Dual-loaded nanoemulsion as a promising strategy for *Candidozyma auris* systemic infections

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Introduction

Candidozyma auris (formerly *Candida auris*) is an emerging yeast that has been increasingly associated with infections outbreaks, mainly systemic. It represents nowadays a global threat due to its high lethality, contagiousness, and resistance to major first-line antifungals like micafungin and amphotericin B. Therefore, the study of new therapeutic options is necessary. [1]

Aim

The present study sought to develop a nanoemulsion (NE) loaded with micafungin and amphotericin B (NEMA) as a resistance-free therapeutic option.

Methods

NEMA were prepared as previously published.[2] To be representative, the in vitro assays (minimal inhibitory concentration (MIC), Checkerboard assay, anti-biofilm efficacy evaluation)were performed with two strains from each *C. auris* clades (India, CLADE I—InP13 and AL1; Japan, CLADE II—JAP1 and Kro; Spain, CLADE III—SP94 and SP96; Venezuela, CLADE IV—VENC6 and BRA). For the in vivo assay one strain from each clade (InP13, JAP1, SP96, and VENC6) was used. Figure 1 represents the timeline of this study experimental stages.

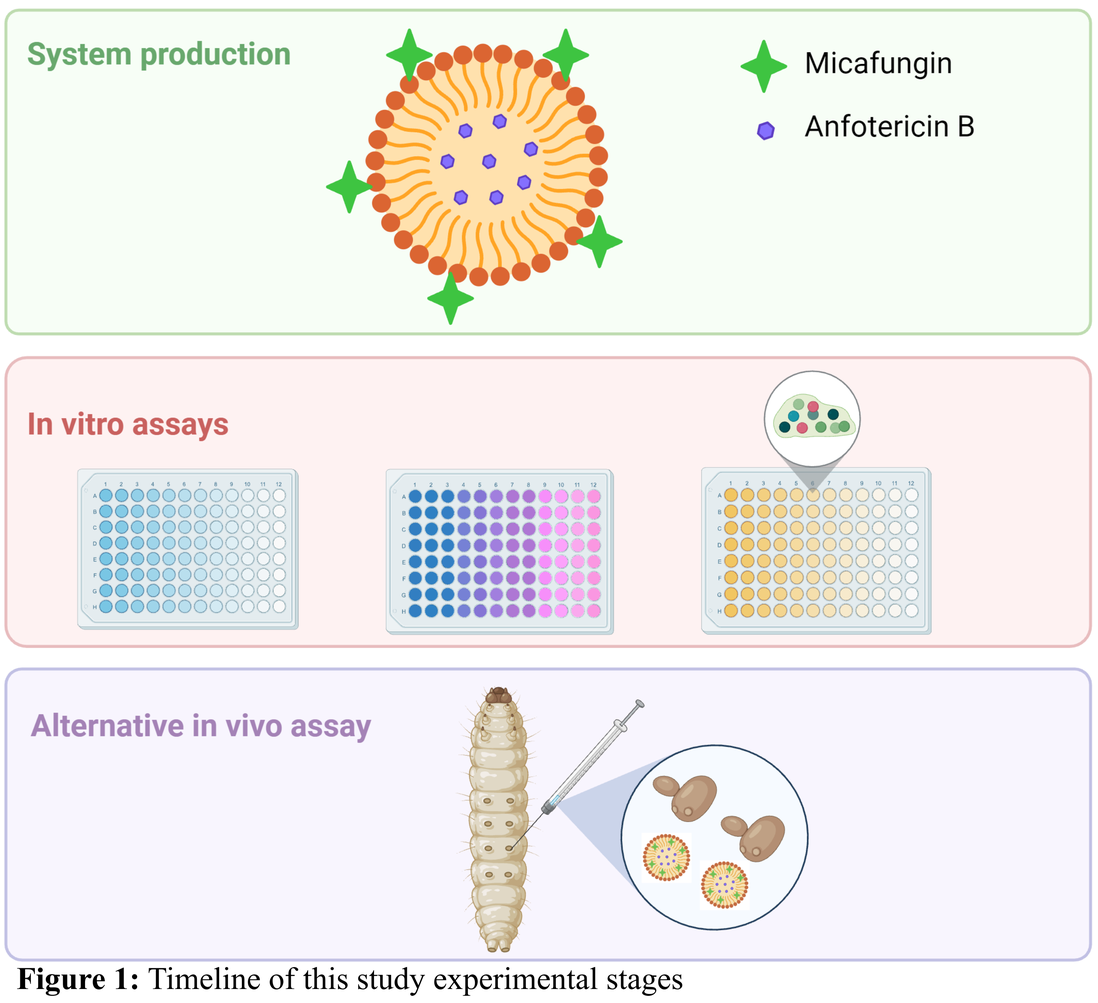

Figure 1: Timeline of this study experimental stages

Results

The mean MIC values for the tested strains were 0.7 µg/mL for amphotericin B and 3.0 µg/mL for micafungin. Checkerboard assay showed drug synergism for all strains once the mean combined concentrations capable to inhibit 90% of growth was 0.07 µg/mL for amphotericin B and 0.301 µg/mL for micafungin. All concentrations of the free or loaded drug combination (50 µg/mL to 0.78 µg/mL), significantly inhibited biofilm metabolic activity, except VENC6 at the lowest NEMA concentration tested.

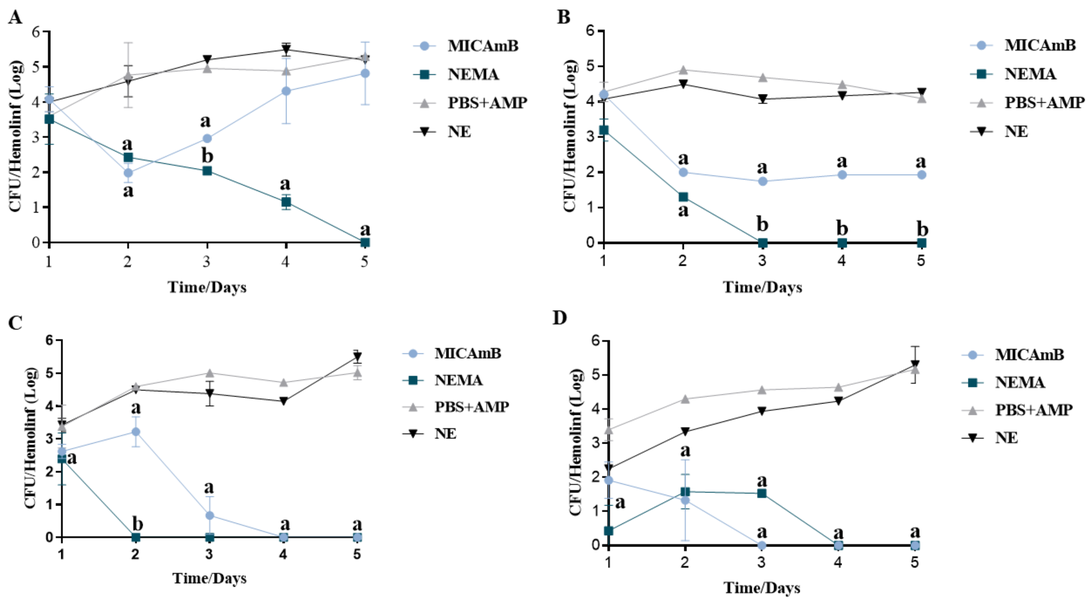

Figure 2: Fungal load in hemolymph after treatment with MICAmB and NEMA in infected *Galleria mellonella* Legend: A: InP13 (clade I); B: JAP 1 (clade II); C: SP96 (clade III); D: VEN C6 (clade IV); MICAmB: Micafungin + Amphotericin B; NEMA: Nanoemulsion dual-loaded with micafungin + amphotericin B; NE: Nanoemulsion; AMP: Ampicillin; absence of letters: no statistical difference; presence of letters (a and b) statistical difference compared to infection control groups; different letters: statistical difference between infection control group and treatment group

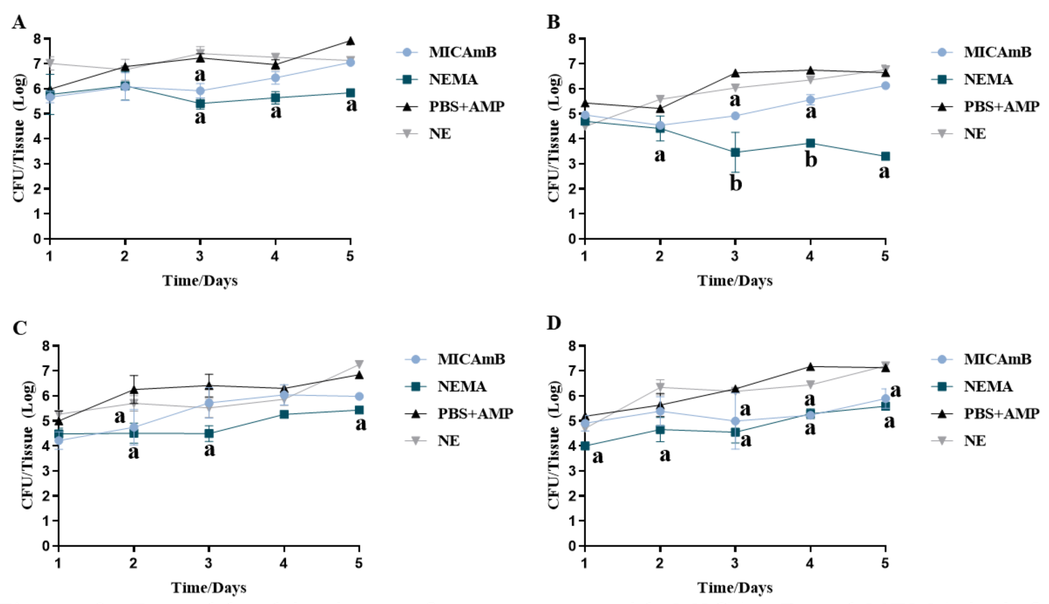

Figure 3: Fungal load in tissue after treatment with MICAmB and NEMA in infected *Galleria mellonella* model Legend: A: InP13 (clade I); B: JAP 1 (clade II); C: SP96 (clade III); D: VEN C6 (clade IV); MICAmB: Micafungin + Amphotericin B; NEMA: Nanoemulsion dual-loaded with micafungin + amphotericin B; NE: Nanoemulsion; AMP: Ampicillin; absence of letters: no statistical difference; presence of letters (a and b) statistical difference compared to infection control groups; different letters: statistical difference between infection control group and treatment group

Conclusion

Amphotericin B and micafungin combination demonstrated a potential synergistic effect against *C. auris*, justifying their co-encapsulation in a NE. Furthermore, dual loading potentiated the drugs anti-fungal activity in both in vitro and in vivo assays. So, amphotericin B and micafungin dual-loading may represent a promising hope for the control of systemic infections caused by *C. auris*. However, further studies are needed to reinforce the conclusions of this study.

Acknowledgments

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References

[1] Marena, G.D., et al., Mycosphere. 2022; [2] 820-861;2-Marena, G.D., et al., Biofouling. 2024: 602-616; [3] Marena, G.D., et al., A. Pathogens. 2024: 549; [4]Marena, G.D., et al., Microorganisms. 2023: 1626.