Pan-resistant *Candida auris*: New York Sub-cluster Susceptible to Antifungal Combinations

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Abstract

Four pan-resistant *Candia auris* strains from New Yrok outbreak were 100% inhibited *in vitro* by combinations of two antifungal drugs using fixed concentrations achievable *in vivo*. Pan-resistant *C. auris* strains have mutations in eleven gene targets associated with major antifungal drugs, and constituted a distinct sub-cluster among NY strains.

We recently reported the emergence of pan-resistance in *Candida auris* from New York [1]. Since 2016, New York hospitals and healthcare facilities have faced the highest number of clinical and surveillance cases of *C. auris* in the United States[2]. Effective strategies for the prevention, control, and treatment of *C. auris* are still being developed, all of which could be complicated by the observed pan-resistance. A conceptual framework supports deploying drug combinations to combat the threat of antimicrobial resistance[3]. Therefore, we studied if strains of pan-resistant *C. auris* are susceptible to combinations of current antifungal drugs and what genetic features distinguish NY pan-resistant *C. auris*. Details of methods are provided in the Supplementary Appendix. Four pan-resistant *C. auris* strains were 100% inhibited *in vitro* by combinations of two antifungal drugs using fixed concentrations achievable *in vivo*. Expectedly, flucytosine combinations with amphotericin B, azoles, or echinocandins were most effective (Figure 1A). In time-kill analysis, two-drug combinations caused greater than two log reduction in growth relative to the respective single drugs suggestive of a fungicidal action (Figure 1B). The results are consistent with our recent publication on the efficacy of antifungal combinations for NY *C. auris* strains with various multi-drug resistance patterns. Based on a comparative genomic analysis, we found four pan-resistant *C. auris* strains have mutations in eleven gene targets associated with major antifungal drugs. These findings are similar to other reports for drug-resistant *C. auris* strains. All four pan-resistant strains constituted a distinct sub-cluster among NY strains (Figure 1C) [4,5]. Two different, non-synonymous mutations in the predicted sequence of FKS1p were observed. *C. auris* 19-4 and 19-61 exhibited FKS1 S635P,

whereas *C. auris* 19-42 and 19-43 showed FKS1 S635Y (Figure 1D). These mutations are in a known hotspot of FKS1, a glucan synthase gene, and the target of echinocandin antifungal drugs. Finally, pan-resistance appears to exact fitness cost in at least two *C. auris* strains (19-42 and 19-43), which showed a prolonged lag growth phase (Figure 1E); these strains have a high resistance to caspofungin (>16 mg/L). Further results are presented in the Supplementary Appendix. Our findings suggest that pan-resistant *C. auris* strains remain susceptible to antifungal combinations, which might help expand the available therapeutic options. The genetic analysis suggests ongoing mutations in response to antifungal drug pressure are the likely drivers of emerging pan-resistance in NY *C. auris* strains.

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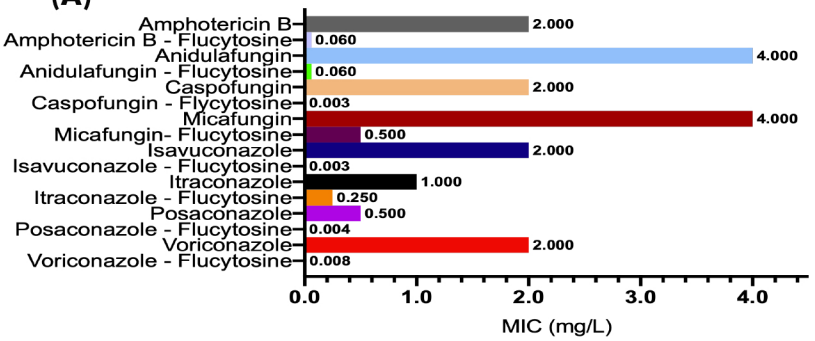
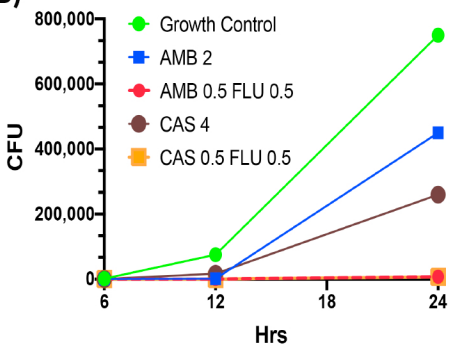
Figure 1. Characterization of pan-resistant *Candida auris*. (A) Pan-resistant *C. auris* strains are susceptible to two-drug combinations containing flucytosine and amphotericin B, azole or echinocandins, data for C. auris 19-4, (B) A representative time-kill curve with amphotericin B or caspofungin alone or in combination with flucytosine. Two-drug combinations caused three log reduction in growth of *C. auris* 19-4 relative to either drug alone, (C) Four pan-resistant *C. auris* strains (19-4, 19-42, 19-43, 19-61) have gene variants associated with resistance to a variety of antifungal drugs; Notably, mutations in the hotspot of glucan synthase gene FKS1, the target of echinocandins, as found in *C. auris* 19-4, 19-42, 19-43, and 19-61. (D), (F) A profound lag in growth of two pan-resistant strains, *C. auris* 19-42 and *C. auris* 19-43 suggesting a fitness cost.

Figure 1

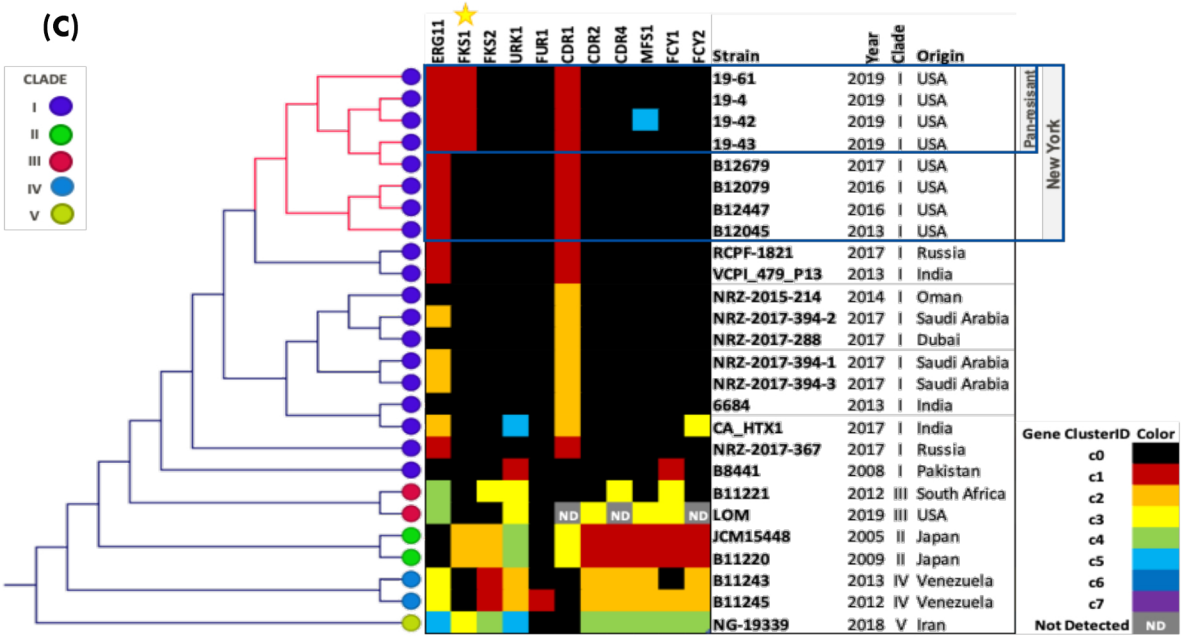
(A)



(B)

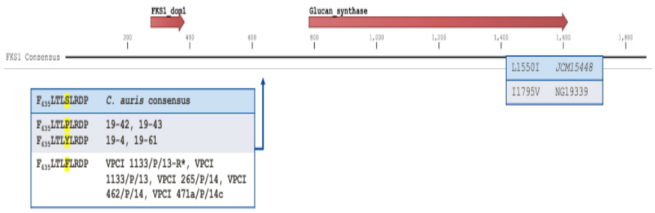
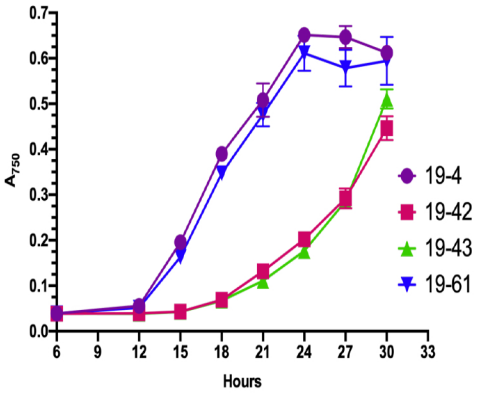


(C)



2

(D)



(E)