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 York 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). These 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 (Figure 1C). These findings are similar to other reports for drug-resistant *C. auris* strains. 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. All four pan-resistant strains constituted a distinct sub-cluster among NY strains (Figure 1E) [4,5]. 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 1F); 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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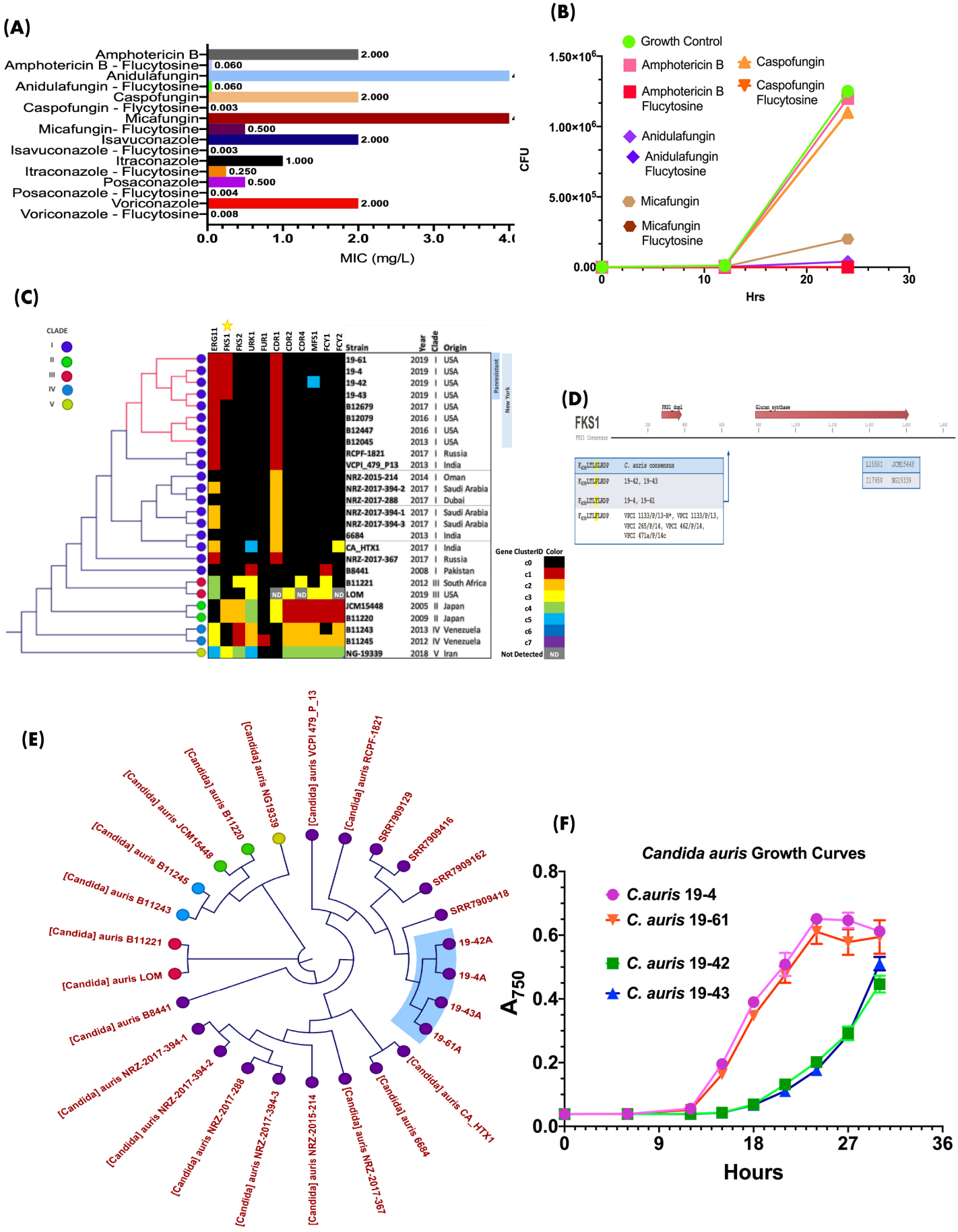
Figure 1. Characterization of pan-resistant *Candida auris*. (A) Pan-resistant *C. auris* strains are susceptible to two-drug combinations containing either amphotericin B, an azole or an echinocandin in combination with flucytosine; representative data shown for *C. auris* 19-4, (B) Time-kill curve with single drug alone or two-drug combination with flucytosine. Two-drug combinations caused more than two log reduction in growth; representative data shown for *C. auris* 19-43, (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, (D) Notably, mutations in the hotspot of glucan synthase gene FKS1, the target of echinocandins are found in *C. auris* 19-4, 19-42, 19-43, and 19-61, (E) Four pan-resistant *C. auris* strains constituted a distinct sub-cluster among NY strains; the unrooted Neighbor-Joining tree was derived from whole genome assemblies, (F) A profound lag in growth of two pan-resistant strains, *C. auris* 19-42 and *C. auris* 19-43 suggesting a fitness cost; RPMI broth at 35℃.

Figure 1


(A)
Amphotericin B 2.000
Amphotericin B - Flucytosine 0.060
Anidulafungin
Anidulafungin - Flucytosine 0.060
Caspofungin 2.000
Caspofungin - Flycytosine 0.003
Micafungin
Micafungin- Flucytosine 0.500
Isavuconazole 2.000
Isavuconazole - Flucytosine 0.003
Itraconazole 1.000
Itraconazole - Flucytosine 0.250
Posaconazole 0.500
Posaconazole - Flucytosine 0.004
Voriconazole 2.000
Voriconazole - Flucytosine 0.008
0.0 1.0 2.0 3.0
MIC (mg/L)
(B)
Growth Control
Amphotericin B
Amphotericin B Flucytosine
Anidulafungin
Anidulafungin Flucytosine
Micafungin
Micafungin Flucytosine
Caspofungin
Caspofungin Flucytosine
1.50×10^6
1.00×10^6
5.00×10^5
0.00
CFU
0 10 20 30
Hrs
(C)
CLADE
I
II
III
IV
V
ERG11 FKS1 FKS2 URK1 FUR1 CDR1 CDR2 CDR4 MFS1 FCY1 FCY2
Strain Year Clade Origin
19-61 2019 I USA
19-4 2019 I USA
19-42 2019 I USA
19-43 2019 I USA
B12679 2017 I USA
B12079 2016 I USA
B12447 2016 I USA
B12045 2013 I USA
RCPF-1821 2017 I Russia
VCPI_479_P13 2013 I India
NRZ-2015-214 2014 I Oman
NRZ-2017-394-2 2017 I Saudi Arabia
NRZ-2017-288 2017 I Dubai
NRZ-2017-394-1 2017 I Saudi Arabia
NRZ-2017-394-3 2017 I Saudi Arabia
6684 2013 I India
CA_HTX1 2017 I India
NRZ-2017-367 2017 I Russia
B8441 2008 I Pakistan
B11221 2012 III South Africa
LOM 2019 III USA
JCM15448 2005 II Japan
B11220 2009 II Japan
B11243 2013 IV Venezuela
B11245 2012 IV Venezuela
NG-19339 2018 V Iran
Pansensitant
New York
ND
Gene ClusterID Color
c0
c1
c2
c3
c4
c5
c6
c7
Not Detected
(D)
FKS1
FKS1_domi
Glucan_synthase
FKS1 Consensus
C. auris consensus
19-42, 19-43
19-4, 19-61
VPCI 1133/P/13-R*, VPCI 1133/P/13, VPCI 265/P/14, VPCI 462/P/14, VPCI 471a/P/14c
L15S01 JCM15448
I1795V NG19339
(E)
[Candida] auris NG19339
[Candida] auris VCPI 479_P_13
[Candida] auris RCPF-1821
[Candida] auris B11220
[Candida] auris JCM15448
[Candida] auris B11245
[Candida] auris B11243
[Candida] auris B11221
[Candida] auris LOM
[Candida] auris B8441
[Candida] auris NRZ-2017-394-1
[Candida] auris NRZ-2017-394-2
[Candida] auris NRZ-2017-288
[Candida] auris NRZ-2017-394-3
[Candida] auris NRZ-2015-214
[Candida] auris NRZ-2017-367
[Candida] auris 6684
[Candida] auris CA_HTX1
SRR7909129
SRR7909416
SRR7909162
SRR7909418
19-42A
19-4A
19-43A
19-61A
(F)
Candida auris Growth Curves
C.auris 19-4
C. auris 19-61
C. auris 19-42
C. auris 19-43
A750
0.8
0.6
0.4
0.2
0.0
0 9 18 27 36
Hours