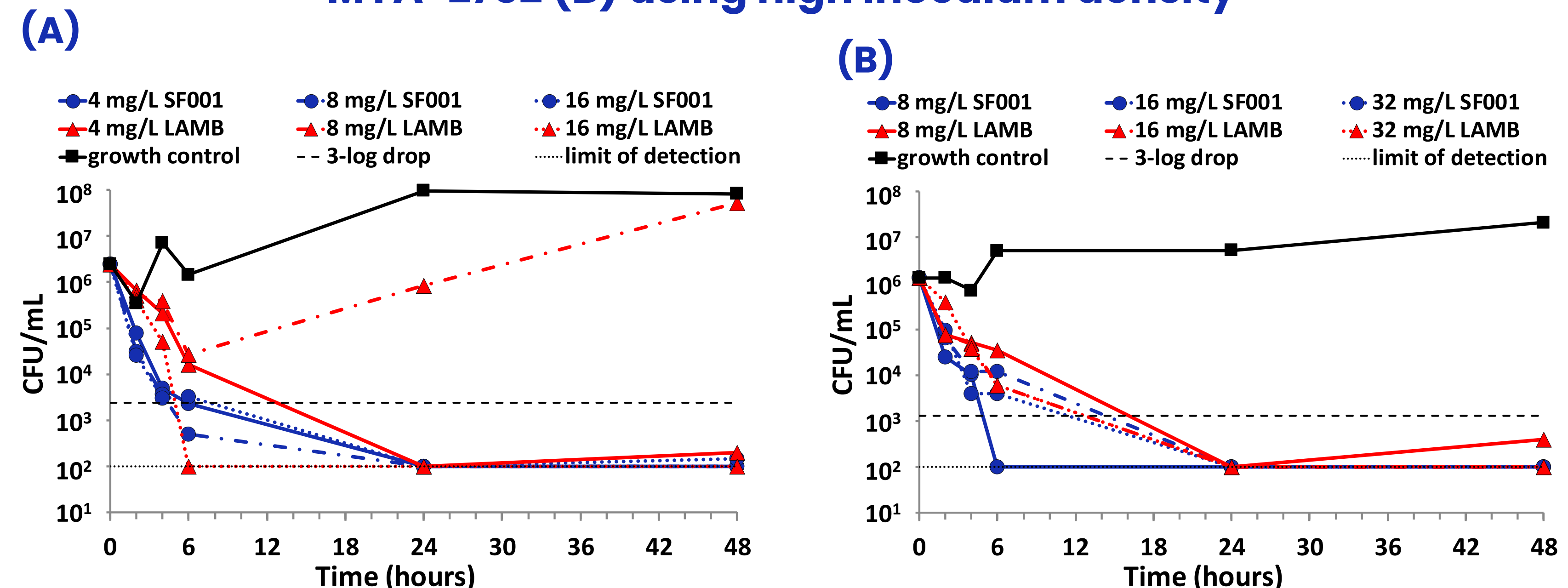


Figure 2. Kill-curves for SF001 and LAmB using equivalent concentrations based on LAmB MIC against ATCC 90028 (A) and ATCC MYA-2732 (B) using high inoculum density



CONCLUSIONS

- The MIC of SF001 against *C. albicans* was generally 4-fold lower than that of LAmB MIC.
- Fungal regrowth observed with SF001 at later timepoints (24 and 48 hr) was diminished by either increasing the SF001 test concentration or by decreasing inoculum density.
- These results confirm concentration-dependent killing by SF001 and indicate that stoichiometric imbalances between SF001 and its target (ergosterol) can be overcome by either decreasing inoculum density or increasing test concentration.

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