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Multidrug-resistant *Candida auris* Isolates from New York Outbreak are Highly Virulent & Responsive to Antifungal Treatment in a *Galleria mellonella* Experimental Model

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Abstract

Background: *Candida auris* causes prolonged colonization and bloodstream infections in hospitalized patients. Different *C. auris* clades vary in their geographic origin, disease spectrum, and antifungal resistance but the biological basis underlying such variations needs further investigation. In this study, we used wax moth *Galleria mellonella* as a model system to understand the virulence and antifungal susceptibility profile of *C. auris* South Asia Clade I and East Asia Clade II as part of the New York outbreaks.

Methods: Six clinical isolates of *C. auris,* including five South Asia (Clade I), and one East Asia (Clade II) were selected for this study. They also vary in their primary source and antifungal susceptibility profiles. These strains were cultured, dilution series were prepared with phosphate buffered saline (PBS), and 10 µl containing 10^4 to 10^7 CFU were injected into the proleg of *Galleria mellonella* larvae using a VICI Pressure-Lok C-160 liquid microsyringe. These larvae were incubated at 37ºC for 10 days to determine survival curve, host pigmentation, in vivo growth of *C. auris* in larvae hemolymph, and overall larvae tissue response against *C. auris*. The larvae injected with three South Asia (Clade I) isolates were treated with drug combinations with fluconazole (FLC) and monitored their survival over 10-day period. Additionally, larvae were injected with two pan-drug resistant isolates of South Asia Clade I and treated with single drug to evaluate fitness and survival of infected versus treated larvae.

Results: We found South Asia Clade I to be more virulent than the East Asia Clade II in the *Galleria mellonella* model system based on survival curve, significant induction of pigmentation in larvae, increased CFU in the hemolymph, intense tissue and immune responses. Overall, the drug combination improved larvae survival and health index (pigmentation, cocoon formation and mobility) infected with South Asia (Clade I) isolates compared to untreated control larvae. The larvae infected with pan-resistant isolates when treated with individual antifungal drugs, also showed improved survival.

Conclusions: Overall, our results indicate that *G. mellonella* serves as an important invertebrate model system to understand virulence of *Candida auris.* Additionally, it can also be utilized for in vivo understanding of drug interaction with *C. auris*.

Introduction

Candida auris is a multidrug-resistant fungal pathogen classified as an urgent public health threat by the Centers for Disease Control and Prevention (CDC). Since its first outbreak in 2009, it has been reported from more than 40 countries and 6 continents with mortality rate of 66% [1]. It was first identified in the United States in 2013 and first detected in New York State in 2016. As of 2020, CDC confirmed 1,678 clinical cases in the United States and approximately 55% were reported from New York and New Jersey[2]. Thus, *C. auris* is considered a serious global public health threat because of its multidrug resistance, nosocomial outbreak potential, healthcare and intensive care unit-associated transmission and the ability to persist for long durations in the hospital, nursing home and clinical environments[3].

To better understand *C. auris* infection and host response, we need to explore host defense mechanisms and pathogen virulence properties as well as the response to different drug treatment. It is also extremely important to gain molecular insight of host-pathogen interactions for developing new strategies to prevent and control *C. auris* infection. As a mammalian model systems, the mouse is a remarkable, well characterized, and a reproducible model system for understanding fungal pathogenesis[4]. However, the potential drawback of this model system is that it requires special sets of skills, instruments and high cost[5]. Therefore, in this study, we investigated the suitability of *G. mellonella* as an invertebrate model system to understand *C. auris*-host interactions at the cellular and molecular levels. Additionally, we have investigated if *G. mellonella* can be used as a host to understand drug treatment against *C. auris* infection.

Acknowledgments

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Methods

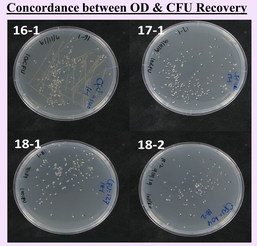
***C. auris* Isolates with Antifungal Resistance Profile**

Isolates	Clade	Source	MALDI-TOF	VOR	AND	CAS	FZ	IZ	ISA	PZ	MF	AP	FC
16-1	East Asia	Ear	*C. auris* (2.014/2.00)	0.25	0.12	0.12	8	0.5	0.25	0.25	0.12	0.5	0.5
17-1	South Asia	Blood	*C. auris* (2.383/2.53)	2	4	4	>256	0.5	0.5	0.25	4	1	0.094
18-1	South Asia	Wound swab	*C. auris* (2.22/2.27)	0.5	0.25	0.03	128	0.12	0.12	0.03	0.12	1	>32
18-2	South Asia	Ascites fluid	*C. auris* (1.95/2.23)	2	8	2	>256	1	1	0.25	4	1.5	0.064
20-32	South Asia	Abdomen tissue	*C. auris* (2.52/2.59)	8	4	16	>256	1	4	1	8	2	8
20-34	South Asia	Nares	*C. auris* (2.50/2.45)	2	0.25	0.25	>256	1	2	0.5	0.12	1	0.094

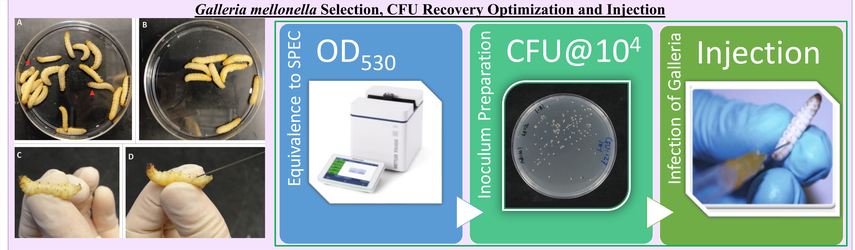
Equivalence CFU and OD_{530}

CFU	OD /Trans(16-1)	OD /Trans(17-1)	OD /Trans(18-1)	OD /Trans(18-2)
$1*10^4$	0.020/95.7%	0.019/96.0%	0.020/95.6%	0.019/95.0%
$1*10^5$	0.025/94.1%	0.026/94.5%	0.026/94.0%	0.26/94.2%
$1*10^6$	0.095/81.0%	0.096/80.0%	0.095/81.0%	0.096/81.3%
$1*10^7$	0.25/55.7%	0.24/56.0%	0.24/56.8%	0.25/56.0%
$1*10^8$	0.45/34.8%	0.44/35.6%	0.43/36.0%	0.44/36%
$1*10^9$	1.15/15.1%	1.10/15.3%	1.20/14.7%	1.19/14.3%

Concordance between OD & CFU Recovery



***Galleria mellonella* Selection, CFU Recovery Optimization and Injection**



Results

Kaplan-Meyer Survival Curve

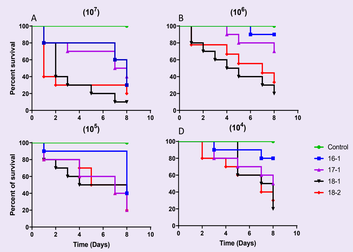


Figure 1. Thirty *G. mellonella* larvae were infected with different *C. auris* isolates ranging from 10^4 to 10^7 cells. A group of 10 *G. mellonella* larvae were injected with PBS, which served as control. All larvae were placed at 37°C incubator for 10-days to monitor survival. Figures A to D represent Kaplan-Meyer survival curve with distinct differences within doses and strains tested ($p<0.0001$). Overall, results revealed that East Asia Clade II (16-1) was low in virulence than South Asia Clade I (17-1, 18-1, and 18-2).

***G. mellonella* Melanization**

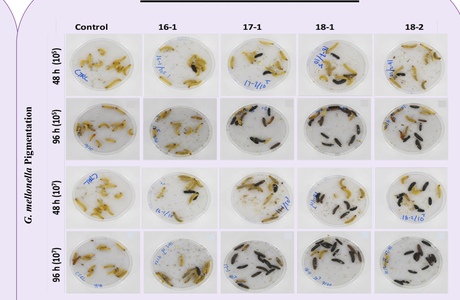


Figure 2. Visual appearance of *G. mellonella* larvae infected with different stains of *C. auris* at a dose of 10^5 and 10^7 cells. The larvae injected with PBS served as control. The degree of melanization was higher in larvae infected with South Asia Clade I (17-1, 18-1, and 18-2) than melanization observed in East Asia Clade II (16-1).

Immuno-genes Expression in *G. mellonella*

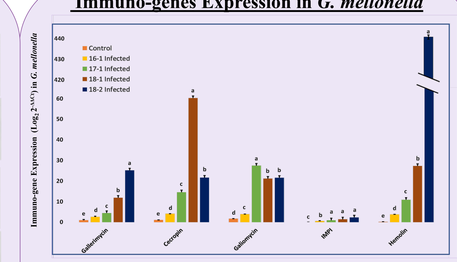


Figure 3. Quantitative RT-PCR analysis of immuno-genes (*ceropin*, *galiomycin*, *gallerimycin*, *IMPI*, and *hemolin*) transcripts in total RNA isolated from the *Galleria mellonella* larvae at 24-h post infection with 10^7 cells of different *Candida auris* isolates. The expression of each gene was normalized to the expression of the housekeeping gene. The immune response gene expression was significantly enhanced ($p < 0.05$) against larvae infected with South Asia Clade I isolates compared to the East Asia Clade II isolate.

***C. auris* CFU Load in Hemolymph**

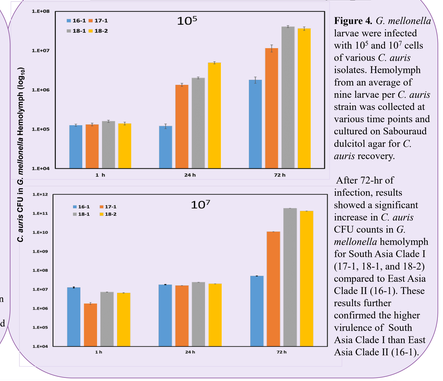


Figure 4. *G. mellonella* larvae were infected with 10^5 and 10^7 cells of various *C. auris* isolates. Hemolymph from an average of nine larvae per *C. auris* strain was collected at various time points and cultured on Sabouraud dulcitol agar for *C. auris* recovery.

After 72-hr of infection, results showed a significant increase in *C. auris* CFU counts in *G. mellonella* hemolymph for South Asia Clade I (17-1, 18-1, and 18-2) compared to East Asia Clade II (16-1). These results further confirmed the higher virulence of South Asia Clade I than East Asia Clade II (16-1).

Histopathology of *G. mellonella*

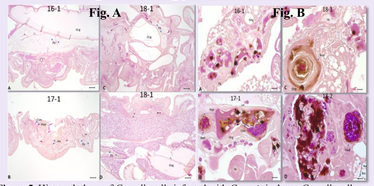


Figure 5. Histopathology of *G. mellonella* infected with *C. auris* isolates. *G. mellonella* were infected with low (10^5) and high (10^7) doses of *C. auris*. At a low dose (Fig. A) of *C. auris* infection, cross-sections of *G. mellonella* organelles (Org) showed fat bodies (Fb) with minor amount of melanization in the hemocoel and granuloma-like hemocyte nodules formation (arrow heads). At the high dose (Fig. B) of *C. auris* infection in *G. mellonella,* there are numerous lakes of dense eosinophilic material with melanin deposition (*) around organelles (Org) and expanding in the fat body (Fb), hemolymph lake with numerous PAS-positive hyphae (Hyp) and conidia (Con) with granuloma-like hemocyte nodules (arrow heads) with prominent melanin deposition expanding in the wall of organelle (Org). Overall, South Asia Clade I (17-1, 18-1 and 18-2) isolates caused severe organelle damage in *G. mellonella* compared to East Asia Clade II (16-1) isolate (Image size:50x; scale 10µm).

Antifungal Combination Improved *C. auris* Infected *G. mellonella* Survival

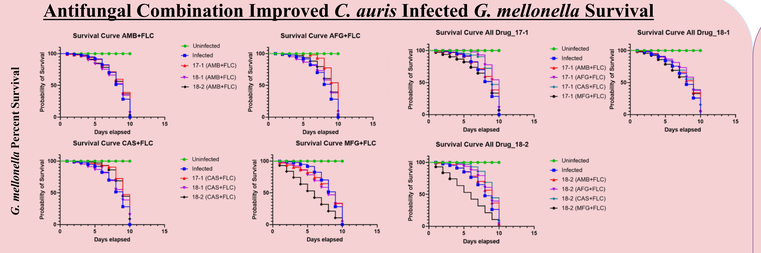


Figure 6. Five antifungal drugs (AMB, AFG, CAS, MFG, and FLC) were selected for the drug combination study. The final working drug combination was AMB+FLC, (0.06+1) mg/L ; AFG+FLC (0.12+1) mg/L ; CAS+FLC (0.12+1) mg/L ; and MFG (0.12+1) mg/L. A group of 10 *G. mellonella* larvae was infected with 10^5 cells of *C. auris* (17-1, 18-1, and 18-1) for each drug combination. The controls included a set of 10 larvae injected with 10^7 cells of *C. auris* as infected control and ten larvae injected with PBS as uninfected control. All larvae were incubated at 37°C for 10-days to monitor survival curve. The results revealed that drug combination improved the survival of *C. auris* infected larvae compared to the untreated but infected larvae.

Antifungal Enhanced *G. mellonella* Health Score *&* Survival

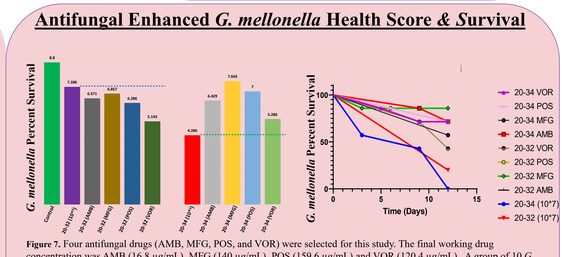


Figure 7. Four antifungal drugs (AMB, MFG, POS, and VOR) were selected for this study. The final working drug concentration was AMB (16.8 µg/mL), MFG (140 µg/mL), POS (159.6 µg/mL) and VOR (120.4 µg/mL). A group of 10 *G. mellonella* larvae was infected with 10^5 cells of fluconazole-resistant *C. auris* (20-34) or pan-drug-resistant *C. auris* (20-32) for each drug treatment. The controls included a set of 10 larvae either injected with PBS as uninfected or infected with 20-34 or 20-32 without drug treatment. All *G. mellonella* larvae were incubated at 37°C for 10-days to monitor health index (pigmentation, cocoon formation, and mobility) and survival rate. The drug treatment helped the overall health index of larvae infected with 20-34 but not with 20-32. These results revealed that the antifugals used in the study were effective against fluconazole-resistant but not against pan-drug-resistant *C. auris* isolate.

Conclusions

We established *Galleria mellonella* as a model system to study *Candida auris* virulence and drug interaction studies. The South Asia Clade I was highly virulent, while East Asia Clade II was least virulent. The single or two-drug combination improved the survival and health of larvae infected with fluconazole-resistant or susceptible *C. auris* isolates but not the survival or heath of larvae infected with pan-drug-resistant isolate. These results suggest that *G. mellonella* can be used as an important invertebrate model system to understand the virulence of *Candida auris*. Additionally, it can also be utilized for in vivo understanding of drug interaction with *C. auris*.

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